RNA 2018

The 23rd Annual Meeting of the RNA Society

PROGRAM & ABSTRACTS

May 29 – June 3, 2018
University of California, Berkeley

Adrian Ferré-D'Amaré — *National Institutes of Health, Bethesda*
Atlanta Cook — *Wellcome Centre for Cell Biology, Edinburgh*
Anne Ephrussi — *EMBL, Heidelberg*
Don Rio — *University of California, Berkeley*
Mihaela Zavolan — *Biozentrum, University of Basel*

GENERAL INFORMATION

Throughout the Program listing, the numbers next to the titles refer to corresponding oral or poster abstract numbers in the Abstract section of this book. These abstracts should not be cited in bibliographies. Material contained herein should be treated as personal communication, and should be cited only with the consent of the author.

NO UNAUTHORIZED PHOTOGRAPHY IN SESSIONS: To encourage sharing of unpublished data at the RNA Society Meeting, taking of photographs and/or videos during scientific sessions (oral or poster), or of posters outside of session hours, is strictly prohibited. Violators of this policy may have their equipment confiscated (cameras, cell phones, etc.) and/or they may be asked to leave the conference and have their registration privileges revoked without reimbursement.

USE OF SOCIAL MEDIA: The official hashtag of the 23rd Annual Meeting of the RNA Society is #RNA2018. The organizers encourage attendees to tweet about the amazing science they experience at the meeting, so that those who could not come to Berkeley can join in from afar. However, please respect these few simple rules when using the #RNA2018 hash tag or talking about the meeting on Twitter and other social media:

1. Be polite and respectful of others in all of your messages.
2. Do not transmit photographs of slides or posters under any circumstances.
3. Do not transmit photographs of conference attendees without their clear consent.
4. Tweeters should respect requests of presenters who ask attendees to refrain from tweeting the content of their talks and posters.

Front cover image: The front page shows a sunset view of the city of Berkeley, California. The tower in the foreground is the famed Campanile on the University of California, Berkeley, campus. The water in the background is San Francisco Bay, with the Bay Bridge (left), San Francisco skyline (center left), and Golden Gate Bridge (right). Photo by Adrian Ferré-D'Amaré.

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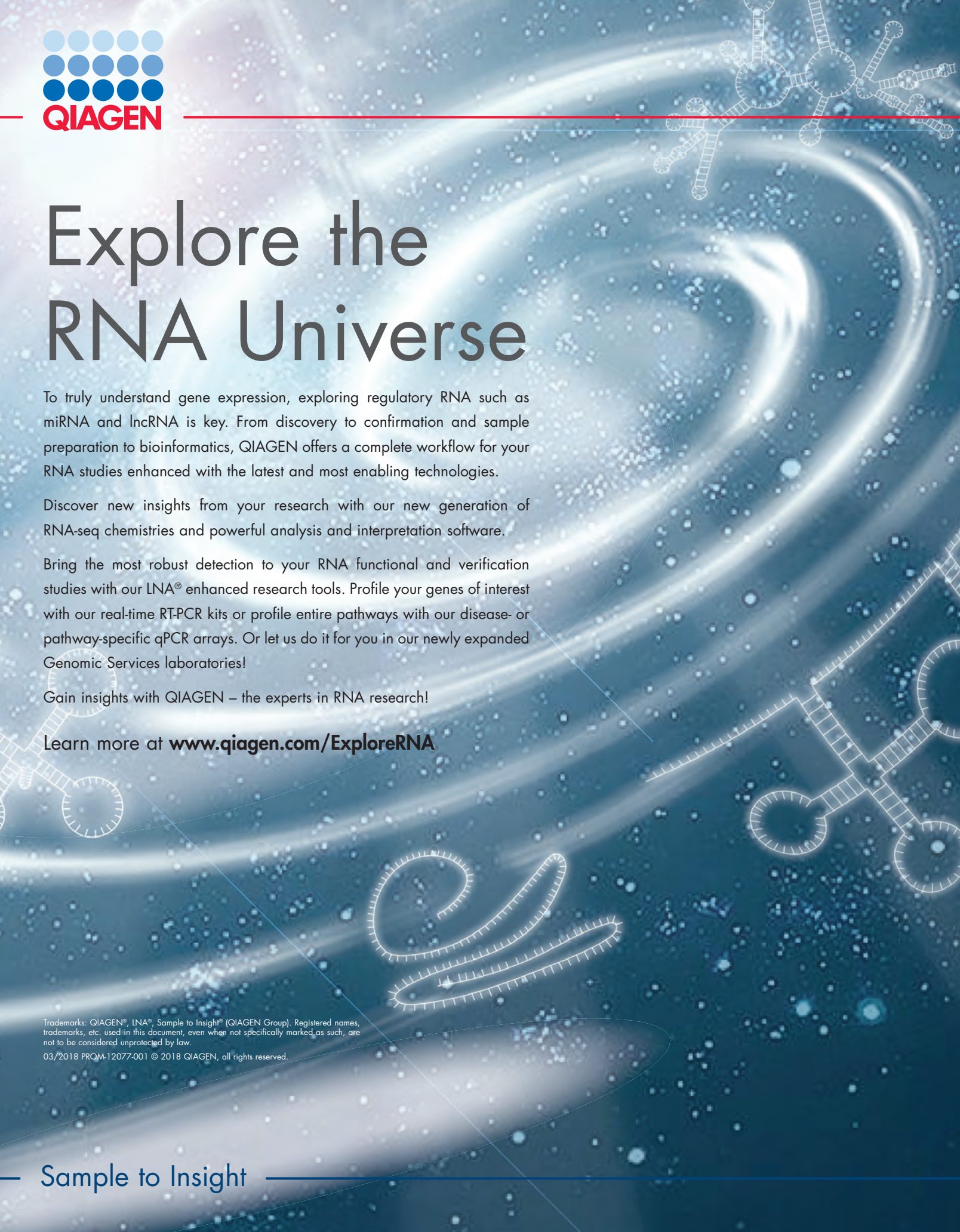
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RNA 2019

The 24th Annual Meeting of the RNA Society will be held in Krakow, Poland from June 11 to June 16, 2019, at the ICE Kraków Congress Centre.

2019 Organizers

Witold Filipowicz, *Friedrich Miescher Institute, Germany*
Brenda Bass, *University of Utah, USA*
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March 21, 2018

Juan Valcárcel
Centre de Regulacio Genomica
Dr Aiguader 88
ES-08003 Barcelona
Spain

Dear Participants,

It is my great pleasure and honor to remind you that this year is the RNA Society's 25th birthday as a non-profit legal entity. It has been a long road built on community effort– and inspired by the scientific vision of the Society's founders, Tom Cech, Joan Steitz and Olke Uhlenbeck. That effort has shown that RNA research is of wide interest, not only to provide key scientific insights into cellular function, but also–in due time–to generate biotechnological products and therapeutic applications. Clearly, this is best accomplished through intellectual boldness and rigorous science, but also through personal friendship– and fun. These values remain at the heart of the Society, and we strive to maintain them as our defining principles.

Enjoy the meeting!

Juan Valcárcel
RNA Society President 2017-2018

Invitation to Membership

The RNA Society was established in 1993 to facilitate sharing and dissemination of experimental results and emerging concepts in RNA science. The Society is an interdisciplinary, cohesive intellectual home for those interested in all aspects of RNA research. We welcome new members from all disciplines and we look forward to sharing the new perspectives they bring to the Society.

Our members work in numerous areas of RNA science including but not limited to:

RNAi and miRNA
Ribosomes and Translation Regulation
Splicing Regulation and Alternative Splicing
RNA Turnover and Surveillance
Integration of Nuclear Gene Expression Processes
RNA Regulation in Neurons and Specialized Cells
RNA Structure and Folding
RNA and Disease: Therapeutic Strategies
Viral RNA Mechanisms
Methods in RNA and RNP Research
Noncoding RNA
Splicing Mechanisms
3'End Formation and Riboregulation of Development
RNA Transport and Localization
RNP Biosynthesis and Function
RNP Structure and RNA-Protein Interactions
RNA Catalysis
Heterochromatin Silencing
Telomerases
Bioinformatics

Benefits of RNA Society membership include:

- Print or on-line subscription to the RNA Society journal, *RNA* (IF 6.05)
- Reduced charges for those who publish in *RNA*
  - o A $500 discount on the manuscript publication fee ($1000 instead of $1500)
  - o An additional $500 discount for those members who wish to provide open access to their articles immediately upon publication ($1500 instead of $2000)
  - o Free color figures
- Reduced registration fees for the annual meeting of the Society (a savings of ~$200)
- Access to the *RNA Society Newsletter*, a biannual forum for disseminating information to members and discussing issues affecting the Society and RNA science
- Numerous professional development opportunities for junior scientists and the potential for greater involvement in the RNA Society
- Access to a Directory of Members (available on-line)
- Free job postings on the RNA Society website
- Opportunities to request Travel Fellowships to the RNA Society Annual Meeting, as well as financial support for RNA-related conferences and events organized by you

These benefits more than offset the cost of a one-year RNA Society membership. Additionally, two- and three-year memberships (as well as a lifetime membership) are also available at a further discounted rate. Please see our on-line membership registration system for full details.

Please take a moment to start or renew your RNA Society membership at
http://rnasociety.org/become-a-member

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NOTES

PROGRAM–RNA 2018

The 23rd Annual Meeting of the RNA Society
University of California, Berkeley
May 29 – June 3, 2018

(See building locations on the campus map at the back of the book.)

Tuesday May 29

14:00 – 20:00	**Registration**	Zellerbach Lobby
16:30 – 19:00	**Welcome reception** *Featuring music by Harry Noller and the RiboBand*	Pauley Ballroom & Patio
19:00 – 21:30	**Opening session: Award talks** (1-5) *Scaringe Award: Fuguo Jiang, University of California Berkeley* *Scaringe Award: Madeline Sherlock, Yale University* *Scaringe Award: Boxuan Zhao, Stanford University* *Early-Career Award: Andrei Korostelev, UMass Medical School* *Mid-Career Award: Erik Sontheimer, UMass Medical School*	Zellerbach Auditorium

Wednesday May 30

08:00 – 08:45	**Sponsored Seminar** [page 25] *Sponsored by Oxford Nanopore Technologies*	Berkeley City Club
08:00 – 18:00	**Registration**	Zellerbach Lobby
09:00 – 10:15	**Plenary session 1: Splicing** (6-14) *Chair: Patricia Coltri, Universidade de São Paulo*	Zellerbach Auditorium
10:15 – 10:45	Coffee break	Zellerbach Lobby
10:45 – 11:45	**Plenary session 1 continues**	
11:45 – 12:30	**Keynote 1** (15) *Mikiko Siomi,University of Tokyo*	Zellerbach Auditorium
12:30 – 14:00	Lunch	Crossroads Dining Hall
14:00 – 15:15	**Concurrent session 1: RNA Turnover** (16-24) *Chair: Katherine Berry, Mount Holyoke College*	Zellerbach Playhouse
	Concurrent session 2: Regulatory RNAs (25-34) *Chair: Ayelet Lamm, Technion - Israel Institute of Technology*	Zellerbach Auditorium
15:15 – 15:45	Coffee break	Zellerbach Lobby
15:45 – 16:45	**Concurrent sessions 1 and 2 continue**	
16:45 – 17:00	Break	

Note: Numbers in parenthesis in session listings correspond to abstract numbers.

17:00 – 18:30	**Workshop 1: Capturing Dynamic RNPs** (35-41) *Chair: Julian König, Institute of Molecular Biology, Mainz*	Zellerbach Auditorium
	Workshop 2: Single-cell RNAseq (44, 42, 43, 45, 46) *Chair: Jeremie Breda, University of Basel*	Hertz Concert Hall
	Workshop 3: Probing RNA Structure (49, 47, 50, 48, 51) *Chair: Sharon Aviran, University of California, Davis*	Zellerbach Playhouse
18:30 – 20:00	Dinner	Crossroads Dining Hall
18:30 – 20:00	Meetings Committee dinner/meeting	Anna Head Alumnae Hall
19:00 – 20:30	Junior Scientists Social	Tilden Room & Patio
20:00 – 22:30	**Poster session 1** *Sponsored by: MDPI Journals – Cells, Biomolecules, Non-Coding RNA*	Pauley Ballroom & Patio

Poster	Topic
166 - 175	Biology and Mechanism of Small RNAs
195 - 198	Biology and Mechanism of Transcription
210 - 213	Chemical Biology of RNA
222 - 230	Emerging and High-throughput Techniques
248 - 254	Interconnections Between Gene Expression Processes
271 - 281	Long Non-coding RNAs
303 - 306	Regulatory RNAs in Bacteria
317 - 322	Regulatory RNAs in Eukaryotes
333 - 337	Ribosome Biogenesis
347 - 349	RNA and Epigenetics
358 - 365	RNA Bioinformatics
384 - 385	RNA Catalysis
390 - 404	RNA Editing and Modification
431 - 445	RNA Structure and Folding
479 - 481	RNA Synthetic Biology and Systems Biology
486 - 491	RNA Transport and Localization
504 - 514	RNA Turnover
534 - 550	RNA-protein Interactions
584 - 597	RNAs in Disease
625 - 627	RNP Structure
632 - 650	Splicing Mechanism and Regulation
689 - 692	Therapeutic RNAs
703 - 723	Translational Mechanism and Regulation
766 - 770	tRNA
781 - 783	Viral RNAs

Thursday May 31

07:45 – 08:45	**Sponsored Seminar** [page 27] *Sponsored by Lexogen*	Berkeley City Club
08:00 – 16:00	Registration	Zellerbach Lobby
09:00 – 10:15	**Plenary session 2: From Oligo to RNP** (52-62, 135) *Chair: Andrea Rentmeister, Universität Münster*	Zellerbach Auditorium

10:15 – 10:45	Coffee break	Zellerbach Lobby
10:45 – 11:45	**Plenary session 2 continues**	
11:45 – 12:30	**Keynote 2** (63) *Geraldine Seydoux, Johns Hopkins University School of Medicine*	Zellerbach Auditorium
12:30 – 14:00	Lunch	Crossroads Dining Hall
12:30 – 14:00	Mentoring Lunch	International House and Anna Head Alumnae Hall
14:00 – 16:00	**Concurrent session 3: Splicing Mechanisms** (64-72) *Chair: Stephen Rader, University of Northern British Columbia*	Zellerbach Auditorium
	Concurrent session 4: Interconnected RNA Processes (73-81) *Chair: Mary O'Connell, CEITEC-Masaryk University*	Zellerbach Playhouse
16:00 – 16:30	Coffee break	Hertz Hall Lobby
16:30 – 18:30	**Industry session: Careers Beyond Academia** [page 23] *Organized by the RNA Society Junior Scientist Committee* *Chair: Fadi Marayati, Wake Forest University*	Hertz Concert Hall
18:30 – 20:00	Dinner	Crossroads Dining Hall
18:30 – 20:30	Board of Directors dinner/meeting	Anna Head Alumnae Hall
20:00 – 22:30	**Poster session 2**	Pauley Ballroom & Patio

Poster	Topic
176 - 185	Biology and Mechanism of Small RNAs
199 - 204	Biology and Mechanism of Transcription
214 - 217	Chemical Biology of RNA
231 - 238	Emerging and High-throughput Techniques
255 - 262	Interconnections Between Gene Expression Processes
282 - 292	Long Non-coding RNAs
307 - 311	Regulatory RNAs in Bacteria
323 - 327	Regulatory RNAs in Eukaryotes
338 - 341	Ribosome Biogenesis
350 - 353	RNA and Epigenetics
366 - 374	RNA Bioinformatics
386 - 387	RNA Catalysis
405 - 417	RNA Editing and Modification
446 - 462	RNA Structure and Folding
482 - 483	RNA Synthetic Biology and Systems Biology
492 - 497	RNA Transport and Localization
515 - 523	RNA Turnover
551 - 568	RNA-protein Interactions
598 - 611	RNAs in Disease
628 - 629	RNP Structure
651 - 669	Splicing Mechanism and Regulation
693 - 697	Therapeutic RNAs
724 - 744	Translational Mechanism and Regulation
771 - 775	tRNA
784 - 786	Viral RNAs

Friday June 1

07:30 – 08:30	**Sponsored Seminar** [page 29] *Sponsored by Cellecta*	Berkeley City Club
08:00 – 18:00	Registration	Zellerbach Lobby
09:00 – 10:15	**Plenary session 3: The Life and Times of a Ribosome** (82-92) *Chair: Kristin Koutmou, University of Michigan*	Zellerbach Auditorium
10:15 – 10:45	Coffee break	Zellerbach Lobby
10:45 – 11:45	**Plenary session 3 continues**	
11:45 – 12:30	**Keynote 3** (93) *Jonathan Weissman, University of California, San Francisco*	Zellerbach Auditorium
12.30 – 14:00	Lunch	Crossroads Dining Hall
14:00 – 15:15	**Concurrent session 5: Splicing Regulation** (94-101) *Chair: Julia Salzman, Stanford University*	Zellerbach Auditorium
	Concurrent session 6: Emerging Technologies (102-111) *Chair: Jane Jackman, Ohio State University*	Zellerbach Playhouse
15:15 – 15:45	Coffee Break	Zellerbach Lobby
15:45 – 16:45	**Concurrent sessions 5 and 6 continue**	
16:45 – 17:00	Break	
17:00 – 18:30	**Workshop 4: Single-molecule Analysis** (112-117) *Chair: Dan Larson, National Cancer Institute, NIH*	Hertz Concert Hall
	Workshop 5: RNA Editing and Modification (118-120, 149, 392, 121, 123) *Chair: Wendy Gilbert, Yale University*	Zellerbach Auditorium
	Workshop 6: Transcript Isoform Analysis (124-127) *Chair: Qingqing Wang, University of California, Berkeley*	Zellerbach Playhouse
18:30 – 20:00	Dinner	Crossroads Dining Hall
20:00 – 22:30	**Poster session 3**	Pauley Ballroom & Patio

Poster	Topic
186 - 194	Biology and Mechanism of Small RNAs
205 - 209, 795	Biology and Mechanism of Transcription
218 - 221	Chemical Biology of RNA
239 - 247	Emerging and High-throughput Techniques
263 - 270	Interconnections Between Gene Expression Processes
293 - 302	Long Non-coding RNAs
312 - 316	Regulatory RNAs in Bacteria
328 - 332	Regulatory RNAs in Eukaryotes
342 - 346	Ribosome Biogenesis
354 - 356	RNA and Epigenetics
375 - 383	RNA Bioinformatics
388 - 389	RNA Catalysis

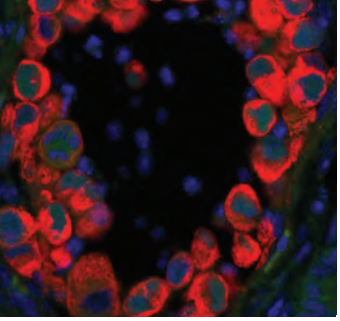
418 - 430	RNA Editing and Modification
463 - 478	RNA Structure and Folding
484 - 485	RNA Synthetic Biology and Systems Biology
498 - 503, 791	RNA Transport and Localization
524 - 533	RNA Turnover
569 - 583, 792, 793	RNA-protein Interactions
612 - 624	RNAs in Disease
630 - 631, 794	RNP Structure
670 - 688	Splicing Mechanism and Regulation
698 - 702	Therapeutic RNAs
745 - 765	Translational Mechanism and Regulation
776 - 780	tRNA
787 - 790	Viral RNAs

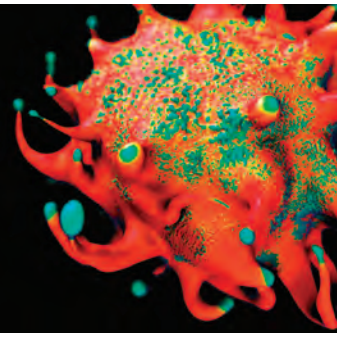
Saturday June 2

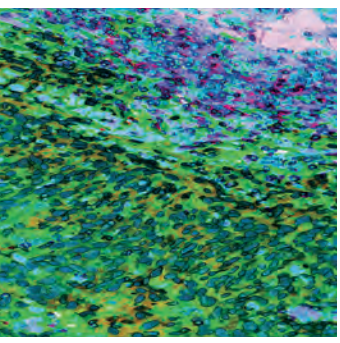
08:00 – 18:00	Registration	Zellerbach Lobby
09:00 – 10:30	**Plenary session 4: 3'-End Formation and RNA Decay** (128-134) *Chair: Bobby Hogg, National Heart, Lung and Blood Institute, NIH*	Zellerbach Auditorium
10:30 – 11:00	Coffee break	Zellerbach Lobby
11:00 – 12:30	**Plenary session 5: Coordinated RNA Processes** (136-140) *Chair: Gloria Brar, University of California, Berkeley*	Zellerbach Auditorium
12:30 – 14:00	Lunch	Crossroads Dining Hall
14:00 – 15:15	**Concurrent session 7: RNA and Disease** (141-148, 150-153) *Sponsored by Storm Therapeutics* *Chair: Julia Kenyon, University of Cambridge*	Zellerbach Auditorium
	Concurrent session 8: RNA Structure (154-165) *Chair: Yanli Wang, Institute of Biophysics, Chinese Academy of Sciences*	Zellerbach Playhouse
15:15 – 15:45	Coffee Break	Zellerbach Lobby
15:45 – 17:00	**Concurrent sessions 7 and 8 continue**	
17:00 – 17:15	Break	
17:15 – 18:45	**Awards Ceremony**	Zellerbach Auditorium
18:45 – 19:00	Break	
19:00 – 23:30	Reception/Dinner/Dance	The Faculty Club

Sunday June 3

Conference concludes







2018 SCIENTIFIC CONFERENCES

Presenting the most significant research on cancer etiology, prevention, diagnosis, and treatment

Cancer Dormancy and Residual Disease
Conference Cochairs: Julio A. Aguirre-Ghiso, Ann F. Chambers, Cyrus M. Ghajar, Christoph A. Klein, and Dorothy A. Sipkins
June 19-22, 2018 | Montreal, QC, Canada

Inaugural AACR International Meeting on Advances in Malignant Lymphoma: Maximizing the Basic-Translational Interface for Clinical Application
Scientific Committee Chair: Ari M. Melnick
June 22-26, 2018 | Boston, MA

Sixth JCA-AACR Special Joint Conference on the Latest Advances in Lung Cancer Research: From Basic Science to Therapeutics
Organizing Committee: Hiroyuki Mano, Seiji Yano, Hiroyoshi Nishikawa, Alice T. Shaw, Roy S. Herbst, and Charles M. Rudin
July 10-12, 2018 | Kyoto, Japan

Pancreatic Cancer: Advances in Science and Clinical Care
Conference Cochairs: Ronald M. Evans, Manuel Hidalgo, Steven D. Leach, Gloria M. Petersen, and Brian M. Wolpin
September 21-24, 2018 | Boston, MA

Second AACR International Conference on Translational Cancer Medicine
Conference Cochairs: Carlos L. Arteaga, Carlos Gil M. Ferreira, and Gabriel A. Rabinovich
September 27-29, 2018 | São Paulo, Brazil

Intestinal Stem Cells and Colon Cancer: Biology to Therapy
Conference Cochairs: Anil K. Rustgi, Johanna Bendell, Hans Clevers, Christina Curtis, and Owen Sansom
September 27-30, 2018 | Washington, DC

Metabolism and Cancer
Conference Cochairs: Ralph J. Deberardinis, Tak W. Mak, Joshua D. Rabinowitz, and M. Celeste Simon
September 28-October 1, 2018 | New York, NY

Fourth CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference: Translating Science into Survival
September 30-October 3, 2018 | New York, NY

EACR-AACR-ISCR Conference: The Cutting Edge of Contemporary Cancer Research
Conference Cochairs: Richard M. Marais, Eli Pikarsky, and Robert A. Weinberg
October 9-11, 2018 | Jerusalem, Israel

30th Anniversary AACR Special Conference Convergence: Systems Biology and Physical Sciences in Oncology
Conference Cochairs: Phillip A. Sharp and William C. Hahn
October 14-17, 2018 | Newport, RI

11th AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
Conference Cochairs: Laura Fejerman, Scarlett Lin Gomez, Augusto C. Ochoa, Brian M. Rivers, and Ivis Sampayo
November 2-5, 2018 | New Orleans, LA

EORTC-NCI-AACR Molecular Targets and Cancer Therapeutics Symposium
Scientific Committee Cochairs: Charles Swanton, James L. Gulley, and Antoni Ribas
November 13-16, 2018 | Dublin, Ireland

AACR-KCA Joint Conference on Precision Medicine in Solid Tumors
Program Committee Cochairs: Tae-You Kim and Charles L. Sawyers
November 15-17, 2018 | Seoul, South Korea

Tumor Immunology and Immunotherapy
Conference Cochairs: James P. Allison, Lisa M. Coussens, Ira Mellman, and Drew M. Pardoll
November 27-30, 2018 | Miami Beach, FL

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RNA 2018 AWARDS

The RNA Society Lifetime Achievement Award

The RNA Society Lifetime Achievement Award acknowledges the impact of an outstanding RNA scientist on the general scientific community. Each year, beginning in 2003, the Board of Directors has selected the recipient who receives a lifetime membership in the RNA Society in recognition of their outstanding contributions. The award is presented at the Annual RNA Meeting, where the recipient gives a special address to the RNA Society.

Previous winners include Joan Steitz (2003), Harry Noller (2004), John Abelson (2005), Christine Guthrie (2006), Walter Keller (2007), Norm Pace (2008), Thomas Cech (2009), Fritz Eckstein (2010), Witold Filipowicz (2011), Olke Uhlenbeck (2012), Phillip Sharp (2013), Reinhard Lührmann (2014), Anita Hopper (2015), Eric Westhof (2016), and Lynne Maquat (2017).

*Congratulations to **Jean Beggs**, Univ of Edinburgh-Inst of Cell Biology, who is the winner of the 2018 RNA Society Lifetime Achievement Award.*

The RNA Society Service Award

The RNA Society Service Award is given in appreciation of outstanding service to the RNA community. The overall mission of the RNA Society is to facilitate sharing and dissemination of experimental results and emerging concepts in RNA research. Each year, the Board of Directors identifies the recipient of this award who has made exemplary contributions to these goals.

Previous winners include Tim Nilsen (2003), Chris Greer (2004), Jean Beggs (2005), Olke Uhlenbeck (2006), Marvin Wickens (2007), Eric Westhof (2008), Anita Hopper (2009), Lynne Maquat (2010), Evelyn Jabri (2011), Brenda Peculis (2012), Ann Marie Micenmacher (2014), David Lilley (2015), Andrea Barta (2016), and Andrew Feig (2017).

*Congratulations to **Elizabeth Tran**, Purdue Univ, who is the winner of the 2018 RNA Society Service Award.*

The RNA Society Mid-Career Award

The RNA Society Mid-Career Award is given in recognition of scientists who have made significant contributions to their field in the first 20 years of their career as an independent investigator.

Previous winners include Karla Neugebauer (2017) and Nils Walter (2017).

*Congratulations to **Erik Sontheimer**, University of Massachusetts Medical School, who is the winner of the 2018 RNA Society Mid-Career Award.*

The RNA Society Early-Career Award

The RNA Society Early-Career Award is given in recognition of scientists who have made significant contributions to their field in the first 10 years of their career as an independent investigator.

Previous winners include Wendy Gilbert (2017) and Gene Yeo (2017).

*Congratulations to **Andrei Korostelev**, University of Massachusetts Medical School, who is the winner of the 2018 RNA Society Early-Career Award.*

The RNA Society/Scaringe Award

The RNA Society/Scaringe Young Scientist Award was established to recognize the achievement of young scientists engaged in RNA research and to encourage them to pursue a career in the field of RNA. In 2004 and 2005, the RNA Society/Scaringe Award was made to the student author(s) of the best paper, as selected by the editors, published during the previous year in RNA. The winners of the 2004 and 2005 awards were Stefano Marzi and Ramesh Pillai, respectively. In 2006, this award was revamped and opened to all junior scientists (graduate students or postdoctoral fellows) from all regions of the world who have made a significant contribution to the broad area of RNA. The award is no longer restricted to authors who have published in the RNA journal. The award includes a cash prize and support for travel and registration costs for the awardee(s) to attend the annual RNA Society meeting.

SCARINGE
Supporting the Future

Previous graduate student winners include: Jeff Barrick (2006), Malte Beringer (2007), Qi Zhang (2008), Jeremey Wilusz (2009), John Calarco (2010), Jasmine Perez (2011), Chenguang Gong (2012), Tatjana Trcek Pulisic (2012), Wenwen Fang (2013), David Weinberg (2014), Samuel Sternberg (2015), Katherine Warner (2015), Ryan Flynn (2016), Nian Liu (2016), and Malik Chaker-Margot (2017).

Previous postdoctoral fellow winners include Megan Talkington (2006), Zefeng Wang (2007), Alexei Aravin (2008), Shobha Vasudevan (2009), Luciano Marraffini (2010), Hani Zaher (2011), Kotaro Nakanishi (2012), Dipali Sashital (2012), Je-Hyun Yoon (2013), Jinwei Zhang (2014), Schraga Schwartz (2015), Basil Greber (2016), Thi Hoang Duong Nguyen (2016), and Zhipeng Lu (2017).

Congratulations to graduate students **Madeline Sherlock***, Yale University, and* **Boxuan Zhao***, University of Chicago, along with postdoctoral fellow* **Fuguo Jiang***, University of California, Berkeley, the winners of the 2018 RNA Society/Scaringe Award.*

Sponsored Poster Prizes

Biochemistry

The journal Biochemistry and ACS Publications are pleased to recognize junior scientists with six poster prizes to be awarded at RNA 2018. The prizes are for 'Excellence in RNA Research', and each includes a $250 cash award.

JBC JOURNAL OF BIOLOGICAL CHEMISTRY

The Journal of Biological Chemistry is pleased to recognize young researchers with two poster prizes to be awarded at RNA 2018. Each includes a $250 cash award.

THE EMBO JOURNAL

The EMBO Journal is pleased to sponsor a 200€ poster prize. A certificate will be awarded at RNA 2018, with the prize to be claimed directly from EMBO.

The RNA Society is pleased to provide additional poster prizes for RNA 2018, as needed, depending on the number of outstanding poster presentations. Each consists of a $250 cash award.

All graduate students and postdoctoral fellows presenting posters at the meeting are eligible for these prizes.

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ADDITIONAL SCHEDULED EVENTS AT RNA 2018

Tuesday, May 29

10:00 **Junior Scientists Pre-Conference Activity**

- Meet at Downtown Berkeley BART Station
- Open to all graduate students and postdocs. Registration required, fees payable for BART and bike rental.

The annual pre-meeting activity provides the opportunity to meet other junior scientists while exploring the beautiful city of San Francisco on a bicycle. We will take BART Red Line to Powell Street Station, where the famous cable-cars make their final stop and are manually turned, as in the old days! From there we will walk through Union Square to Blazing Saddles bike store, where our bikes are waiting for us.

The tour is a round trip to the Golden Gate Bridge by way of the Ferry Building Market, world famous Pier 39 (with its enchanting sea lions and great views of Alcatraz), Fisherman's Wharf (where you can enjoy clam chowder in sourdough bowls, or simply smell freshly baked sourdough bread) and many more historical buildings and scenic vistas until we will finally reach the top of the Golden Gate Bridge! On our way back, we will zig-zag down Russian Hill and bike through Downtown. From there you are free to continue exploring the town on your own or go back to any BART station to return to Berkeley.

The tour will conclude in time to make it back to campus for the Welcome Reception. The registration link can be found on the RNA 2018 website (Junior Scientists page) and through our social media accounts.

16:30 – 19:00 **Welcome Reception**

- Pauley Ballroom and Patio
- Open to all attendees.

Relax and reacquaint with your RNA family as we gear up for four full days of RNA science. Eat, drink, and tap your foot to the sounds of Harry Noller and the RiboBand. Who is the RiboBand?

Mario Guarneri (trumpet) is founder of the Guarneri Jazz Quartet and of the non-profit foundation Jazz in the Neighborhood in San Francisco. Mario has done everything from playing Dixieland with Louis Armstrong at the age of thirteen to performing in the Los Angeles Philharmonic for fifteen seasons under conductors Zubin Mehta and Carlo Maria Giulini. He was principal trumpet for the Los Angeles Chamber Orchestra for a decade, toured Europe with the Los Angeles Brass Quintet, and has performed with the New York Philharmonic, the San Francisco Symphony, Radio City Music Hall Orchestra, and even the Barnum & Bailey Circus.

Denny Berthiaume (piano) is founder of The Trio, a long-standing Bay Area jazz group, after performing for many years as pianist/keyboardist for Randy Masters'

group Solar Plexus, with whom he recorded 5 albums. Denny held a long-standing gig at Garden City, the premier jazz venue on the San Francisco Peninsula, while accompanying legendary jazz vocalists Diane Schuur, Rosemary Clooney and a host of others. His piano skills have also been in evidence at Bay Area productions of West Side Story, Jesus Christ Superstar, Evita and A Chorus Line.

Stephen Auerbach (bass) has performed as sideman with Claire Fischer, Laurindo Almeida, Joe Henderson and many other legendary jazz musicians. He has played with countless Bay Area jazz groups and performs regularly with the Bay Area's Redwood Symphony Orchestra.

George Ballantyne (drums) was drummer/percussionist for Solar Plexus and has performed with numerous Bay Area jazz groups over the past several decades, including appearances at West Coast jazz festivals from Canada to San Diego.

Harry Noller (tenor saxophone) played with Keith Johnson and the Jazz Prophets and the Benny Wilson Blues Band (while in graduate school), with the Marty Gellen Quartet in Cambridge, England and with the Jürgg Lenggenhager Quartet in Switzerland (while a postdoc), and with Solar Plexus, Griffin and other jazz groups in Santa Cruz. He played with Mario Guarneri in the Orindians in the early 1950s.

Wednesday, May 30

18:30 – 20:00 **Meetings Committee Meeting**

- Anna Head Alumnae Hall
- Open to the Meetings Committee, the Board of Directors, meeting sponsors, and (due to space constraints) a small number of additional observers.

This meeting is where potential sites for future RNA Society meetings are reviewed and selected. Any member of the RNA Society is welcome to attend, but due to space constraints one should request participation in advance by sending an email to the Meetings Committee Chair, Benoit Chabot (Benoit.Chabot@USherbrooke.ca).

19:00 – 20:30 **Junior Scientists Social**

- Tilden Room and Patio (top floor, MLK Student Union)
- Open to all graduate students and postdocs.

This junior scientist-only social is a great opportunity to meet your peers, unwind and socialize! After dinner and before posters, come have a drink with us!

Thursday, May 31

12:30 – 14:00 **Mentoring Lunch**

- International House and Anna Head Alumnae Hall
- Open to all attendees with pre-arrangement. Seating is limited, and advance completion of the pre-conference survey is required.

This lunch is an informal gathering that brings together six or seven graduate students and post docs with one to two academic and industry mentors to answer questions about careers. Topics include the pros and cons of academic vs industry careers, finding jobs, grant applications, and of course lots of interesting science. These lunches are

fun for the mentors and hopefully fun and useful for the mentees as well. To the extent possible, mentors and mentees with common career and geographical objectives or experiences are grouped together.

18:30 – 20:30 **RNA Society Board of Directors Meeting**

- Anna Head Alumnae Hall
- *Open to the Board of Directors and (due to space constraints) a small number of additional observers.*

This is the business meeting of the international RNA Society. Topics include an RNA journal update, results of the Meetings Committee deliberations, a report on finances and a vote on the next year's budget, and new initiatives. Any member of the RNA Society is welcome to attend, but due to space constraints one should request participation in advance by sending an email to the CEO, Jim McSwiggen (ceo@rnasociety.org).

Saturday, June 2

17:15 – 18:30 **Awards Ceremony**

- Zellerbach Auditorium

This is our opportunity to honor the people who have made significant contributions to RNA science. This year's awardees include:

- RNA Society Lifetime Achievement Award
  Jean Beggs, University of Edinburgh
- RNA Society Service Award
  Elizabeth Tran, Purdue University
- Poster prize winners
- Remarks on the 25th Anniversary of the RNA Society
  Olke C. Uhlenbeck, Northwestern University

19:00 – 23:30 **Conference Closing Event**

- The Faculty Club, UC Berkeley
- *Open to all registered attendees at no additional charge but tickets are required.*

For our closing party, we'll stay on campus and discover the historic Faculty Club, a casually elegant building with quintessentially Northern California woodsy charm. Mix and mingle, indoors and out, with food and beverage stations for grazing, and music for dancing, of course, to the music of DJ Aari Jethmal.

INDUSTRY SESSION

16:30 – 18:30 **Careers Beyond Academia**

The RNA junior scientists are pleased to host this workshop of leading scientists from the biotechnology sector representing various career stages and trajectories.

16:30 – 16:45 **Welcoming remarks by Fadi Bahjat Marayati**

Wake Forest University, representing the RNA Society Junior Scientists Committee

16:45 – 16:55 **Dr. Rachel Haurwitz,**

President and CEO, Caribou Biosciences

Dr. Rachel Haurwitz earned her Ph.D. in Molecular and Cell Biology from the University of California, Berkeley in Jennifer Doudna's group where she authored a number of high impact research articles characterizing CRISPR-Cas systems. In 2011, Rachel co-founded Caribou Biosciences to develop emerging CRISPR-Cas technologies. Caribou Biosciences is an industry leader in pioneering new applications for CRISPR-Cas gene editing to develop medical therapies and bio-based products. Rachel will discuss her transition from an academic career to her role as president and CEO of a startup company.

16:55 – 17:05 **Dr. Leah Makley**

President and CEO, ViewPoint Therapeutics

Dr. Leah Makley received her Ph.D. in Medicinal Chemistry from the University of Michigan, in the laboratory of Dr. Jason Gestwicki. During her graduate studies, she contributed to the development of biophysical screening strategies to identify small molecule modulators of protein misfolding, leading to ViewPoint's flagship technology. She cofounded the company in July 2014 and serves as President and CEO. The ViewPoint team is passionately committed to developing therapeutics for protein misfolding disorders in ophthalmology, including cataracts and presbyopia. Leah will discuss her transition into industry, the growth and evolution of ViewPoint Therapeutics, and share her perspectives for junior scientists exploring various career paths including entrepreneurship.

17:05 – 17:15 **Dr. Joe Lewcock**

Head of Biology Discovery, Denali Therapeutics

Dr. Joe Lewcock earned his Ph.D. from Johns Hopkins University School of Medicine and conducted his postdoctoral research at the Salk Institute. Prior to joining Denali Therapeutics, Dr. Lewcock spent 9 years at Genentech, where he was instrumental in building the neuroscience research division. Joe now serves as Head of Biology Discovery at Denali where, among other therapeutic strategies, he develops approaches to target RNA-binding protein aggregations that disrupt the cellular stress response in neurodegenerative diseases such as ALS and Alzheimer's. Dr. Lewcock will discuss aspects of his industry career and his collaborations with various academic institutions.

17:15 – 17:25 **Donald McCarthy**

Co-founder and CEO, Empire Biotechnologies

Dr. Donald McCarthy holds a Ph.D. in Biochemistry from the State University of New York at Albany. He did his postdoctoral training at the University of California San Francisco where he studied collagen degradation in fibrotic diseases. He is now founder and CEO at Empire Biotechnologies, a company developing GI therapeutics and Managing Director at the Panorama Research Institute where he manages a portfolio of early stage, product-focused companies. Donnie will discuss his transition from academia to building biotech companies.

17:25 – 17:35 **Dr. James Kiefer**

Senior Scientist and Associate Director, Structural Biology, Genentech

Dr. James Kiefer earned his Ph.D. in Biochemistry from Duke University studying structural determinants of the fidelity of DNA replication. His postdoctoral studies at Monsanto Company analyzed the reaction catalyzed by the membrane proteins cyclooxygenases 1 and 2 and their pharmacologic inhibition. He subsequently worked in structure-based drug design for more than 10 years at Pfizer and its subsidiaries before joining Genentech in 2012, where he is currently Associate Director of Structural Biology. His focus remains small molecule drug discovery, and his research interests include targets that directly or indirectly modulate gene transcription resulting in human disease.

17:35 – 17:45 **Dr. Ron Batra**

Vice President, Research and Development, Locana Bio (previously Verily Life Sciences)

Dr. Ranjan (Ron) Batra was a pharmacist when he became interested in genetics research. He earned his doctoral degree at the lab of Maurice Swanson, a renowned RNA biologist. Ron developed a keen interest in microsatellite repeat diseases as well as genomics during his Ph.D. career and applied that knowledge to study the mechanisms of C9orf72 ALS during his postdoc at the Mayo Clinic. After a second postdoc at Gene Yeo's lab, publishing many high impact publications, and interviewing for several faculty positions, Ron secured a genomics scientist position at Verily Life Sciences (formerly Google Life Sciences, an alphabet company) in 2017 where he applied high throughput genomics and automation to solve various population health problems. Ron recently joined a start-up called Locana Bio founded by his previous mentor and partner Gene Yeo and David Nelles, respectively, as the vice president of research and development to advance RNA-targeting CRISPR based therapeutics. Ron will discuss his transition from an academic to biotech career and compare his roles in academia, industry, and startup environments.

17:45 – 18:30 **Discussion Panel**

Following brief presentations, we will open a discussion panel and focus on various strategies and skillsets necessary for navigating transitions into industry and biotechnology career paths. Please join us for this exciting networking and career development opportunity.

SPONSORED SEMINAR

Date & Time: Wednesday, May 30, 08:00 – 08:45

Location: Members Lounge, Berkeley City Club

Theme: Full-length RNA sequencing with nanopores

Chairperson: *Dan Garalde, Senior Applications Scientist, Oxford Nanopore Technologies*

Sponsored by: Oxford Nanopore Technologies

This session includes three speakers discussing transcriptomics using nanopore sequencing. Methods include direct RNA and cDNA sequencing.

*To find out more, visit: https://register.nanoporetech.com/rnasociety

Measuring the transcriptome of the *C. elegans* lifecycle using direct RNA sequencing

Norah Hilger

Johns Hopkins University

Though regulation of gene expression is central to animal development and disease, there are still many unsolved questions about the dynamic nature of this regulation through development. Here, we take advantage of a new tool, direct RNA nanopore sequencing (dRNA-seq), to explore some aspects of transcriptome regulation, including splicing variation, alternative polyadenylation (APA), and an initial attempt at epitranscriptomics. Here we have generated whole transcriptome for *C. elegans*, a classic biological model for the study of development, across developmental stages (L1-L4, YA, GA) of the N2 strain. *C. elegans* is a relatively simple metazoan with a fully sequenced, compact genome, a well characterized and invariant cell lineage, and an excellent molecular genetic toolbox. It is an ideal system for exploratory development of new genomic technologies such as the full-length transcript sequencing we propose here. Understanding of the functional significance of pervasive transcript isoform diversity requires identifying exactly where and when each isoform is expressed using a model system that is tractable to experimental manipulation. By sequencing full-length transcripts across development, we provide a first look at identification of the diversity and transcript architecture of the transcriptome. We generated 1-3 flowcells of direct RNA sequencing data for each lifecycle point, then aligned it against the reference transcriptome (ce11 RefSeq) using minimap2. We then assessed the prevalence of different splicing isoforms, focusing a few genes, i.e. spp-10, rpt-4. As dRNA-seq uses a spliced 3′ adaptor, the entire length of the poly-A tail is sequenced, but becomes subject to the typical nanopore homopolymer problem. We used a custom hidden markov model, implemented as part of the nanopolish suite of tools, to estimate the length of the poly-A tail by the length of the time of the poly-A segment of the current trace. Finally, we characterized regions of the transcriptome which different substantially from the expected canonical base signal to find areas with modifications.

A reference human transcriptome based on native RNA sequencing

Miten Jain

UC Santa Cruz

The Nanopore RNA consortium is an international consortium of Oxford Nanopore MinION and GridION users. In 2017, the consortium generated a dataset consisting of 13 million native RNA and 24 million cDNA strand reads based on poly-A RNA isolated from the human reference cell line GM12878. This dataset is publicly available on GitHub: https://github.com/nanopore-wgs-consortium/NA12878/blob/master/RNA.md. The median read identity for RNA strand reads was around 86%, and we observed aligned read lengths of up to 22 kb (116 exons). We also observed a strong correlation (R=0.875) between native RNA and cDNA datasets, and that 73% of annotated human reference transcripts were captured by the native RNA data. We anticipate this dataset will serve as a resource to the community for native RNA sequencing.

We will present updates from the consortium work on analysis of these data that will include characterization of poly-A tail lengths using nanopore ionic current dwell time, assessment of full-length transcripts, detection of novel isoforms, and detection of base modifications using signal-level analysis.

Full-length transcripts associated with splicing factor mutations in cancer

Angela Brooks

UC Santa Cruz

We will present our work using Nanopore sequencing of cDNA to identify transcript alterations associated with mutations in the splicing factors U2AF1 and SF3B1. These are some of the most frequently mutated splicing factors across multiple cancer types. For the SF3B1 project, we've sequenced primary chronic lymphocytic leukemia samples with and without an SF3B1 K700E mutation. For the U2AF1 project, we've sequenced isogenic lung cell lines with and without U2AF1 S34F mutation, in biological replicates, in order to perform differential isoform analysis. For this talk, I will highlight our computational approaches for splice site detection, full-length isoform detection, alternative splicing analysis, and isoform quantification. I will also highlight new insights into altered transcripts associated with these splicing factor mutations that could not be observed with short-read data.

SPONSORED SEMINAR

LEXOGEN
Enabling complete transcriptome sequencing

Date & Time: Thursday, May 31, 07:45 – 08:45

Location: Ballroom, Berkeley City Club

Theme: Alternative adenylation, differential gene expression, blood samples, and cost-efficiency: When to switch to 3′ mRNA-Seq

Chairperson: *Lukas Paul, Senior Manager of Scientific Affairs, Lexogen, Vienna, Austria*

Sponsored by: Lexogen

Tag-based profiling of transcriptomes reduces some of the most limiting aspects of RNA-Seq: sequencing depth, scale of NGS data evaluation and overall experimental costs. In return, analysis robustness is enhanced and low-input & degraded RNA samples become accessible. At the heart of this development is the QuantSeq 3' mRNA-Seq library preparation kit from Lexogen; more than 70 publications in 2017 & 2018 alone feature this method with applications including differential gene expression and alternative polyadenylation. The ability to block globin mRNAs from entering the libraries now extends the input range to blood samples, and QuantSeq is the library preparation of choice for SLAMseq, a new and advanced metabolic RNA-Seq technology. Full automation and a free, cloud-based data evaluation further simplify the move from standard RNA-Seq to 3′ mRNA-Seq.

Comparing 3′TAG-seq to more traditional RNA-seq and when its best to switch

Matthew L. Settles

Bioinformatics Core Facility, Genome Center, University of California, Davis, Davis, CA, 95664

TAG-seq techniques, such as the Lexogen QuantSeq 3′mRNA-Seq assay, have fast become a cheaper and viable alternative to more traditional RNAs-seq, for the purpose of differential gene expression. Further, with the addition of the Globin blocking module and Unique Molecular identifiers, the TAG-seq protocol is an option for nearly all experiment types. This presentation discusses a core facilities experience transitioning differential expression studies from many different organisms to the TAG-seq protocol, including experience with the hemoglobin module and unique molecular indexes.

Landscape of Alternative Polyadenylation in Lung Cancer

Adriana Zingone[1], Gregor Rot[2], Michael Ante[3], Elise Bowman[1], Dalia Daujotyte[3], Khadijah Mitchell[1], Bríd M. Ryan[1]

[1]Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD, 20892;

[2]Institute of Molecular Life Sciences and Swiss Institute of Bioinformatics, Winterthurerstrasse 190, 8057 Zurich, Switzerland;

[3]Lexogen GmbH, Campus Vienna Biocenter 5, 1030 Vienna, Austria

Alternative polyadenylation (APA) involves the selection of an alternate poly(A) site (PAS) on a pre-mRNA, creating isoforms of various lengths. Several normal physiological processes are regulated by APA.

In cancer however, APA is emerging as an alternative mechanism for proto-oncogene activation in the absence of somatic mutations. Patterns of APA, in some ways a hidden complexity of cancer transcriptomes, may further our understanding of lung cancer biology, but has not been specifically addressed in lung cancer. We conducted 3′UTR sequencing on a large series of paired tumor and normal samples (n=200) from lung cancer patients using the Lexogen QuantSeq 3′mRNA-Seq assay. Our results indicate global shortening of the 3′UTR in lung cancer and is consistent with previous studies of other cancer types. Previously, we described transcriptomic differences between European Americans and African Americans, with pathways sustaining cell proliferation more prevalent among EA lung tumors. Indeed, our study also identifies racial differences in APA in lung cancer. Given that exposures such as temperature and exogenous hormones can also induce APA as a stress-response mechanism we also addressed whether smoking is a driver of APA. Our study highlights the importance of APA in lung cancer and suggests this process as a distinct form of transcriptomic perturbation.

SPONSORED SEMINAR

Date & Time: Friday, June 1, 07:30 – 08:30

Location: Members Lounge, Berkeley City Club

Theme: A Complete Platform for Ultra-Sensitive Single-Cell Expression Profiling

Chairperson: *Alex Chenchik, Ph.D., President and Chief Scientific Officer, Cellecta, Inc., Mountain View, California*

Cellecta is a leading provider of genomic products and services. Our functional genomics portfolio includes products and services for gene knockout, knock-down and knock-in screens, custom and off-the-shelf, genome-wide CRISPR and RNAi libraries, constructs, cell engineering, as well as NGS kits including the DriverMap™ Targeted Expression Profiling assay.

At the seminar, we will present our work using barcoded single cells to phenotypically characterize samples by expression profiling for detection of cellular sub-types, as well as the use of barcodes with CRISPR effector libraries to observe clonal phenotypic changes induced by specific genetic disruptions in progeny cells.

Combining Barcode Libraries with Targeted Gene Expression for Single-Cell Genetic Analysis

Alex Chenchik, Mikhail Makhanov, Costa Frangou

Cellecta, Inc.

Labeling of target cells with lentiviral barcode libraries offers an effective approach for monitoring cell phenotype in time course experiments *in vitro* and *in vivo* using single-cell molecular analysis. Cellecta's CellTracker™ libraries, with millions of different barcodes, enable rapid labeling of each cell in a large population with a unique sequence that is detectable in NGS RNA expression profiling assays such as RNA-seq. These barcodes then provide a key to identify the subpopulation of progeny cells derived from a single barcoded progenitor cell in a single-cell analysis data set. Furthermore, the progeny cells could be phenotypically characterized by expression profiles, such as stem cells, differentiated cells, activated or apoptotic cells, etc. Subsequently, cell barcodes can be incorporated in conjunction with genetic effector libraries, such as CRISPR sgRNA libraries, to identify clonal phenotypic changes induced by specific genetic disruptions in progeny cells derived from the single progenitor cell.

However, while barcoding cells provides an effective way to group cells based on clonal origin in heterogenous cell populations, comprehensive single-cell expression profiling of large numbers of cells remains challenging. Generating genome-wide quantitative RNA-seq data from thousands of cells is prohibitive. With standard RNA-seq analysis of single cells, the depth of sequencing is limited, and only highly expressed transcripts can be reliably measured. To address this limitation, quantitative single-cell gene expression profiling requires the use of a targeted method that focuses on key genes involved in cell-specific biomarkers, signaling pathways or biological responses of interest.

We will demonstrate how the DriverMap™ Targeted RNA Expression Profiling assay with CellTracker™ lentiviral barcoded libraries, provide a complete platform for highly sensitive single-cell expression profiling. Data will be presented showing how targeted DriverMap RNA expression profiling of single cells combined with cell barcoding could significantly improve phenotyping of distinct cell populations.

ORAL ABSTRACT LISTING

TUESDAY, MAY 29, 2018: 19:00 – 21:30
Opening Session – Zellerbach Auditorium
Abstracts 1 – 5

1 **Structural Insights into CRISPR and Anti-CRISPR Systems for Precise Genome Editing**
Fuguo Jiang, Jennifer Doudna

2 **Revealing Hidden Biology by Identifying Ligands for the *ykkC* Orphan Riboswitches**
Madeline Sherlock, Ruben Atilho, Sarah Malkowski, James Nelson, Narasimhan Sudarsan, Shira Stav, Ronald Breaker

3 **Specialized guides of m^{6}A regulatory pathway: YTHDF reader proteins and their functions**
Boxuan Zhao, Xiao Wang, Alana Beadell, Zhike Lu, Hailing Shi, Robert Ho, Chuan He

4 **Visualizing translation by ensemble cryo-EM**
Andrei Korostelev

5 **Compact, High-Accuracy Cas9 Orthologs and their Natural Inhibitors**
Erik Sontheimer, Nadia Amrani, Alireza Edraki, Xin Daniel Gao, Raed Ibraheim, Jooyoung Lee, Aamir Mir, Tomás Rodriguez

WEDNESDAY, MAY 30, 2018: 09:00 – 11:45
Plenary Session 1: Splicing – Zellerbach Auditorium
Patricia Coltri, Chair
Abstracts 6 – 14

6 **Misregulation of an activity-dependent splicing network impacts neuronal translation and underlies autism spectrum disorders**
Thomas Gonatopoulos-Pournatzis, Mathieu Quesnel-Vallieres, Rieko Niibori, Shen Zhang, Ulrich Braunschweig, Robert J. Weatheritt, Manuel Irimia, Nahum Sonenberg, Anne-Claude Gingras, Sabine P. Cordes, Benjamin J Blencowe

7 **Prespliceosome structure provides insight into spliceosome assembly and regulation**
Clemens Plaschka, Pei-Chun Lin, Clément Charenton, Kiyoshi Nagai

8 **CryoEM structure of the yeast spliceosomal P complex at 3.3 Å resolution**
Rui Zhao, Shiheng Liu, Xueni Li, Lingdi Zhang, Jiansen Jiang, Ryan Hill, Yanxiang Cui, Kirk Hansen, Hong Zhou

9 **Structural basis for specific pre-mRNA splicing correction at the 5'-splice site induced by small molecules**
Sébastien Campagne, Simon Rüdisser, Hasane Ratni, Frédéric Allain

10 **Quantitative activity profile and context dependence of all human 5' splice sites**
Mandy Wong, Justin Kinney, Adrian Krainer

11 **Decoding extensive regulation of *RON* alternative splicing with a high-throughput mutagenesis screen**
Julian König

12 **DDD40800- a novel modulator of pre-mRNA Splicing**
Andrea Pawellek, Ursula Ryder, Marek Gierlinski, Andrew Woodland, David Gray, Angus Lamond

13 **DDX17 specifically, and independently of DDX5, controls the use of the HIV A4/5 splice acceptor cluster and is essential for efficient replication of the virus**
Nyaradzai Sithole, Claire Williams, Aisling Vaughan, Julia Kenyon, Andrew Lever

14 Smu1 and RED are required for the activation of spliceosomal B complexes assembled on short introns
Sandra Keiper, Panagiotis Papasaikas, Ilya Komarov, Juan Valcárcel, Holger Stark, Cyrille Girard, Reinhard Lührmann

WEDNESDAY, MAY 30, 2018: 11:45 – 12:30
Keynote 1: Mikiko C. Siomi – Zellerbach Auditorium
Abstract 15

15 piRNA Biogenesis and Function
Mikiko C. Siomi

WEDNESDAY, MAY 30, 2018: 14:00 – 16:45
Concurrent Session 1: RNA Turnover – Zellerbach Playhouse
Katherine Berry, Chair
Abstracts 16 – 24

16 Loss of *Sfpq* causes long-gene transcriptopathy in the brain
Akihide Takeuchi, Kei Iida, Motoyasu Hosokawa, Masatsugu Denawa, Mikako Ito, Kinji Ohno, Masatoshi Hagiwara

17 ICP27-mediated suppression of mRNA 3' processing as a key host shut-off mechanism for Herpes Simplex Virus
xiuye Wang, Rozanne Sandri-Goldin, Yongsheng Shi

18 m6A mRNA methylation controls the innate immune response to infection by targeting IFNB
Roni Winkler, Ella Abram, Aharon Nachshon, Modi Safra, Clara Soyris, Shay Geula, Lior Lasman, Michal Mandelboim, VuThuy Khanh Le-Trilling, Mirko Trilling, Yaqub (Jacob) Hanna, Schraga Schwartz, Noam Stern-Ginossar

19 The ribonuclease Regnase-1 maintains iron homeostasis via the destabilization of iron-regulatory transcripts
Masanori Yoshinaga, Takashi Mino, Osamu Takeuchi

20 Living life on the edge: a high-resolution map of the mammalian RNA decay landscape
Alex Tuck, Aneliya Rankova, Philip Knuckles, Greggory Rice, Jason Borawski, Fabio Mohn, Matyas Flemr, Alice Wenger, Kedar Natarajan, Sarah Teichmann, Razvan Nutiu, Marc Buehler

21 The exon junction complex undergoes a compositional switch that alters its nonsense-mediated mRNA decay activity and overall mRNP structure
Justin Mabin, Lauren Woodward, Robert Patton, Mengxuan Jia, Vicki Wysocki, Ralf Bundchuh, Guramrit Singh

22 Isoform-specific catalytic activity of the NMD RNA helicase UPF1: how alternative splicing introduces structural changes to differentially regulate an enzyme
Manjeera Gowravaram, Fabien Bonneau, Joanne Kanaan, Vincent D. Maciej, Francesca Fiorini, Saurabh Raj, Vincent Croquette, Hervé Le Hir, Sutapa Chakrabarti

23 Unique repression domains in Pumilio accelerate destruction of target mRNAs
René Arvola, Isioma Enwerem, Joseph Buytendorp, Aaron Goldstrohm

24 How piRNAs make planarians regenerate
Iana Kim, Elizabeth Duncan, Eric Ross, Alejandro Sánchez Alvarado, Kuhn Claus

WEDNESDAY, MAY 30, 2018: 14:00 – 16:45
Concurrent Session 2: Regulatory RNAs – Zellerbach Auditorium
Ayelet Lamm, Chair
Abstracts 25 – 34

25 Structures of ykkC riboswitches bound to ppGpp and PRPP reveal a novel principle of ligand recognition
Alla Peselis, Alexander Serganov

26 Structures of riboswitch aptamers that recognize phosphoribosyl pyrophosphate (PRPP) and guanosine tetraphosphate (ppGpp) reveal ruggedness in the functional landscape of the *ykkC* RNA
Caroline Reiss, Andrew Knappenberger, Scott Strobel

27 ciRS-7 exonic sequence is embedded in a long non-coding RNA locus
Steven Barrett, Kevin Parker, Caroline Horn, Miguel Mata, Julia Salzman

28 The small non-coding vault RNA1-1 regulates autophagy by direct interaction with the autophagy receptor p62
Rastislav Horos, Magdalena Büscher, Anne-Marie Alleaume, Roos Kleinendorst, Abul K. Tarafder, Thomas Schwarzl, Elisabeth M. Zielonka, Asli Adak, Alfredo Castello, Wolfgang Huber, Carsten Sachse, Matthias W. Hentze

29 Dicer cleaves 5′-extended microRNA precursors originating from RNA Polymerase II transcription start sites
Peike Sheng, Christopher Fields, Kelsey Aadland, Tianqi Wei, Oralia Kolaczkowski, Tongjun Gu, Bryan Kolaczkowski, Mingyi Xie

30 Cardioprotective microRNA-574-Fam210a axis maintains mitochondrial translational homeostasis
Jiangbin Wu, Kadiam C Venkata Subbaiah, Qiuqing Wang, John Ashton, Eric Small, Chen Yan, Peng Yao

31 LIN28 selectively modulates a subclass of let-7 microRNAs
Dmytro Ustianenko, Hua-Sheng Chiu, Sebastien M. Weyn-Vanhentenryck, Pavel Sumazin, Chaolin Zhang

32 Mechanism of internalization of extracellular vesicle (EV) derived single stranded microRNAs in mammalian cells
Bartika Ghoshal, Suvendra N. Bhattacharyya

33 Transgenerational inheritance masks a critical role for piRNA methylation in C. elegans
Josh Svendsen, Kristen Brown, Rachel Tucci, Brooke Montgomery, Tai Montgomery

34 The ribosome dependent function of the universally conserved translational ATPase YchF
Hans-Joachim Wieden, Binod Pageni, Dora Capatos, Fan Mo, Senthilkumar Kailasam, Harland Brandon

WEDNESDAY, MAY 30, 2018: 17:00 – 18:30
Workshop 1: Capturing Dynamic RNPs – Zellerbach Auditorium
Julian König, Chair
Abstracts 35 – 41

35 TRAPPing the RNA-associated proteome
Stefan Bresson, Vadim Shchepachev, Christos Spanos, Juri Rappsilber, David Tollervey

36 KIN-CLIP: transcriptome-wide kinetics for RNA-protein interactions in cells
Deepak Sharma, Leah Zagore, Matthew Brister, Carlos Crespo Hernandez, Donny Licatalosi, Eckhard Jankowsky

37 Deconvolving the RNA life cycle from high-resolution time-resolved protein-RNA interaction data
David Schnoerr, Alina Selega, Rob van Nues, Stuart McKellar, Edward Wallace, Sander Granneman, Guido Sanguinetti

38 Spliceosome profiling visualizes operations of a dynamic RNP at nucleotide resolution
Jordan Burke, Adam Longhurst, Daria Merkurjev, Jade Sales-Lee, Beiduo Rao, James Moresco, John Yates 3rd, Jingyi Jessica Li, Hiten Madhani

39 **Spliceosome profiling reveals mega-RNPs active in splicing in human cells**
Byung Ran So, Chao Di, Christopher Venters, Zhiqiang Cai, Jiannan Guo, Jung-Min Oh, Chie Arai, Gideon Dreyfuss

40 **Transcriptome-wide identification and validation of AGO-RBP co-regulation on mRNA targets**
Erin Sternburg, Yahui Li, Jason Estep, Fedor Karginov

41 **N6-methyladenosine-dependent regulation of the pre-replicated Chikungunya viral genome**
Sarah Arcos, Byungil Kim, Katie Rothamel, Yuqi Bian, Seth Reasoner, Manuel Ascano

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WEDNESDAY, MAY 30, 2018: 17:00 – 18:30
Workshop 2: Single-cell RNAseq – Hertz Concert Hall
Jeremie Breda, Chair
Abstracts 44, 42, 43, 45, 46

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44 **Direct RNA sequencing to detect modified nucleotides using Oxford Nanopore**
Mark Akeson, Timothy Gilpatrick, Miten Jain, Nicholas Loman, Matthew Loose, Hugh Olsen, Joshua Quick, Norah Sadowski, Jared Simpson, Terrance Snutch, Winston Timp, John Tyson, Rachael Workman, Philip Zuzarte

42 **Inferring gene regulatory landscapes from single-cell RNA-seq data**
Jeremie Breda, Mihaela Zavolan, Erik van Nimwegen

43 **Quantifying regulatory factors transcriptome-wide utilizing gene co-expression networks inferred from single-cell sequencing**
Marcel Tarbier, Sebastian D. Mackowiak, Inna Biryukova, Marc R. Friedländer

45 **Spatial reconstruction of single enterocytes uncovers broad zonation along the intestinal villus axis**
Andreas Moor, Yotam Harnik, Shani Ben-Moshe, Efi Massasa, Keren Bahar Halpern, Shalev Itzkovitz

46 **The NMD Factor UPF3B Shapes Olfactory Neurogenesis and the Olfactory Receptor Repertoire**
Samantha Jones, Jennifer Dumdie, Blue Lake, Kun Zhang, Miles Wilkinson

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WEDNESDAY, MAY 30, 2018: 17:00 – 18:30
Workshop 3: Probing RNA Structure – Zellerbach Playhouse
Sharon Aviran, Chair
Abstracts 49, 47, 50, 48, 51

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49 **Light-Activated Probing of RNA Structure Inside Cells**
Chao Feng, Dalen Chan, Jojo Joseph, Mikko Muuronen, William Coldren, Nan Dai, Ivan Corrêa, Filipp Furche, Christopher Hadad, Robert Spitale

47 **RNA structure prediction with interaction constraints (RNA SPICs)**
Marta Gabryelska, Tomasz Turowski, Omer Ziv, Guido Sanguinetti, Eric Misca, Grzegorz Kudla

50 **Elucidating Signal Transduction Pathways in RNA Mediated Gene Regulation**
Debapratim Dutta, Ivan Belashov, Joseph Wedekind

48 **Rapid transcriptome-wide search for functional RNAs *in vivo* via structural data signatures**
Mirko Ledda, Pierce Radecki, Sharon Aviran

51 **Shift of Monomer-Dimer Equilibrium Reveals An Irreversible RNA Structural Rearrangement**
Botros Toro, Dan Fabris, Pan Li

THURSDAY, MAY 31, 2018: 09:00 – 11:45
Plenary Session 2: From Oligo to RNP – Zellerbach Auditorium
Andrea Rentmeister, Chair
Abstracts 52 – 62, 135

52 **Enzymatic or *in vivo* installation of propargyl groups in combination with click chemistry enables enrichment and detection of methyltransferase target sites in RNA**
Katja Hartstock, Benedikt Nilges, Anna Ovcharenko, Nicolas Cornelissen, Nikolai Püllen, Ann-Marie Lawrence-Dörner, Sebastian Leidel, Andrea Rentmeister

53 **Novel molecular tools for targeted labeling of RNA**
Giedrius Vilkaitis, Migle Tomkuviene, Alexandra Plotnikova, Aleksandr Osipenko, Milda Mickute, Viktoras Masevicius, Saulius Klimašauskas

54 **Orientation-dependent FRET using rigid fluorogenic RNA aptamers**
Sunny C.Y. Jeng, Robert J. Trachman III, Adrian R. Ferré-D'Amaré, Peter J. Unrau

55 **Detection of Ligand-Induced Conformational Changes in Oligonucleotides by Second-Harmonic Generation**
Margaret Butko, Gabriel Mercado, Noreen Rizvi, Elliott Nickbarg, Hai-Young Kim, Mark McCoy, Thierry Fischmann, Corey Strickland

56 **OTTER, a new method for measuring absolute quantity of tRNAs**
Akihisa Nagai, Kouhei Mori, Yuma Shiomi, Tohru Yoshihisa

57 ***De novo* computational RNA modeling into cryoEM maps of large ribonucleoprotein complexes**
Kalli Kappel, Shiheng Liu, Kevin P. Larsen, Georgios Skiniotis, Joseph D. Puglisi, Elisabetta Viani Puglisi, Z. Hong Zhou, Rui Zhao, Rhiju Das

58 **Structure of the Ago2:miR-122:HCV complex**
Luca Gebert, Ian MacRae

59 **Cryo-EM structure of human Dicer and its complexes with a pre-miRNA substrate**
Hong-Wei Wang, Zhongmin Liu, Jia Wang, Hang Cheng, Xin Ke, Lei Lei, Qiangfeng Cliff Zhang

60 **Repetitive DNA reeling by the Cascade-Cas3 complex in nucleotide unwinding steps**
Luuk Loeff, Stan Brouns, Chirlmin Joo

61 **Cas4-dependent prespacer processing ensures high-fidelity programming of CRISPR arrays**
Hayun Lee, Yi Zhou, David Taylor, Dipali Sashital

62 **Recruitment of a Type III-A CRISPR-Cas Csm complex to the transcription elongation complex by recognition of the nascent RNA transcript**
Tina Liu, Jun-jie Liu, Abhishek Aditham, Eva Nogales, Jennifer Doudna

135 **Cryo-EM structure of human telomerase holoenzyme and new insight into its assembly and function**
Thi Hoang Duong Nguyen, Jane Tam, Robert Alexander Wu, Basil Greber, Daniel Toso, Eva Nogales, Kathleen Collins

THURSDAY, MAY 31, 2018: 11:45 – 12:30
Keynote 2: Geraldine Seydoux – Zellerbach Auditorium
Abstract 63

63 **Co-assembly of liquid and gel-like phases in an RNA granule**
Geraldine Seydoux

THURSDAY, MAY 31, 2018: 14:00 – 16:00
Concurrent 3: Splicing Mechanisms – Zellerbach Auditorium

Stephen Rader, Chair

Abstracts 64 – 72

64 Genome-wide determination of 1st and 2nd step rates of pre-mRNA splicing in vivo using rapid metabolic RNA labeling time courses coupled with a novel targeted sequencing approach
Michael Gildea, Hansen Xu, Ben Jung Fair, Zachary Dwyer, Jeffrey A. Pleiss

65 Structural characterization of the UsnRNP assembly machinery and its regulation by post-translational modifications
Jyotishman Veepaschit, Aravindan Viswanathan, Remy Bordonne, Oliver Gruss, Archana Prusty, Clemens Grimm, Utz Fischer

66 U6 snRNA m^6A stabilizes 5' splice site recognition
Yuma Ishigami, Takayuki Ohira, Yutaka Suzuki, Tsutomu Suzuki

67 Global Interplay of A-to-I RNA editing and pre-mRNA Splicing
Utkarsh Kapoor, Konstantin Licht, Fabian Amman, Michael Jantsch

68 Conserved interaction between yeast Hsh155 and Cus2 enforces ATP-dependent prespliceosome assembly by antagonizing Prp5 ATPase activity
Jason Talkish, Oarteze Hunter, Haller Igel, Steven Horner, Nazish Jeffrey, Clara L. Kielkopf, Manuel Ares Jr.

69 High-throughput suppressor analysis identifies molecular surfaces and contacts that likely control activation of the B complex spliceosome
David Brow

70 Both U4 and U6 snRNAs promote Brr2 dependent U4/U6 unwinding
Klaus Nielsen, Jon Staley

71 Structural accommodations accompanying splicing of a group II intron RNP
Xiaolong Dong, Srivathsan Ranganathan, Guosheng Qu, Carol Lyn Piazza, Marlene Belfort

72 Structural Insights into Thermostable Group II Intron Reverse Transcriptases (TGIRTs) and their Functions in RNA Splicing, Intron Retromobility, and RNA-seq
Jennifer L. Stamos, Alfred M. Lentzsch, Seung Kuk Park, Georg Mohr, Alan M. Lambowitz

THURSDAY, MAY 31, 2018: 14:00 – 16:00
Concurrent 4: Interconnected RNA Processes – Zellerbach Playhouse

Mary O'Connell, Chair

Abstracts 73 – 81

73 A Dual-Activity Topoisomerase Interacts with RNAi Machinery to Promote Heterochromatin Formation and Transcriptional Silencing
Seung Kyu Lee, Yutong Xue, Weiping Shen, Yongqing Zhang, Muzammil Ahmad, Yuyoung Joo, Yi Ding, Wai Lim Ku, Supriyo De, Elin Lehrman, Kevin Bechker, Elyssa Lei, Keji Zhao, Sige Zou, Alexei Sharov, Weidong Wang

74 SWI/SNF-regulated alternative splicing produces metabolic-state specific protein isoforms, dynamically controlling Coenzyme Q_6 biosynthesis and expression of stress-response genes
Srivats Venkataramanan, Agape Awad, Anish Nag, Anoop Galivanche, Michelle Bradley, Lauren Neves, Stephen Douglass, Catherine Clarke, Tracy Johnson

75 The Conserved RNA Binding Protein ZFR Represses Innate Immune Signaling by Controlling Splicing and Decay of Histone Variant macroH2A mRNAs
Nazmul Haque, Ryota Ouda, Chao Chen, Keiko Ozato, J. Robert Hogg

76 **The spliceosomal component Sf3b1 protects embryonic neurons from R-loop mediated DNA damage**
Sara Nik, Shelly Sorrells, Mattie Casey, Rosannah Cameron, Harold Truong, Cristhian Toruno, Michelle Gulfo, Albert Lowe, Cicely Jette, Rodney Stewart, Teresa Bowman

77 **The regulation of mRNA nuclear export and translation by RanBP2-dependent sumoylation**
Alexander Palazzo, Qingtang Shen, Mathew Truong

78 **tRNA synthetases as novel mRNA binding proteins: target mRNAs, mode of recognition and regulatory role**
Ofri Levi, Yoav Arava

79 **The structure of yeast tRNA ligase reveals a competition between non-conventional mRNA splicing and RNA decay**
Jirka Peschek, Peter Walter

80 **Formation of tRNA wobble inosine in humans is perturbed by a primeval mutation linked to intellectual disability**
Jillian Ramos, Lu Han, Yan Li, Fowzan Alkuraya, Eric Phizicky, Dragony Fu

81 **PRMT7-dependent arginine methylation regulates the stability and function of RNA-binding proteins**
Tiago R. Ferreira, Adam A. Dowle, Eliza C. Alves-Ferreira, Tony R. Larson, Michael Plevin, Angela K. Cruz, Pegine B. Walrad

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FRIDAY, JUNE 1, 2018: 09:00 – 11:45
Plenary Session 3: The Life and Times of a Ribosome – Zellerbach Auditorium
Kristin Koutmou, Chair
Abstracts 82 – 92

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82 **Diverse roles of RNA helicases in driving structural transitions and compositional changes in pre-ribosomal complexes**
Lukas Brüning, Philipp Hackert, Roman Martin, Katherine E. Sloan, Markus T. Bohnsack

83 **Ribosome Biogenesis Coming Into Focus: Remodeling Events Driving Middle Stages of Large Subunit Assembly in Yeast**
John Woolford, Stephanie Biedka, Jelena Micic, Amber LaPeruta, Daniel Wilson

84 **Ltv1 facilitates and monitors assembly of the 40S ribosome beak structure during ribosome biogenesis**
Jason Collins, Homa Ghalei, Joanne Doherty, Rebecca Culver, Katrin Karbstein

85 **5' UTR RNA Elements Confer Translational Specificity to Shape Vertebrate Embryonic Development**
Kathrin Leppek, Nick Quade, Kotaro Fujii, Nenad Ban, Maria Barna

86 **Global Identification of Cap-Independent Translation Initiation Sites in Human Transcriptome**
Yun Yang, Xiaojuan Fan, Sirui Zhang, Zefeng Wang

87 **Translation elongation dynamics by ensemble cryo-EM**
Anna B. Loveland, Gabriel Demo, Andrei A. Korostelev

88 **mRNA modifications alter translation elongation and fidelity**
Daniel Eyler, Mehmet Tardu, Monika Franco, Kristin Koutmou

89 **Regulated translation elongation as a mechanism of differential protein synthesis during stress**
Alex Harvey, Raghav Chanchani, Jingxiao Zhang, Brian Zid

90 **Structure of a pre-handover mammalian ribosomal SRP•SRP receptor targeting complex**
Kan Kobayashi, Ahmad Jomaa, Jae Ho Lee, Sowmya Chandrasekar, Daniel Boehringer, Shu-ou Shan, Nenad Ban

91 **mRNA secondary structures allosterically bias ribosomes into a kinetically altered slower pathway**
Varsha Desai, Filipp Frank, Maurizio Righini, Antony Lee, Ignacio Tinoco, Carlos Bustamante

92 Accurate design of translational output by a neural network model of ribosome distribution
Robert Tunney, Nicholas McGlincy, Monica Graham, Nicki Naddaf, Lior Pachter, Liana Lareau

FRIDAY, JUNE 1, 2018: 11:45 – 12:30
Keynote 3: Jonathan Weissman – Zellerbach Auditorium
Abstract 93

93 Monitoring protein synthesis in time and space with ribosome profiling
Jonathan Weissman

FRIDAY, JUNE 1, 2018: 14:00 – 16:45
Concurrent 5: Splicing Regulation – Zellerbach Auditorium
Julia Salzman, Chair
Abstracts 94 – 101

94 Full-length characterization of transcript isoforms to investigate cancer-associated mutations
Alison Tang, Cameron Soulette, Marijke van Baren, Kevyn Hart, Catherine Wu, Angela Brooks

95 Deciphering the splicing code of coordinated RNA binding proteins PSI and hrp48 in sculpting the Drosophila transcriptome
Qingqing Wang, Lucas Horan, J. Matthew Taliaferro, Donald Rio

96 Structural and functional mechanism of alternative splicing regulation of the immune cell protease MALT1
Alisha Jones, Isabel Meininger, Annalisa Schaub, Arie Geerlof, Michael Sattler, Daniel Krappmann

97 RBM5 and RBM10 interact with components of the 17S U2 snRNP
Andrey Damianov, Jeffrey Huang, Yi Ying, William Barshop, James Wohlschlegel, Douglas Black

98 Cdc2-like kinases 1/4 (CLK1/4) act as cellular thermometer to control body temperature dependent rhythmic alternative splicing in mammals
Tom Haltenhof, Ana Kotte, Francesca De Bortoli, Marco Preußner, Florian Heyd

99 Plant hnRNPs participate in light-regulated alternative splicing
Chueh-Ju Shih, Bou-Yun Lin, Hsiang-Wen Chen, Yung-Hua Lai, Hsin-Yu Hsieh, Shih-Long Tu

100 Splice-Switching Antisense Oligonucleotides to Correct CLN3 Gene Expression in Juvenile Batten Disease
Jessica Centa, Francine Jodelka, Anthony Hinrich, Frank Rigo, Michelle Hastings

101 *HRAS* exon 2 is a vulnerable exon which can be targeted by SSOs to abolish RAS activity in cancer cells
Anne-Mette Hartung, Jeanne Mari Vejen Bang, Barbara Guerra, Brage Storstein Andresen

FRIDAY, JUNE 1, 2018: 14:00 – 16:45
Concurrent 6: Emerging Technologies – Zellerbach Playhouse
Jane Jackman, Chair
Abstracts 102 – 111

102 Proteome-wide and quantitative identification of RNA-dependent protein complexes – a novel concept to discover unexpected functions of RNAs
Maiwen Caudron-Herger, Scott F. Rusin, Mark E. Adamo, Jeanette Seiler, Vera Schmid, Arminja N. Kettenbach, Sven Diederichs

103 Using customizable Pentatricopeptide Repeat (PPR) proteins as affinity tags to purify specific RNPs for proteomic analysis
James McDermott, Rafael Miranda, Alice Barkan

104 An enhanced hybridization capture approach reveals different modes of lncRNA spreading on chromatin
Martin Machyna, Matthew Simon

105 Biophysical and biological implications of a quantitative and comprehensive model for RNA binding by human Pumilio proteins
Inga Jarmoskaite, Sarah Denny, Pavan Vaidyanathan, Winston Becker, Johan Andreasson, Curtis Layton, Kalli Kappel, Varun Shivashankar, Raashi Sreenivasan, Rhiju Das, Daniel Herschlag, William Greenleaf

106 Modeling and assessing the CRISPR-Cas9 off-targeting potential by nucleic acid duplex interactions
Ferhat Alkan, Anne Wenzel, Jakob Hull Havgaard, Jan Gorodkin

107 Heavily and Fully Modified RNAs Guide Efficient Cas9-Mediated Genome Editing
Aamir Mir, Julia Alterman, Matthew Hassler, Alexandre Debacker, Edward Hudgens, Dimas Echeverria, Michael Brodsky, Anastasia Khvorova, Jonathan Watts, Erik Sontheimer

108 Argonaute-based programmable RNase as a tool for cleavage of highly-structured RNA
Daniel Dayeh, William Cantara, Jonathan Kitzrow, Karin Musier-Forsyth, Kotaro Nakanishi

109 How mRNA wraps the 30S at the beginning of translation
Yi-Lan Chen, Jin-Der Wen

110 Probing Mechanisms of RNA Chaperones with a Bacterial Three-Hybrid Assay
Smriti Pandey, Kelly Chambers, Clara Wang, Courtney Hegner, Katherine Berry

111 When antisense makes sense: exploring the role of RNA polymerase-binding RNA aptamers in control of bacterial antisense transcription
Andrés Magán García, Nadezda Sedlyarova, Fatinah El-Isa, Natascha Hartl, Renée Schroeder

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FRIDAY, JUNE 1, 2018: 17:00 – 18:30
Workshop 4: Single-molecule Analysis – Hertz Concert Hall
Dan Larson, Chair
Abstracts 112 – 117

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112 Site-specific two-color labeling of long RNAs for single-molecule FRET
Fabio D. Steffen, Meng Zhao, Richard Börner, Michelle F. Schaffer, Roland K.O. Sigel, Eva Freisinger

113 Kinetic Selection of Spliceosome Substrates by the Yeast U1 snRNP
Sarah Hansen, Mark Scalf, Lloyd Smith, Aaron Hoskins

114 Direct measurements of protein synthesis kinetics in live cells using fluorescently labeled tRNAs
Ivan Volkov, Martin Lindén, Javier Aguirre Rivera, Ka-Weng Ieong, Mikhail Metelev, Johan Elf, Magnus Johansson

115 Real-time quantification of single RNA frameshifting dynamics in living cells
Timothy Stasevich, Kenneth Lyon, Tatsuya Morisaki, Luis Aguilera, Brian Munsky

116 Intracellular single particle tracking of miRNA induced silencing complexes and mRNAs reveals sub-stoichiometric, transient binding and induced target aggregation
Ameya Jalihal, Hui Li, Nils Walter

117 Real-time single-molecule imaging of riboswitch dynamics during transcription elongation
Adrien Chauvier, Patrick St-Pierre, Jean-François Nadon, Jonathan Grondin, Cibran Perez-Gonzalez, Sébastien Eschbach, Anne-Marie Lamontagne, Juan-Carlos Penedo, Daniel Lafontaine

FRIDAY, JUNE 1, 2018: 17:00 – 18:30
Workshop 5: RNA Editing and Modification – Zellerbach Auditorium
Wendy Gilbert, Chair
Abstracts 118 – 120, 149, 392, 121, 123

118 An RNA Binding Protein that Directs Substrate Binding of an Adenosine-to-Inosine RNA Editing Enzyme *in vivo* and Expands the Number of Editing Sites in the Transcriptome
Suba Rajendren, Aidan C. Manning, Yuichiro Takagi, Heather A. Hundley

119 BioID identifies novel regulators of ADARs and A-to-I RNA editing
Emily Freund, Qin Li, Anne Sapiro, Sandra Linder, James Moresco, John Yates, Jin Billy Li

120 Regulation of A-to-I RNA Editome by ADARs-interacting Partner Death Associated Protein 3 (DAP3) in Human Esophageal Cancer
Jian HAN, Ömer AN, Henry YANG, Polly Leilei CHEN

149 Decreased A-to-I RNA editing as a source of keratinocytes dsRNA in psoriasis
Leah Shallev, Eli Kopel, Ariel Feiglin, Gil S Leichner, Dror Avni, Yechezkel Sidi, Eli Eisenberg, Aviv Barzilai, Erez Y. Levanon, Shoshana Greenberger

392 Pseudouridine synthase 7-like protein PUS7L is required for infection by hepatitis C virus
Erin Borchardt, Wenyu Lin, Raymond Chung, Brett Lindenbach, Wendy Gilbert

121 Uridylation by TUT4/7 restricts retrotransposition of human LINE-1
Zbigniew Warkocki, Pawel Krawczyk, Dorota Adamska, Jose Garcia Perez, Andrzej Dziembowski

122 Withdrawn

123 Transcriptome-wide identification of substrates of radical SAM RNA methylating enzymes
Vanja Stojkovic, Tongyue Chu, Gabriel Therizols, David Weinberg, Danica Galonic Fujimori

FRIDAY, JUNE 1, 2018: 17:00 – 18:30
Workshop 6: Transcript Isoform Analysis – Zellerbach Playhouse
Qingqing Wang, Chair
Abstracts 124 – 127

124 Polypyrimidine Tract Sequence Determines Splicing Efficiencies of Long Non-Coding RNAs
Zuzana Krchnakova, Prasoon Kumar Thakur, Michaela Krausova, Nicole Bieberstein, Michaela Müller-McNicoll, David Stanek

125 An important class of intron retention events in human erythroblasts is regulated by cryptic exons proposed to function as splicing decoys
Marilyn Parra, Ben Booth, Richard Weiszmann, Brian Yee, Gene Yeo, James Brown, Susan Celniker, John Conboy

126 Widespread accumulation of ribosome-associated isolated 3' UTRs in specific neuronal cell populations of the aging brain
Peter Sudmant, Hyeseung Lee, Daniel Dominguez, Myriam Heiman, Christopher Burge

127 NMD-Degradome Sequencing Reveals Ribosome-Bound Decay Intermediates Tailored with Nontemplated Nucleotide Heterogeneity at Their 3'-Ends
Tatsuaki Kurosaki, Keita Miyoshi, Jason Myers, Lynne Maquat

SATURDAY, JUNE 2, 2018: 09:00 – 10:30
Plenary Session 4: 3′-end Formation and RNA Decay – Zellerbach Auditorium
Bobby Hogg, Chair
Abstracts 128 – 134

128 A Hub for 3'-end Processing: Structural Insights into mRNA Polyadenylation
Ana Casañal, Ananthanarayanan Kumar, Christopher H Hill, Ashley D Easter, Gianluca Degliesposti, Balaji Santhanam, Mark Skehel, Paul Emsley, Carol V. Robinson, Lori A. Passmore

129 Molecular basis for the recognition of the human AAUAAA polyadenylation signal
Yadong Sun, Yixiao Zhang, Keith Hamilton, James Manley, Yongsheng Shi, Thomas Walz, Liang Tong

130 Structural basis of AAUAAA polyadenylation signal recognition by the human CPSF complex
Marcello Clerici, Marco Faini, Lena Muckenfuss, Ruedi Aebersold, Martin Jinek

131 The structural basis of mRNA decapping: cap recognition and activation of the Edc1-Dcp1-Dcp2-Edc3 mRNA decapping complex with substrate analog poised for catalysis
Jeffrey Mugridge, Ryan Tibble, Marcin Ziemniak, Jacek Jemielity, John Gross

132 Structure of the nuclear exosome captured on a maturing pre-ribosome
Jan Michael Schuller, Sebastian Falk, Lisa Fromm, Ed Hurt, Elena Conti

133 Helicase-Assisted Degradation of Structured RNA by the Mtr4-Exosome Complex
John Zinder, Eva-Maria Weick, M Rhyan Puno, Kurt Januszyk, Michael DiMattia, Elizabeth Wasmuth, Laurent Cappadocia, Christopher Lima

134 Control of global mRNA turnover by protein acetylation
Fabian Poetz, Sahil Sharma, Georg Stoecklin

SATURDAY, JUNE 2, 2018: 11:00 – 12:30
Plenary Session 5: Coordinated RNA Processes – Zellerbach Auditorium
Gloria Brar, Chair
Abstracts 136 – 140

136 Direct observation of transcription and splicing dynamics in single human cells
Yihan Wan, Joseph Rodriguez, Murali Palangat, Gianluca Pegoraro, Gudla Prabhakar, George Zaki, Daniel Larson

137 Pervasive, coordinated protein level changes driven by transcript isoform switching during meiosis
George Otto, Ze Cheng, Emily Powers, Abdurrahman Keskin, Philipp Mertins, Steve Carr, Marko Jovanovic, Gloria Brar

138 Global changes in mRNA abundance drive differential shuttling of RNA binding proteins, linking cytoplasmic RNA degradation to transcription
Sarah Gilbertson, Joel Federspiel, Ileana Cristea, Britt Glaunsinger

139 Physiological relevance of the nucleo-cytoplasmic shuttling of the SR protein SRSF1
Fiona Haward, Magda Maslon, Ian Adams, Javier Caceres

140 Adenovirus co-opts cellular factors to mediate epitranscriptomic m6A modification of viral RNAs important for splicing and protein production
Alexander Price, Alexa McIntyre, Katharina Hayer, Nandan Gokhale, Kevin Janssen, Benjamin Garcia, Stacy Horner, Chris Mason, Matthew Weitzman

SATURDAY, JUNE 2, 2018: 14:00 – 17:00
Concurrent 7: RNA and Disease – Zellerbach Auditorium

Sponsored by Storm Therapeutics

Julia Kenyon, Chair

Abstracts 141 – 148, 150 – 153

141 Mechanisms of ALS/FTD-linked RNA binding protein aggregation
Yueh-Lin Tsai, Dinghai Zheng, Tristan H. Coady, Bin Tian, James L. Manley

142 The effect of CUG repeat binding molecule on the alternative splicing in myotonic dystrophy type 1
Jinxing Li, Masayuki Nakamori, Jun Matsumoto, Asako Murata, Chikara Dohno, Agnieszka Kiliszek, Kasia Taylor, Krzysztof Sobczak, Kazuhiko Nakatani

143 Mechanstic consequences of mutations in *DDX3X* in patients with medulloblastoma or global developmental delay
Ruiji Jiang, Lindsey Suit, Brieana Fregeau, Stephen Floor, Elliott Sherr

144 Analysis of oxidatively modified RNA in human neurons reveals the correlation between NAT8L mRNA damage and multiple sclerosis pathophysiology
Prakash Kharel, Lindsey Smith, Naveen Singhal, Ansuman Chattopadhyay, Jennifer McDonough, Soumitra Basu

145 The oncofetal RNA binding protein IGF2BP1 enhances an aggressive tumor cell phenotype by impairing miRNA-directed downregulation of oncogenic factors
Simon Mueller, Nadine Bley, Markus Glaß, Bianca Busch, Vanessa Rousseau, Danny Misiak, Tommy Fuchs, Marcell Lederer, Stefan Huettelmaier

146 Translation control of the immune checkpoint in cancer and its therapeutic targeting
Yichen Xu, Hyun Yong Jin, Mauro Poggio, Zhen Shi, Craig Forester, Craig Stompf, Maria Barna, Kevin Webster, John Cunningham, Davide Ruggero

147 Oncogenic Variation of Ribosomal RNA in Human Cancer
Artem Babaian, Dylan Girodat, Hans-Joachim Wieden, Gregg Morin, Dixie Mager

148 Spatial and developmental regulation of A-to-I RNA editing in the brain
Victor Karlström, Marie Öhman

150 Engineered RNase P ribozymes effectively inhibit the infection of murine cytomegalovirus in animals
Wei Tang, Wei Li, Yujun Liu, Gia-Phong Vu, Michael Reeves, Xu Sun, Xiwen Xing, Phong Trang, Fenyong Liu

151 Therapeutic targeting of a thrifty microRNA in metabolic diseases
Anders Naar

152 High UGA nonsense mutation correction by a new readthrough molecule
Catherine Leroy, Carole Trzaska, Hana Benhabiles, Fabrice Lejeune

153 A translation inhibitor targets a bimolecular cavity between eIF4A and polypurine RNA
Shintaro Iwasaki, Wakana Iwasaki, Mari Takahashi, Ayako Sakamoto, Yuichi Shichino, Stephen N. Floor, Koichi Fujiwara, Mari Mito, Kosuke Dodo, Mikiko Sodeoka, Takuhiro Ito, Nicholas T. Ingolia

SATURDAY, JUNE 2, 2018: 14:00 – 17:00
Concurrent 8: RNA Structure – Zellerbach Playhouse

Yanli Wang, Chair

Abstracts 154 – 165

154 How ubiquitous and important are transient "invisible" RNA states for biological function?
Theodore Dayie

155 High temporal- and spatial resolution studies of a helix-to-coil transition that controls the switching mechanism of a riboswitch
Nathan S. Daly, Jason Hon, Scott M. Trocchia, Colin Nuckolls, Kenneth L. Shepard, Ruben L. Gonzalez Jr.

156 Co-transcriptional folding of a riboswitch controls the fate of the transcriptional machinery
Adrien Chauvier, Julia Widom, Jason Porta, Melanie Ohi, Nils G. Walter

157 Structural basis of tRNA-mediated transcription anti-termination by a T-box riboswitch
Shuang Li, Zhaoming Su, Jean Lehmann, Vasiliki Stamatopoulou, Constantinos Stathopoulos, Wah Chiu, Jinwei Zhang

158 New tRNA interaction sites in the US T-box riboswitch
Jane K. Frandsen, Anna V. Sherwood, Frank J. Grundy, Tina M. Henkin

159 A structural basis of gene regulation by guanidine riboswitches
Lin Huang, David Lilley

160 Crystal structure of an essential RNA domain from IRES of hepatitis A virus in complex with a synthetic antibody fragment
Deepak Koirala, Yaming Shao, Yelena Koldobskaya, James Fuller, Sandip Shelke, Phoebe Rice, Evgeny Pilipenko, Joseph Piccirilli

161 The Structure of an Extended RNA Kink-Turn Reveals a Potential Tool for RNA Design
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162 A structurally complex fluorescent aptamer is improved with rational design and structure guided engineering
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316 In Vivo Expression Studies of the V. cholerae Glycine Riboswitch including Mutational Analysis of Ligand-Binding and Dimerization Regions
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321 Selective export into exosomes and function of tRNA fragments during T cell activation
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332 Nuclear Ago2 Participates MicroRNA Regulation of Viral RNA Splicing by Interacting with RNA C*is*-elements
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339 New transcription of 18S and 25S ribosomal RNA components by RNA Polymerase II, in Candida albicans during nutritional depletion
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341 Microexons alternatively spliced in the transcript of a human ribosomal protein gene.
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342 Modular assembly of the nucleolar pre-60S ribosomal subunit
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345 Bcp1 and methyltransferase Rkm1 regulate the stability of ribosomal protein Rpl23 (uL14) coordinately
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348 RNA demethylase FTO influences nuclear pre-mRNA processing events
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349 Genome-wide analyses reveal the coordination between alternative splicing and DNA methylation for light response in plants
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350 Control of RNA structure and RNP assembly by N^6-methylation of adenine in sheared basepairs
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351 RNA binding to a chromatin modifier - Polycomb Repressive Complex 2
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352 RNA-mediated chromatin alterations in cellular dormancy
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353 Identification and characterization of RNAs that interact directly with the human LSD1 complex.
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354 Determining the effects of mutations in the chromatin remodeler subunit SMARCA4 on alternative splicing in lung adenocarcinoma
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355 Regulation of alternative splicing by p300-mediated acetylation of splicing factors
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356 Novel Oncogenic Function of ARID1A-L in Ewing Sarcoma
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358 Profiling of recurrent 5' UTR mutations reveals altered regulatory elements and structural stability in cancer genomes
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359 Computational Tools for 2D and 3D Structure Modeling of RNA
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360 Functional RNA interactions as defined by RNA binding protein data and RNA secondary structure probing under *in vitro* and *in vivo* conditions
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361 Divergence of singleton and tandem glycine riboswitch aptamers
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362 PyRy3D: software for modeling macromolecular complex structures and its application to the *Trypanosoma brucei* 20S editosome
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363 Understanding the Basis and Evolution of tRNA Gene Regulation with Comparative Epigenomics
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364 Transcriptomic splicing information may leak personal private information by computationally linked to the genome
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365 Comprehensive portrait of canonical and non-canonical splicing in cancer
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366 Rfam: The transition to a genome-centric sequence database
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367 miRTrace: a tool for quality control and tracing taxonomic origins of microRNA sequencing data
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368 A human-specific switch of alternatively spliced AFMID isoforms contributes to TP53 mutations and tumor recurrence in hepatocellular carcinoma
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369 rna-pdb-tools: a toolbox to analyze structures and simulations of RNA & dataset of curated structures submitted to the RNA-Puzzles experiment
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370 Heteroformity: a measure of diversity for alternative splicing
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371 Systematic characterization of reverse transcriptases-related proteins and their diversities in prokaryotes
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372 Withdrawn

373 Withdrawn

374 The codon stabilization coefficient is a reliable parameter to indicate the most biologically accurate mRNA half-life measurements in *S. cerevisiae.*
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375 Computer-aided design of RNA structures and targeting
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376 RNAcentral: The unified database of ncRNA sequences with comprehensive genome mapping and improved quality controls
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378 Discovery and characterization of putative non-coding RNAs regulating methylases
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379 R2C2: Increasing Accuracy of MinION Sequencing Reads
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380 The Junction Usage Model (JUM): A method for comprehensive annotation-free analysis of tissue-specific alternative pre-mRNA splicing patterns
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381 Prospects for recurrent neural network models to learn RNA biophysics from high-throughput data
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382 Transcription contributes modestly to the regulation of genome-wide differential microRNA expression in mammals
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383 Mining the miRBase: what are we talking about when we talk about miRBase
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385 Activity-dependent modulation of cytoplasmic polyadenylation element binding protein 3 ribozyme
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386 Tandem hammerhead ribozymes in metazoan genomes give rise to abundant circRNAs involved in new types of retrotransposons
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387 Fitness landscapes and genotype networks for understanding RNA evolution
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388 Directed Evolution of a Novel L-Ribonuclease Ribozyme against HIV-1 D-TAR Element
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389 Specificity for adenine is established during the hydrolytic cycle of poly(A)-specific ribonuclease (PARN)
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390 Probing the Cpf1-crRNA Interaction with Ribochemical Modification
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391 The m^{6}A RNA methyltransferase METTL16 targets the U6 snRNA, various non-coding RNAs and pre-mRNAs
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392 Pseudouridine synthase 7-like protein PUS7L is required for infection by hepatitis C virus
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394 Enzymatic properties of Vaccinia RNA capping enzyme
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395 Genome-wide analysis of RBP targets in plants
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397 Roles in rRNA methylation for the C-terminal domain of erythromycin resistance methyltransferases, members of the rRNA-Adenine Dimethylase family
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398 TRMT1-catalyzed RNA modifications modulate translation to ensure proper proteostasis and neurodevelopment
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399 PseudoU writers of the Epitranscriptome: Identification and Characterization of Pseudouridine synthases
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402 Maize Active RNA Editing Complexes Include PPR, DYW, OZ, RIP/MORF, and ORRM Proteins
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404 Structual and functional study of tRNA 2'-phosphotransferase Tpt1p in *Saccharomyces cerevisiae*
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405 Roles of Fragile X proteins in RNA editing regulation
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406 Components of human mitochondrial RNase P act together to bind, methylate and process mitochondrial pre-tRNAs
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407 Deciphering effects of *Adar* on *Drosophila* metamorphosis.
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408 Surveillance of hypomodified tRNAs promotes bacterial tRNA quality control
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409 Expression and function of an oxidative stress induced RNA repair system in *Escherichia coli*
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410 tRNA processing in human mitochondria
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411 RNA editing is important for worm development and regulates RNAi
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412 The landscape of miRNA editing in animals and its impact on miRNA biogenesis and targeting
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413 Dynamic RNA Splicing and Gene-Specific Edited Isoforms identified in human PBMCs using high-throughput sequencing and alternate allele specific quantification methods.
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414 Deciphering the code of structure-function relationship in A-to-I RNA editing
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415 Structure and mechanism of a bacterial t6A biosynthesis system
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416 Engineering an RNA-targeting CRISPR/Cas9 system to edit transcripts in living cells
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417 Mutagenesis screens identify editosome protein domains that differentially affect RNA editing between life cycle stages of *Trypanosoma brucei*
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418 PRIMA-seq: novel method for enrichment and sequencing of the RNAs with primary aliphatic amines
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419 Human Pus10 protein produces pseudouridine 54 in select tRNAs
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420 Structural features of archaeal box C/D guide sRNAs required for dual 2'-*O*-methylation function
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421 A-to-I RNA editing regulates skeletal muscle specific exonization of Alu element
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422 Structural basis for preferential deadenylation of U6 snRNA by Usb1
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424 A new tool for sequence-specific manipulation of N^6-methyladenosine
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425 Metabolic and chemical regulation of tRNA modification associated with taurine deficiency and human disease
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429 Investigating the ADAR interactome
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430 Profile of m6A RNA modification identified age-associated regulation of AGO2 mRNA stability
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432 Analysis of HCV RNA genome structure
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433 Structural and functional study of *Flaviviridae* and *Picornaviridae* untranslated region in the pre-initiation complex .
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435 Diverse structural ensembles of the MALAT1 triple helix reveal putative target sites for drug discovery.
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436 A new classification and nomenclature of RNA conformation connecting backbone conformers and mutual position of nucleobases
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437 Seed length drives structural flexibility of miR-34a-SIRT1 mRNA complex
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438 Dissection of the Energetic and Conformational Properties of a Ubiquitous Tertiary Structural Motif through High-throughput and Single-molecule Studies
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439 Impact of Modified Nucleobases on Base Pairing in RNA Experimental Structures
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440 SHAPE-JuMP: Detecting RNA-RNA interactions via covalent linkage and reverse transcription
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441 Fold and protein binding of the U12-U6$_{atac}$ snRNA complex of the minor spliceosome
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442 Capturing The Light Triggered Release of Adenine Inside Adenine Riboswitch Crystals
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443 Influence of conformationally restricted nucleosides on model RNAs structure.
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445 Suppression of alternate pseudoknot fold expands the human telomerase template repertoire.
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446 Simulating the miR-34a-mSIRT1 conformational rearrangement using Replica Exchange Molecular Dynamic simulations to assess miRNA activity
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447 Monitoring the 30S binding dynamics to a tbpA riboswitch in real time
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448 Altering the rate of translation by ribosome-induced conformational switching of a structured 3'UTR.
Erik Hartwick, David Costantino, Andrea MacFadden, Jay Nix, Siqi Tian, Rhiju Das, Jeffrey Kieft

449 RNA Duplex Destabilization Due to Cosolvent Effects
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450 Structural insights into improved riboswitch regulation from natural variation
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451 Nucleobase-Water Stacking Interactions in RNA Molecules: Bioinformatics and Quantum Mechanics Analysis
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452 NMR studies of an RNA thermosensor
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453 Chemo-enzymatic synthesis of position-specifically modified RNA for biophysical studies including light control and NMR spectroscopy
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454 Computer Folding of guanine quadruplexes
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455 The formation of intramolecular secondary structure brings mRNA ends in close proximity
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456 Withdrawn

457 Co-crystal structure of a T-box riboswitch 3' domain in complex with its cognate tRNA
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458 Utilizing Chemical Tools to Explore the Role of Secondary and Tertiary Structural Motifs in MALAT1-driven Cancer Processes
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459 Screening assays to identify small molecule inhibitors targeting the MALAT1 triple helix
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460 An RNA Pseudoknot Stimulates Human T-cell Lymphotropic Virus Type 1 *pro-pol* -1 Programmed Ribosomal Frameshifting
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461 Use of SHAPE to study *in vivo* RNA folding in *Entamoeba histolytica*
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462 Ligand-driven Changes in Spinach2 and Broccoli Aptamer Affinities.
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464 Global structural remodeling as an evolutionary response to sequence duplication in RNA
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465 Crystal structure determination of riboswitches and BsMiniIII endoribonuclease-assisted production of recombinant RNA
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466 Unwinding the double pseudoknot of the HDV-like ribozyme family
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467 Relating thermodynamics to structure: A comparative NMR analysis of an RNA duplex before and after adenosine deamination
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468 Probing RNA secondary structure in the activated yeast spliceosome
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470 Time-resolved crystallographic studies of the adenine riboswitch aptamer domain
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471 Structural determinants of exoribonuclease-resistant RNA elements
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472 Versatile Tools Towards Real-time Single-molecule Biology
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473 Is there a riboSNitch in the 3' UTR of Lipoprotein Lipase?
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474 An mRNA structure that affects aberrant expression of a proto-oncogene by eIF3 recognition
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475 Blind prediction of noncanonical RNA structure at atomic accuracy
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476 Effects of *in vivo-like* Conditions on G-quadruplex Formation
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477 Dissecting binding modes of near-cognate ligand analogs of the GTP class II RNA aptamer
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478 An automated protocol to identify RNA sequence families that adopt similar tertiary structures: GNRA and UNCG tetraloops as a test case
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479 RNA-based regulation of gene expression to improve the biotechnologically relevant bacteria *Methylobacterium extorquens*
Emilie Boutet, Roqaya Imane, Fallou Wade, Martin Lamarche, Carlos Miguez, Jonathan Perreault

480 Expanding the RNA Mango Toolbox Through Multi-stem and Split Designs
Steven Burden, Hagen Shults, Sabrina Zerrade, Matthew Ferguson, Eric Hayden

481 A Study of Transcriptional Activation by the Transcription Factor Gal4 in Saccharomyces cerevisiae by 3D Orbital Tracking and in vivo RNA labelling
Anh Huynh, Micah Buckmiller, Daniel R. Larson, Tineke Lenstra, Matthew L. Ferguson

482 Chemical modifications on synthetic guide RNA for improved RNA stability and cellular viability in CRISPR-Cas9 genome editing
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483 Applying riboregulator to knock down chromosomal gene *cyabrB2* in *Synechocystis* sp.PCC 6803 for higher glycogen production
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484 Non-canonical translation initiation signals for synthetic biology
Justin Vigar, Erik Holmqvist, Lars Barquist, Jalyce Heller, Graeme Glaister, Astha, Adriana Zyla, Trushar Patel, Janusz Bujnicki, Jörg Vogel, Hans-Joachim Wieden

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Joseph Yesselman, Daniel Eiler, Erik Carlson, Alexandra Ooms, Wipapat Kladwang, Xuesong Shi, David Costantino, Daniel Herschlag1, Michael Jewett, Jeffrey Kieft, Rhiju Das

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486 Naturally intronless mRNAs are targeted to nuclear speckles for assemblingexport-competent mRNPs
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487 Regulation of mRNA export *in vivo* by the DEAD-box ATPase Dbp5
Stephanie Heinrich, Maria Hondele, Désirée Marchand, Carina Patrizia Derrer, Pascal Vallotton, Ben Montpetit, David Grunwald, Karsten Weis

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Michelle Ho, John Rossi, John Burnett

489 Withdrawn

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Kyoung Mi Kim, Krystyna Mazan-Mamczarz, Madhu Lal, Kotb Abdelmohsen, Myriam Gorospe

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Baohui Zhu, Qian Zhang, Shuai Hou, Weijie Wang, Haixin Lei

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Ying Li, Mahima B. Aggarwal, Ke Ke, Kim Nguyen, Robert C. Spitale

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Alena L. Drayton, B. Fadi Marayati, James F. Tucker, Reid H. Huckabee, James B. Pease, Clifford W. Zeyl, Ke Zhang

494 Stress granules are heterogeneous assemblies composed of distinct core substructures that contain unique RNA and protein compositions
Tyler Matheny, Anthony Khong, Briana Van Treeck, Roy Parker

495 Nopp140 is required for targeting Cajal body-specific (sca)RNPs to Cajal bodies and for proper modification of spliceosomal snRNAs
Jonathan Bizarro, Svetlana Deryusheva, Joseph G. Gall, U. Thomas Meier

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Matthew Taliaferro, Gary Bassell, Christopher Burge

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Richard Taylor, Corinne Houart

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Tatjana Trcek, Tyler Douglas, Hari Shroff, Ruth Lehmann

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Tatsuhisa Tsuboi, Matheus Viana, Evelina Tutucci, Robert Singer, Susanne Rafelski, Brian Zid

501 RNA self-assembly contributes to stress granule formation and defining the stress granule transcriptome
Briana Van Treeck, David Protter, Tyler Matheny, Anthony Khong, Christopher Link, Roy Parker

502 Coordinated regulation of *hunchback* mRNA by Pumilio, Nanos and Brain Tumor Protein in *Drosophila*
Zhenyu Yu, Yi Wang, Na Yang, Ruiming Xu

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Binyamin Zuckerman, Igor Ulitsky

791 Unraveling the function and subcellular localization of DDX6 in Human cells
Jo-Hsi Huang, Wei-Chi Ku, Yen-Chun Chen, Yi-Ling Chang, Chia-Ying Chu

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504 Global nuclear mRNA retention by the FUBP family of RNA binding proteins as a novel mechanism of posttranscriptional gene regulation
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505 The NEXT complex controls the proper levels of miRNA precursors in plants
Mateusz Bajczyk, Lukasz Szewc, Dawid Bielewicz, Agata Stepien, Heike Lange, Zofia Szweykowska-Kulinska, Dominique Gagliardi, Artur Jarmolowski

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Alicia Bao, Anita Hopper

507 Genome-Wide Analysis of mRNA Degradation Kinetics
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508 Phosphorylation-dependent regulation of deadenylation by the Tristetraprolin CNOT1-interacting motif
Alberto Carreno, Jens Lykke-Andersen

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Clementine Delan-Forino, Christos Spanos, Juri Rapsilber, David Tollervey

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Mohamed El-Brolosy, Andrea Rossi, Zacharias Kontarakis, Carsten Kuenne, Stefan Günther, Nana Fukuda, Carter Takacs, Shih-Lei Lai, Ryuichi Fukuda, Caludia Gerri, Khrievono Kikhi, Antonio Giraldez, Didier Stainier

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Elena Galimberti, Marius Garmhausen, Andreas Lackner, Veronika Herzog, Stefan Ameres, Martin Leeb

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Pooja Gangras, Thomas L. Gallagher, Kiel T. Tietz, Natalie C. Deans, Sharon L. Amacher, Guramrit Singh

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Ewa Grudzien-Nogalska, Huijuan Cui, Megerditch Kiledjian, Xinfu Jiao

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Sujatha Jagannathan, Stephen Tapscott, Robert Bradley

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Aparna Kishor, Zhiyun Ge, J. Robert Hogg

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Yujin Lee, Ok Hyun Park, Hongseok Ha, Kwon Jeong, Yoon Ki Kim

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Joseph Lobel, Ryan Tibble, John Gross

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Mikhail Mesitov, Takuma Suematsu, Liye Zhang, Tian Yu, Inna Aphasizheva

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Aneliya Rankova, Alex Tuck, Daniel Hess, Vytautas Iesmantavicius, Matyas Flemr, Marc Bühler

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Nadra Al-Husini, Dylan Tomares, Seth Childers, Jared Schrader

522 Assessing the role of UPF1 during premature translation termination
Lucas D. Serdar, DaJuan L. Whiteside, Sarah Nock, Kristian E. Baker

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Ang Gao, Nikita Vasilyev, Joel Belasco, Alexander Serganov

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Irina St. Louis, Yeseul Ahn, Calandra Sagarsky

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Aleksandra Sulkowska, Andor Auber, Dániel Silhavy, Joanna Kufel, Izabela Wawer

526 The human homolog of a bacterial endonuclease is essential for mitochondrial gene expression
Sabrina Summer, Alexandre Smirnov, Anna Smirnova, Nina Entelis, Ivan Tarassov, Walter Rossmanith

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Akinori Takahashi, Shohei Takaoka, Haytham Mohamed, Tomokazu Yamaguchi, Keiji Kuba, Tadashi Yamamoto

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Ryan Tibble, David Paquette, Tristan Daifuku, John Gross

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Kiel Tietz, Thomas Gallagher, Zachary Morrow, Nicolas Derr, Sharon Amacher

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531 Withdrawn

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Masashi Yamaji, Miki Jishage, Cindy Meyer, Hemant Suryawanshi, Evan Der, Misaki Yamaji, Aitor Garzia, Pavel Morozov, Sudhir Manickavel, Hannah McFarland, Robert G Roeder, Markus Hafner, Thomas Tuschl

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Akiko Yanagiya, Dina Mostafa, Tadashi Yamamoto

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535 Functions of LARP4 RNA Binding Proteins in Vertebrate Development
Emily Baldwin, Triona Fielding, Maria Conte, Corinne Houart

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Vandita Dattatraya Bhat, Kathleen L. McCann, Yeming Wang, Dallas Fonseca, Te-Wen Lo, Marvin Wickens, Traci M. Tanaka Hall, Zachary T. Campbell

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Kristopher Brannan, Kevin Dong, Erica Wolin, Marko Jovanovic, Gene Yeo

539 "High VaultAge" - Molecular studies on the vault RNA1-1 – p62 interaction
Magdalena Büscher, Rozemarijn Kleinendorst, Mandy Rettel, Frank Stein, Kevin Haubrich, Janosch Hennig, Mikhail Savitski, Carsten Sachse, Rastislav Horos, Matthias Hentze

540 Exploring the characteristics and functions of RNA duplexes bound in vivo by Staufen
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541 Single molecule study of the dynamics of RNA unfolding by protein S1: Conformational stability of the RNA matters most
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Anna Desai, Zhiqiang Hu, James Lloyd, Steven Brenner

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Daniel Dominguez, Peter Freese, Maria S. Alexis, Amanda Su, Tsultrim Palden, Myles Hochman, Nicole Lambert, Gene Yeo, Eric L. Van Nostrand, Breton Graveley, Christopher B. Burge

545 Investigating the Interactions of Splicing Factor SF3A1 with the Stem-loop 4 of U1 snRNA and RNA helicase UAP56
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546 Molecular basis of function of the RNA-binding protein PSF
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Solomon Haizel, Somdeb Mitra, Usha Bhardwaj, Ruben L. Gonzalez, Jr., Dixie J. Goss

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Frances E. Henderson, Jackson M. Gordon, Jinwei Zhang

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Min Jia, Heinz Gut, Jeffrey Chao

550 Genome-wide mapping RNA targets of *Enterococcus faecalis* Small Alarmone Synthetase RelQ using *in vivo* UV crosslinking
Steffi Jimmy, Liis Andresen, Vallo Varik, Gemma C. Atkinson, Vasili Hauryliuk

551 Structural basis of Dnmt2 stimulation by queuine tRNA modification
Sven Johannsson, Piotr Neumann, Alexander Wulf, Luisa Mathilde Welp, Hans-Dieter Gerber, Matthias Krull, Ulf Diederichsen, Henning Urlaub, Ralf Ficner

552 In-gel probing to examine RNA structures within RNA-protein complexes of varying stoichiometry
Ziqi Long, Jingwei Zeng, Andrew Lever, Julia Kenyon

553 Systematic investigation of RNA-targeting determinants for CRISPR-Cas13
Gavin J. Knott, Akshay Tambe, Brittney W. Thornton, Jennifer A. Doudna

554 Interactomic and Enzymatic Analyses of Affinity Isolated Human LINE-1 Retrotransposons
Martin Taylor, Ilya Altukhov, Vikram Deshpande, John LaCava

555 Genome-wide identification of DEAD-box RNA helicase targets reveals roles for RNA secondary structure remodeling in transcriptional termination
Yu-Hsuan Lai, Krishna Choudhary, Zheng Xing, Sharon Aviran, Elizabeth Tran

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Audrone Lapinaite, Jennifer A. Doudna, Jamie H. D. Cate

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Yeon Lee, Qingqing Wang, Bo Li, Lior Pachter, Donald Rio

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Neil Lloyd, Deborah Wuttke

560 The effects of SF3B1 inhibitors on U2 snRNP
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Prabuddha Madubashitha, Nisansala Muthunayake, Christine Chow

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Ewan McRae, Dave Davidson, Sean McKenna

564 Deciphering human ribonucleoprotein regulatory networks
Neelanjan Mukherjee, Svetlana Lebedeva, Marcin Sajek, Hans-Hermann Wessels, Thalia Farazi, Aitor Garzia, Mahsa Ghanbari, Alina Munteanu, Jessica Spitzer, Kemal Akat, Thomas Tuschl, Uwe Ohler

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566 RNA Aptamers and HIV-1 Reverse Transcriptase: The Molecular Basis of Their Interface and Its Impact on the Reverse Transcriptase-Protease Interaction.
Phuong Nguyen, Jie Zheng, Steve Tuske, Margaret Lange, Patrick Griffin, Eddy Arnold, Donald Burke

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Stephanie Oerum, Clément Dégut, Marjorie Catala, Pierre Barraud, Ciaran Condon, Carine Tisné

568 *circSamD4* promotes myogenesis
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Yeonkyoung Park, Hyunjung Hwang, Joori Park, Yoon Ki Kim

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Nicholas Parsonnet, Jon Markert, Robert Batey, Deborah Wuttke

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Katherine Rothamel, Byungil Kim, Mauel Ascano

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Davide Tavella, Katianna Antkowiak, Reyyan Bulut, Sean Ryder, Francesca Massi

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Sarah Safran, Brenda Bass

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Samantha Schwartz, Graeme Conn

575 Global Identification of Non-Coding RNA Targets of 3' Processing Enzymes
Timothy Shaw, Rea Lardelli, Jens Lykke-Andersen

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Yatendra pratap Singh, Shashi shekhar Singh, Alok Bhattacharya, Sudha Bhattacharya

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Erin Sternburg, Jason Estep, Yahui Li, Daniel Nguyen, Fedor Karginov

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Maxim Tollenaere, Julie Nielsen, Anna Vind, Melanie Blasius, Tanveer Batth, Niels Mailand, Jesper Olsen, Matthias Gaestel, Christopher Tiedje, Simon Bekker-Jensen

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Christopher Venters, Byung Ran So, Chao Di, Zhiqiang Cai, Jiannan Guo, Jung-Min Oh, Chie Arai, Gideon Dreyfuss

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Jyotsna Vinayak, Stefano Marrella, Rawaa Hussain, Karine Solomon, Leonid Rozenfeld, Mark Bayfield

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Xuan Ye, Fan Yang, Gabriele Varani, Eckhard Jankowsky

583 How the Modular RelA Architecture Controls Bacterial Stringent Response
Ying Zhang, Anna Loveland, Eugene Bah, Andrei Korostelev

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Aaron Mendez, Carolin Vogt, Jens Bohne, Britt Glaunsinger

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Yalemi Morales, Keith Olsen, Jacqueline Bulcher, Sean Johnson

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584 Therapeutic Effect of RECTAS for Familial Dysautonomia by Activating SRSF6 on IKBKAP Intronic Splicing Enhancer
Masahiko Ajiro, Young-Jin Kim, Tomonari Awaya, Lorenz Studer, Adrian Krainer, Masatoshi Hagiwara

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Benjamin Akiyama, J. David Beckham, Jeffrey Kieft

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Peter Androvic, Lukas Valihrach, Denisa Kirdajova, Martin Valny, Miroslava Anderova, Mikael Kubista

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Marcel Bach Pages, Farnusch Kaschani, Alfredo Castello, Renier A. L. van der Hoorn, Gail Preston

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Maria Barton, Kushal Rohilla, Katy Ovington, Anthony Henke, Courtney Slavich, Keith Gagnon

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Daphne A. Cooper, Pedro Miura

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Elizabeth DeLaney, Rosemary Dietrich, James Hiznay, David Symer, Richard Padgett

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Jonathan Lou Esguerra, Jones Ofori, Mototsugu Nagao, Shuto Yuki, Joao Fadista, Hitoshi Sugihara, Leif Groop, Lena Eliasson

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Rita F. Marques, Jan Broder Engler, Katrin Küchler, Ross A. Jones, Thomas Lingner, Gabriela Salinas-Riester, Tom H. Gillingwater, Manuel A. Friese, Kent E. Duncan

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Harry Fischl, Jonathan Neve, Zhiqiao Wang, Radhika Patel, Alastair Louey, Bin Tian, Andre Furger

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Heather Geissel, Hannah Steber, Stephen Blazie, Kasuen Kotagama, Jason Newbern, Alan Rawls, Marco Mangone

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Sethu Madhava Rao Gunja, Sangeetha Bupalan, Beata Filipek-Gorniok, Johan Ledin, Anders Virtanen

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Sven Hagemann, Marcell Lederer, Jessica Bell, Tao Liu, Stefan Hüttelmaier

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Zachary C. E. Hawley, Danae Campos-Melo, Kathryn Volkening, Michael J. Strong

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Dawon Hong, Sunjoo Jeong

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Joseph Iacona, Nicholas Monteleone, Carol Lutz

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Amina Jbara, Chani Stossel, Miri Danan-Gotthold, Maria Raitses-Gurevich, Erez Y. Levanon, Talia Golan, Rotem Karni

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Makenzie Saoura, Kyla-Gaye Pinnock, Christopher Powell, Joanna Rorbach, Louis Levinger, Michal Minczuk

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Qianyun Lu, Xuerong Yang, Daniel Dominguez, Zefeng Wang

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Mary McMahon, Adrian Contreras, Tamayo Uechi, Craig Forester, Bin Chen, David Quigley, Kate Kelley, John Gordan, Ryan Gill, Davide Ruggero

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Nicholas Monteleone, Joseph Iacona, Carol Lutz

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Nagammal Neelagandan, Giorgio Gonnella, Stefan Dang, P. Christoph Janiesch, Rita Marques, Katherine K Miller, Katrin Kuechler, Daniela Indenbirken, Malik Alawi, Adam Grundhoff, Stefan Kurtz, Kent E Duncan

611 Characterization of human disease-causing mutations affecting U12 snRNP function
Antto Norppa, Tuuli Kauppala, Harri Heikkinen, Bhupendra Verma, Hideo Iwaï, Mikko Frilander

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Rebecca Pötschke, Markus Glaß, Christian Mawrin, Christian Strauss, Caspar Kühnöl, Stefan Hüttelmaier

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Archana Prusty, Anja Hirmer, Verena Surrey, Florian Erhard, Ulf-Peter Günther, Bettina Boettcher, Sibylle Jablonka, Utz Fischer

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Ankur Gadgil, Agata Stepien, Pawel Czubak, Marc-David Ruepp, Katarzyna Dorota Raczynska

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Mohammad Alinoor Rahman, Kuna-Ting Lin, Omar Abdel-Wahab, Adrian Krainer

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Christy Rhine, Kamil Cygan, Rachel Soemedi, Samantha Maguire, Michael Murray, Sean Monaghan, William Fairbrother

617 THE SNORNA U3 ALTERS THE CELLULAR PHENOTYPE OF CHONDROCYTES IN OSTEOARTHRITIS
M. Peffers, E.G.J. Ripmeester, M.M.J. Caron, G. van den Akker, M.M.F. Steinbusch, P. Balaskas, A. Cremers, D.A.M. Surtel, T.J.M. Welting

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Sathya Selvarajan, Jothimalar Ramalingam, Jaya Vijayaraghavan

620 Modeling microcephaly using MOPD I patient-derived iPS cells and cerebral organoids to study neuro-developmental defects
Jagjit Singh, Milena Pirozzi, Anthony Wynshaw-Boris, Richard A. Padgett

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Justin Welden, Peter Nelson, Stefan Stamm

623 eCLIP-Seq reveals differential recognition of RNA targets by mutant SRSF2 in stem cell derived hematopoietic progenitor-like cells
Emily Wheeler, Andriana Kotini, Chan-Jung Chang, Eirini Papapetrou, Gene Yeo

624 Development of an inhibitor against Musashi-1 and its use in glioblastoma therapy
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625 Structural insights into the RNA methyltransferase domain of METTL16
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626 Crosslinking of segmentally isotope labeled RNA and MS/MS on large multi-molecular complexes and different RNA-binding domains
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627 Determining RNA target binding specificities of *Marinitoga piezophila* Argonaute (MpAgo) using a structural approach
Angélica M. González Sánchez, Jamie Cate

628 Mutational studies of the Adenoviral Virus-Associated RNA I support a functionally important pseudoknot
Jackson Gordon, Iris Hood, Frances Henderson, Soheila Bahmanjah, Charles Bou-Nader, Jinwei Zhang

629 An atomic structure of the yeast Ribonuclease P
Pengfei Lan, Ming Tan, Jian Wu, Yuebin Zhang, Shuangshuang Niu, Shuwan Qiu, Xuejuan Wang, Gang Cai, Hong Cheng, Guohui Li, Ming Lei

630 Structural architecture of human RNase P holoenzyme by cyro-EM
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631 Structural Insight into the Catalytic Mechanism of Yeast RNase P
Ming Tan, Pengfei Lan, Jian Wu, Guohui Li, Ming Lei

794 Mechanistic Insights into the Regulation of let-7 Biogenesis by hnRNP A1
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Splicing Mechanism and Regulation

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632 Defining an exon commitment window in Exon Definition: insights from Designer Exons
Mauricio Arias Hernandez, Lawrence Chasin

633 Structure of the Post-catalytic Spliceosome from *Saccharomyces cerevisiae*
Rui Bai, Chuangye Yan, Ruixue Wan, Jianlin Lei, Yigong Shi

634 Characterization of regulatory mechanisms for *BIM* exon 3 alternative splicing
Malini Bhadra, Jun Liu, Cheryl Tan, Wan Lin Yue, S.Tiong Ong, Xavier Roca

635 Novel self-splicing introns discovered in genomes of ubiquitous, but little-known Candidate Phyla Radiation bacteria
Christopher Brown, Jillian Banfield, Jamie H. D. Cate

636 In search of kinetic changes in splicing in response to mutations in SF3b1 and Prp8
Tucker Carrocci, Karla Neugebauer

637 mRNA processing regulates the unfolded protein response.
Patrick Cherry, Sally Peach, Jay Hesselberth

638 A global binding map of hnRNP A2/B1
Lise Lolle Christensen, Thomas Koed Doktor, Akio Masuda, Kinji Ohno, Brage Storstein Andresen

639 Identification of protein components in *Trypanosoma cruzi* spliceosome
Rosicler Barbosa, Maria Carolina Elias, Ariel Silber, Patricia Coltri

640 Characterizing the role of Prp16 in spliceosome dynamics and disease
Christopher Craddock, Rafal Ploski, Jonathan Staley

641 Investigating the Role of DDX41 in pre-messenger RNA Splicing and Leukemogenesis
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642 Structural and Functional Characterization of the Spliceosomal Protein Prpf39
Francesca De Bortoli, Bernhard Loll, Alexander Neumann, Markus Wahl, Florian Heyd

643 Using Rapid Nuclear Depletion of Srp2 in *Schizosaccharomyces pombe* with Targeted Sequencing of Splice Junctions from Nascent RNA to Better Understand the Role of SR Proteins in Splicing Regulation
Zachary Dwyer, Jeffrey Pleiss

644 Yeast to human: Yeast U1 snRNP as a model to understand human alternative splicing
Sara Espinosa, Xueni Li, Lingdi Zhang, Rui Zhao

645 When the Arabidopsis *sr45-1* mutant meets environmental challenges
Steven Fanara, Marc Hanikenne, Patrick Motte

646 Crystallographic snapshot of the pre-catalytic state of the spliceosomal DEAH-box ATPase Prp2 in complex with RNA
Florian Hamann, Ralf Ficner

647 Characterization of a group IIc intron with its intron encoded maturase protein
Stephanie Hamill, Chen Zhao, Anna Marie Pyle

648 Investigating alternative RNA processing during mammalian germ cell development
Molly M. Hannigan, Thomas J. Sweet, Leah L. Zagore, Donny D. Licatalosi

649 Evidence Suggesting Prp22 Activates 3' Splice Sites Sequestered in RNA Secondary Structure
Cody Anthony Hernandez, Jonathan P. Staley

650 Growth temperature phenotypes of chimeric yeast/human HSH155/SF3B1 genes uncover a relationship between thermo-stability and Pladienolide B-resistance in HSH155/SF3B1 function.
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651 The role of Npl3p in pre-mRNA splicing in yeast
Agata Jaskulska, Magda Konarska

652 Conserved regulation of RNA processing in somatic cell reprogramming
Alexander Kanitz, Afzal Pasha Syed, Keisuke Kaji, Mihaela Zavolan

653 New Insights into Spliceosome Activation using CoSMoS
Harpreet Kaur, Margaret Rodgers, Aaron Hoskins

654 Antisense oligonucleotides correct the familial dysautonomia splicing defect in IKBKAP transgenic mice.
Rahul Sinha, Young Jin Kim, Tomoki Nomakuchi, Kentaro Sahashi, Yimin Hua, Frank Rigo, C. Frank Bennett, Adrian Krainer

655 NOVA2-mediated regulation of circRNAs during mammalian neurogenesis
David Knupp, Daphne A. Cooper, Simon Pieraut, Pedro Miura

656 Knock out of human variant U2 snRNAs affects alternative splicing
Brian Kosmyna, Varun Gupta, Charles Query

657 A novel multi-domain protein CCAR-1 regulates transcription and co-transcriptional splicing in *C. elegans*.
Hidehito Kuroyanagi, Marina Togo-Ohno, Eichi Watabe, Yohei Watanabe, Yutaka Suzuki, Masatoshi Hagiwara

658 Esrp1 splicing regulation is required for tight junction integrity through epithelial Arhgef11 isoforms that activate RhoA and myosin phosphorylation
SungKyoung Lee, Russ Carstens, Natoya Peart, Yueqin Yang, Benjamin Ciepley

659 Dissecting the mechanism of H3K36 methylation in regulating RNA splicing
Calvin Leung, Stephen Douglass, Marco Morselli, Matthew Obusan, Matteo Pellegrini, Tracy Johnson

660 Withdrawn

661 Cryo-EM structure of the yeast prespliceosome
Clemens Plaschka, Pei-Chun Lin, Clément Charenton, Kiyoshi Nagai

662 Coupled Yeast transcription and splicing *in vitro*
Hsin-I Liu, Chun-Shu Yeh, Tung Luh, Then-Hsien Chang

663 Estimating the Genome-wide Prevalence of SF3A1-dependent Splice Site Pairing
William Martelly, Shalini Sharma

664 Roles of Fission Yeast SR Protein Phosphorylation During Stress
Alexander Piening, Moriah Davis, Michael Marvin

665 Yeast protein Cwc2 and the N-terminal domain of Prp8 stabilize the spliceosomal catalytic center in the first-step conformation.
Katarzyna Matylla-Kulinska, Katarzyna Eysmont, Magda Konarska

666 U2AF is replaced by SPF45 as general splicing factor to recognize weak pyrimidine tract and promotes splicing of human short introns
Kazuhiro Fukumura, Rei Yoshimoto, Tetsuro Hirose, Kunio Inoue, Akila Mayeda

667 RBPMS: A Master Regulator Of The Differentiated Smooth Muscle Cell Alternative Splicing Program
Erick Nakagaki Silva, Clare Gooding, Aishwarya Jacob, Chris Smith

668 Determining the Function of Yeast Ecm2 and its Role in Splicing
Brandon Nikolai, Clarisse van der Feltz, Charles Schneider, Brexton Turner, Margaret Rodgers, Aaron Hoskins

669 Prp19 is an autoinhibited ubiquitin ligase activated by stepwise assembly of three splicing factors
Tales De Moura, Csaba Kibedi, Jana Schmitzova, Olexandr Dybkov, Constantin Cretu, Michael Kachala, Dmitry Svergun, Henning Urlaub, Reinhard Lührmann, Sina Mozaffari-Jovin, Vladimir Pena

670 Study of the effect of post-translational modifications on splicing factor Cwc24 in *Saccharomyces cerevisiae*
M. Griselda Perona, Carla C. Oliveira, Bruno Manadas

671 Single molecule assays resolve ATP dependent and ATP independent binding modes of yeast spliceosomal helicase Prp16
Sujay Ray, Zhenyu Tan, Nils Walter

672 Co-transcriptional splicing during erythroid development
Kirsten Reimer, Karla Neugebauer

673 Alternative splicing analysis in human monocytes and macrophages reveals MBNL1 as major regulator
Hongfei Liu, Paolo Lorenzini, Fan Zhang, Shaohai Xu, Jie Zheng, Xavier Roca

674 Self-regulatory network of the core spliceosome
Malgorzata Rogalska, Estefania Mancini, Caterina Coli, Juan Valcárcel Juárez

675 Intron retention redefines post-transcriptional gene regulation in vertebrate species
Ulf Schmitz, Natalia Pinello, William Ritchie, Justin Wong, John Rasko

676 Functional analysis of RNF113A in human splicing
Guilherme Henrique Gatti Silva, Patricia Pereira Coltri

677 Engineered U1 snRNPs redefine a disabled exon from distance
Natalia Singh, José Del Rio-Malewski, Eric Ottesen, Matthew Howell, Ravindra Singh

678 Sam68 interaction with U1A modulates mTOR pre-mRNA splicing
Suryasree Subramania, Laurence M Gagné, Sébastien Campagne, Karel Mocaer, Miki Feldmüller, Frédéric H.T. Allain, Samer Hussein, Marc-Étienne Huot

679 Evidence for differential protein-protein interactions as a mechanism underlying cell type-specific regulation of alternative last exon splicing by CDK12
Jerry Tien, Shane Colborne, Alvin Liu, Joshua Bray, Sophie Moggridge, Karl Angelo Abuan, Grace Cheng, Christopher Hughes, Genny Trigo-Gonzalez, Gregg Morin

680 Near-atomic Structures of the Spliceosomal Complexes: Insights into the Mechanism of Pre-mRNA Splicing
Ruixue Wan, Chuangye Yan, Rui Bai, Yigong Shi

681 Systematic Identification and Analysis of pre-mRNA Splicing Regulators in Saccharomyces cerevisiae
Hsuan-Kai Wang, Yu-Le Wu, Ming-Jing Hwang, Tien-Hsien Chang

682 Cryo-EM shows mechanism of 3' splice site recognition
Max Wilkinson, Sebastian Fica, Wojciech Galej, Christine Norman, Andrew Newman, Kiyoshi Nagai

683 Conformational Dynamics of Group II Intron Splicing
Timothy Wiryaman, Navtej Toor

684 Targeting the splice factor kinase SRPK1 in leukaemic cells
Chigeru Wodi, Ruth Morse, Sebastian Oltean, Michael Ladomery

685 Genome-wide CRISPR-Cas9 interrogation of splicing networks reveals a mechanism for recognition of autism-misregulated neuronal microexons
Thomas Gonatopoulos-Pournatzis, Mingkun Wu, Ulrich Braunschweig, Jonathan Roth, Hong Han, Andrew Best, Bushra Raj, Michael Aregger, Dave O'Hanlon, Jonathan Ellis, John Calarco, Jason Moffat, Anne-Claude Gingras, Benjamin Blencowe

686 Genetic suppressors of cold-sensitive substitutions that stabilize the U6 internal stem-loop may act on distinct splicing defects
Allyson D. Yake, Sean T. Hinds, Erik D. Anderson, Ian S. Norden, Christine N. Kujak, David A. Brow

687 Caught in Action: Identification of factors sufficient for ATPase activity activation of yeast DEAD-box protein Prp28p
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688 Coordinated alternative splicing and alternative polyadenylation of *Dscam1* regulated by ELAV is essential for neural development in *Drosophila*
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689 Small molecule inhibitors targeting the MALAT1 triple helix exhibit distinct interaction modes
Abeer Ageeli, Michael Yonkunas, Fardokht Abulwerdi, Stuart Legrice, Nathan Baird

690 Exploring Dumbbell-shaped DNA Minimal Vectors for RNA-Guided Genetic Reversal of Glucose-6-phosphate Dehydrogenase Deficiency
Avantika Ghosh, Volker Patzel

691 Targeting Translation Termination Machinery with Antisense Oligonucleotides for Diseases Caused by Nonsense Mutations
Lulu Huang, Audrey Low, Susan Murray, Brett Monia, Shuling Guo

692 Chemically modified siRNAs targeting the RNA structure of the influenza A virus.
Julita Kesy, Marta Soszynska-Jozwiak, Paula Michalak, Elzbieta Lenartowicz, Barbara Szutkowska, Ryszard Kierzek, Elzbieta Kierzek

693 Tumor-targeted delivery of antagomirs by in situ piggybacking on endogenous albumin
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694 AON-induced splice-switching and *DMPK* transcript degradation as therapeutic approaches for Myotonic Dystrophy type 1 (DM1)
Ewa Stepniak-Konieczna, Patryk Konieczny, Julia Zielinska, Krzysztof Sobczak

695 Therapeutic potential of small molecules targeting the MALAT1 lncRNA-encoded triple helix
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696 Exploring the potential of antisense-oligonucleotide-based *PKM* splice-switching as a targeted therapy for liver cancer
Wai Kit Ma, Juergen Scharner, Michaela Norrbom, Frank Rigo, Frank Bennett, Adrian Krainer

697 Hydrophobicity drives the systemic distribution of lipid-conjugated siRNAs via lipid transport pathways
Maire Osborn, Andrew Coles, Annabelle Biscans, Reka Haraszti, Dimas Echeverria, Matthew Hassler, Sarah Davis, Socheata Ly, Anastasia Khvorova

698 A novel heterologous prime/boost vaccine system drives tumor-specific and potent CD8 T cell responses for cancer immunotherapy
Amy Rappaport, Gijsbert Grotenberg, Ciaran Scallan, Renee Greer, Leonid Gitlin, Kieu Lam, James Heyes, Anne Van Abbema, Hadley Hanson, Petra Schreiner, Karin Jooss, Wade Blair

699 Withdrawn

700 Splice-site changing oligonucleotides targeting the serotonin receptor 2C reduce spasticity after spinal cord injury
Stefan Stamm, Samir Patel, Samantha Danyi, Alexander Rabchevsky

701 Maximizing the translational yield of mRNA therapeutics by minimizing 5'-UTRs
Zeljka Trepotec, Manish Aneja, Johannes Geiger, Guenther Hasenpusch, Christian Plank, Carsten Rudolph

702 RNA-based micelles: a novel platform for paclitaxel loading and delivery
Hongran Yin, Yi Shu, Mehdi Rajabi, Hui Li, Mario Vieweger, Sijin Guo, Dan Shu, Peixuan Guo

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703 Translation initiation in bacterial polysome through an mRNA stand-by site
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704 Translational Control by the DEAD-box Helicase Ded1
Peyman Aryanpur, Telsa Mittelmeier, David Renner, Timothy Bolger

705 5'UTR control of developmental gene expression dynamics by conserved novel IRES elements
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706 The landscape of nascent protein synthesis in sensory neurons reveals new insights into pain signaling
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707 Genome-wide analysis reveals that eIF2D coordinates motor neuronal synaptic function and locomotory behavior by modulating translation of specific mRNAs
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708 Biochemical and structural characterization of a novel nucleotide transferase potentially involved in RNA repair
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709 Translational buffering upon splicing inhibition
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710 Understanding the regulation of *inl* gene expression in response to inositol in *Neurospora crassa*
Ananya Dasgupta, Ivaylo Ivanov, Tom Dever, Matthew Sachs

711 EIF3 mediated translation regulation in activated T cells
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712 Structural cross-talk between transcription and translation
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713 Hypoxia-dependent decrease in DDX28 protein levels increases eIF4E2-mediated translation
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714 Most circular RNAs are potentially translated
Xiaojuan Fan, Yun Yang, Zefeng Wang

715 Controlling tissue patterning by translational regulation of signaling transcripts through the core translation factor EIF3C
Kotaro Fujii, Olena Zhulyn, Zhen Shi, Maria Barna

716 Bacterial ribosome conformations probed by ribosome profiling
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717 Measuring the Mechanical Forces During Ribosome Translocation via EF-G Crosslinking
Miriam Gavriliuc, Heng Yin, Shoujun Xu, Yuhong Wang

718 Ribosome heterogeneity and specialization during cellular differentiation
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719 5'-UTR RNA structure modulates protein translation of SERPINA1 in alpha-1 antitrypsin deficiency
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720 Rbfox1 Regulation of Translation in Neurons
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721 How 2'-O-methylation in mRNA disrupts tRNA decoding during translation elongation
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722 Lethal effect of denatured RRF on *E.coli* during the lag phase—Formation of 40S particles and upregulation of ArfA.
Sandeep Sharma, Akira Kaji, Hideko Kaji

723 Translational readthrough in *Drosophila melanogaster*
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724 The Non-Canonical RNA-Binding Protein, Pyruvate Kinase, Regulates the Translation of Protein Synthesis Machinery
Nevraj Kejiou, Lena Ilan, Stefan Aigner, Enching Luo, Nishani Rajakulendran, Masroor Kahloon, Eliza Lee, Hui Zhang, Iryna Kharchuk, Stephane Angers, Gene Yeo, Alexander Palazzo

725 Evolutional conservation of genome-wide eIF4F dependency in protein synthesis
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726 Alternative splicing of cytoplasmically recapped mRNAs
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727 Multiple ways to frameshifting in human immunodeficiency virus type 1
Natalia Korniy, Ekaterina Samatova, Frank Peske, Marina V. Rodnina

728 Mutually Exclusive amino acid Residues Of L13a Regulate its Role in Ribosomal Incorporation and Transcript-Specific Translational Silencing.
Ravinder Kour, Anton A. Komar, Barsanjit Mazumder

729 A CRISPRi screen in eIF4E heterozygous cells reveals a new genetic interaction landscape for the major cap binding protein
Duygu Kuzuoglu-Ozturk, Luke A. Gilbert, Davide Ruggero

730 Genome-encoded heterogeneous rRNAs regulate gene expression by preferentially selecting a subset of mRNAs
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731 Cellular response to small molecules that selectively stall protein synthesis by the ribosome
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732 Single-molecule FRET study of prolyl peptide bond formation
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733 Ribosome binding by yeast eIF4B drives stress-induced changes in translation
Xiaozhuo Liu, Houtan Moshiri, Sarah Walker

734 Inhibition of Poly(A)-Binding Protein with an RNA mimic reduces pain sensitization in mice
Paulino Barragán-Iglesias, Tzu-Fang Lou, Vandita Bhat, Salim Megat, Michael Burton, Theodore Price, Zachary Campbell

735 Caloric Restriction Regulates Circadian Rhythms in Protein Translation.
Kuldeep Makwana, Roman Kondratov

736 Human tRNA-ThrUGU contains ncm^{5}U34 and can decode all four synonymous ACN codons in human cells; studies using stable and unstable mRNAs
Sandy Mattijssen, Fan-Yan Wei, Carissa Stover, Kazuhito Tomizawa, Richard Maraia

737 Examining the function of yeast eIF4E using the auxin-inducible degron system.
Nicholas McGlincy, Nicholas Ingolia

738 Measuring Nonsense Suppression Promoted Readthrough in a Simplified Pure Translational System
Martin Ng, Barry Cooperman

739 How tRNA modifications and nucleotide insertions impact decoding and mRNA frame maintenance
Ha An Nguyen, Christine Dunham

740 Shep is a conserved translational regulator that controls dendrite morphogenesis in sensory neurons
Eugenia Olesnicky, Meghan Lybecker, Michael Titus, Shawna Thornton, Racquel Valadez, Paul Derkach, Simona Antonacci, Darrell Killian, Niko Popitsch

741 Mechanistic Analysis of Glorund-Mediated *nanos* Translational Repression during *Drosophila* Late Oogenesis.
Yingshi Peng, Zachary Flamholz, Elizabeth Gavis

742 Orthogonal regulation of ferritin light chain translation by eIF3 and the iron response proteins
Mia Pulos, Nicholas Ingolia, Jamie Cate

743 Cas9-induced DNA double-strand breaks trigger ribosome remodeling and translational shutdown
Celeste Riepe, Elena Zelin, Nicholas Ingolia, Jacob Corn

744 Evidence against RNA structure-based translation initiation in bacteria
Luc Roberts, Hans-Joachim Wieden

745 Thermodynamic study of macrolides using ITC competition experiments
Emma Schenckbecher, Benoit Meyer, Anna Maria Giuliodori, Daniel Wilson, Eric Ennifar

746 The IGF2 mRNA binding protein 1 (IGF2BP1) impairs the formation of adherens junctions in cancer cells by promoting SRC activity
Annekatrin Schott, Nadine Bley, Danny Misiak, Marcell Lederer, Christian Ihling, Andrea Sinz, Stefan Hüttelmaier

747 Identification of Yeast Translation Initiation Regulating Factors using a Fluorescence-Based Reporter Assay
Peter Schuessler, Stephanie Newman, Joseph Barbi, Sarah E. Walker

748 Selective translation mechanism through two paralogs of eukaryotic initiation factor 4A
Yuichi Shichino, Mari Mito, Shintaro Iwasaki

749 The RNA-binding protein Bfr1p transnationally regulates an ergosterol biosynthesis protein Erg4p in *Saccharomyces cerevisiae*.
Manchalu Srinivas, Ralf-Peter Jansen

750 Sign epistasis as a signature of collision-stimulated quality control during translation
Heungwon Park, Arvind Subramaniam

751 Investigating function and evolution of mammalian expansion segments (ES)
Teodorus Theo Susanto, Kotaro Fujii

752 Conformational control of translation termination on the 70S ribosome
Egor Svidritskiy, Andrei Korostelev

753 Dynamic post-transcriptional regulation by the yeast RNA-binding protein Mrn1p
Kendra Swain, Michael Ly, Nicholas Ingolia

754 An alternative translated isoform of MK2 determines functional diversity
Christopher Tiedje, Philipp Trulley, Goda Snieckute, Simon Bekker-Jensen, Matthias Gaestel

755 Control of mammalian limb patterning by the ribosome
Gerald Tiu, Pallavi Krishnarao, Maria Barna

756 Cancer-associated mRNAs regulated by the HLH motif of human eIF3A
Marina Volegova, Jamie H.D. Cate

757 Phosphorylation of eIF4A drives dynamic changes in gene expression in response to diverse environmental stressors
Ansuman Sahoo, Marium Ashraf, Sarah E. Walker

758 Translational Frameshifting Induced by Mutually Jostling Between Ribosomes
Jianzhou Wang, Jin-Der Wen

759 Multi-protein Bridging Factor 1(Mbf1), Rps3 and Asc1 prevent stalled ribosomes from frameshifting.
Jiyu Wang, Jie Zhou, Qidi Yang, Elizabeth Grayhack

760 Studies of an Altered-Substrate, β-Amino Acid Translating Ribosome, P7A7.
Fred Ward, Zoe Watson, Jamie Cate

761 RNA Pseudoknots: Folding and Interaction with the Ribosome
Chiung-Fang Hsu, Kai-Chun Chang, Yi-Lan Chen, Po-Szu Hsieh, An-Yi Li, Yu-Ting Chen, Jin-Der Wen

762 The Ribo-Interactome Reveals Novel Post-Translational Modifications of Ribosomal Proteins
Adele Xu, Deniz Simsek Buck, Theo Susanto, Maria Barna

763 Global analysis of translation processivity in *E. coli*
Lanqing Ying, William Baez, Ralf Bundschuh, Kurt Fredrick

764 Translational regulation in development and regeneration
Olena Zhulyn, Kotaro Fujii, Zhen Shi, Maria Barna

765 Variants of the 5′-terminal region of p53 mRNA influence the ribosomal scanning and translation efficiency
Paulina Zydowicz-Machtel, Agata Swiatkowska, Lukasz Popenda, Agnieszka Gorska, Jerzy Ciesiolka

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766 Potential involvement of La protein in the nuclear pre-tRNA splicing pathway
Nathan Blewett, Dimitrios Anastasakis, Markus Hafner, Richard Maraia

767 Identification of Nuclear-encoded Mitochondrial tRNAs Using Isotype-Specific Models and tRNAscan-SE 2.0
Patricia Chan, Todd Lowe

768 Elucidating the biological significance of multiple, parallel primary tRNA nuclear exporters in yeast cells
Kunal Chatterjee, Shubhra Majumder, Anita K. Hopper

769 In vitro-based workflow for exogenous and endogenous expression of tissue- and disease-specific tRNAs
Jonathan Howard, Andrew Holmes, Todd Lowe

770 Distinct substrate specificities of human tRNA methyltransferases TRMT10A and TRMT10B
Nathan Howell, Jane Jackman

771 Comprehensive sequence analysis of tRNAs in the three domains of life
Satoshi Tamaki, Haruo Suzuki, Akio Kanai

772 Structural and functional characterization of the mammalian tRNA ligase complex
Alena Kroupova, Stefan Weitzer, Marco Faini, Ruedi Aebersold, Javier Martinez, Martin Jinek

773 Monitoring the 5-methoxycarbonylmethyl-2-thiouridine (mcm5s2U) modification in eukaryotic tRNAs via the gamma-toxin endonuclease
Jenna Lentini, Jillian Ramos, Dragony Fu

774 Evidence supporting the role for Mtr10 in tRNA nuclear import
Regina Nostramo, Anita Hopper

775 Large-scale phylogenetic analysis and diversity of Clp1 polynucleotide kinase family protein
Motofumi Saito, Asako Sato, Shohei Nagata, Masaru Tomita, Satoshi Tamaki, Haruo Suzuki, Akio Kanai

776 Characterization of tRNA intronic circular (tric)RNA biogenesis
Casey Schmidt, A. Gregory Matera

777 Detecting novel genetic code reassignments
Yekaterina Shulgina, Sean Eddy

778 Using DNA sequence variation to infer tRNA activity
Bryan Thornlow, Josh Hough, Ian Fiddes, Joel Armstrong, Russell Corbett-Detig, Todd Lowe

779 A gammaherpesvirus modulates host RNAPIII transcription at specific repetitive loci
Jessica Tucker, Britt Glaunsinger

780 From powerhouse to warehouse: novel and conserved role of mitochondrial outer membrane proteins in tRNA splicing
Yao Wan, Anita Hopper

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781 RNA structure in Influenza A virus ribonucleoproteins
Damien Ferhadian, Valérie Vivet-Boudou, Roland Marquet

782 An RNA-Centric Dissection of Host Complexes Controlling Flavivirus Infection
Karim Majzoub, Yaw Shin Ooi, Ryan A Flynn, Jonathan Diep, Neil Rumachik, Miguel A Mata, Nicholas Van Buuren, Jason K Li, Andreas S Puschnik, Caleb D Marceau, Peter Sarnow, Karla Kirkegaard, Carolyn R Bertozzi, Jan E Carette

783 CBP80/20-dependent translation initiation factor (CTIF) inhibits HIV-1 Gag synthesis by targeting the activity of the viral protein Rev
Francisco García-de-Gracia, Bárbara Rojas-Araya, Aracelly Gaete-Argel, Daniela Toro-Ascuy, Ricardo Soto-Rifo

784 Structure-Activity Relationship of Raloxifene Analogs as Potential Hepatitis B Virus Therapeutics, Through Targeting of Encapsidation Signal, Epsilon, of Viral Pregenomic RNA
Stefano Ginocchio, Fardokht Abulwerdi, Regan LeBlanc, Andrew Longhini, T. Kwaku Dayie, Stuart LeGrice

785 N^6-methyladenosine modification and the YTHDF2 reader protein play cell type specific roles in lytic viral gene expression during Kaposi's sarcoma-associated herpesvirus infection
Charles Hesser, John Karijolich, Dan Dominissini, Chuan He, Britt Glaunsinger

786 Revisiting the RNA Genome Architecture of Influenza Virus
Nara Lee, Adalena Nanni, Valerie Le Sage, Seema Lakdawala

787 Biophysical characterization of non-coding regions of Japanese Encephalitis Virus
Tyler Mrozowich, Justin Vigar, Astha N/A, Hans-Joachim Wieden, Janusz Bujnicki, Trushar Patel

788 Kaposi's sarcoma-associated herpesvirus ORF57 protein protects viral transcripts from two host-mediated RNA decay pathways
Julio Ruiz, Nicholas Conrad

789 Screening for novel anti-HCV lead compounds via attenuation of the 40S ribosomal subunit
Luh Tung, Jun-Wei Lee, Shu-Chun Tsai, Tien-Hsien Chang

790 Human RNase, MCPIP1, Degrades Specific Human and Viral Pre-miRNAs and Inhibits Herpesvirus Infection
Insun Kook, Christine Happel, Joseph Ziegelbauer

NOTES

1 Structural Insights into CRISPR and Anti-CRISPR Systems for Precise Genome Editing

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CRISPR/Cas9, originally derived from bacterial adaptive immune systems, has recently been harnessed as a versatile tool for genome editing and holds great promise in treating cancer diseases. Interestingly, bacteriophages encode small inhibitor proteins–"anti-CRISPRs" to actively circumvent bacterial CRISPR immunity. Those naturally specific "anti-CRISPRs" present important tools that can be used to regulate CRISPR/Cas9-mediated genome editing specificity. My research is focused on understanding of the fundamental mechanisms of CRISPR RNA guided DNA targeting and cleavage by Cas9, as well as the viral anti-CRISPR/Cas9 mechanisms. These structural studies provide a framework to develop more effective and precise CRISPR/Cas9 tools for therapeutic applications.

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2 Revealing Hidden Biology by Identifying Ligands for the *ykkC* Orphan Riboswitches

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The *ykkC* RNA motif was discovered over a decade ago by using bioinformatics and, until recently, was the longest standing orphan riboswitch candidate. The cognate ligand of the *ykkC* riboswitch remained unsolved mostly due to the seemingly disparate set of genes under its regulatory control, which encode proteins annotated as urea carboxylases, multi-drug efflux transporters, sulfonate/nitrate/carbonate transporters, purine biosynthesis enzymes and branched-chain amino acid biosynthesis enzymes. We found that guanidine induces expression of a riboswitch-*lacZ* reporter gene fusion using a large screen of various growth conditions and used in-line probing to further confirm guanidine as the cognate ligand. Additionally, representatives of two other RNA motifs, called mini-*ykkC* and *ykkC*-III, bind guanidine *in vitro*. Similar genes are associated with all three guanidine-binding RNA motifs, and therefore these three distinct classes of RNA have been termed the guanidine-I, -II and -III riboswitches. Although little is known about the biological relevance of guanidine, the wide distribution of these riboswitches and the genes they control suggest that free guanidine is a greatly underappreciated molecule in bacteria. While the majority of *ykkC*-I RNAs selectively bind guanidine, the remaining 30% of *ykkC* RNAs carry nucleotide changes in their binding pockets to allow recognition of a different ligand. Analysis of the consensus sequence and structure models as well as the downstream gene associations for these variant *ykkC* RNAs revealed at least four distinct candidate riboswitch classes in addition to the guanidine-I riboswitch class. The first variant class was found to selectively bind guanosine tetraphosphate (ppGpp), a bacterial alarmone derived from the ribonucleotide GTP. The ppGpp riboswitch class primarily regulates transcription of genes involved in branched-chain amino acid biosynthesis. The second of these variant *ykkC* riboswitch classes was determined to bind phosphoribosyl pyrophosphate (PRPP), the initial substrate for the biosynthesis of RNA monomers, to regulate the expression of genes for purine nucleotide biosynthesis. Tandem arrangements are frequently formed between members of these two newly validated riboswitch classes and other RNA-based regulatory elements to form more sophisticated gene control systems, supporting the hypothesis that RNA World organisms could manage a complex metabolic state without the assistance of protein factors.

3 Specialized guides of m^{6}A regulatory pathway: YTHDF reader proteins and their functions

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N^6-methyladenosine (m^{6}A), the most abundant internal modification existing in eukaryotic mRNAs, plays key roles in the dynamic regulation of gene expression profiles and cellular states. It impacts multiple steps throughout the RNA lifecycle including RNA processing, translation, and decay, via the recognition by selective reader proteins. YTH (YT521-B homology) domain family proteins are the first m^{6}A readers characterized by our lab: YTHDF1 facilitates the translation of m^{6}A-modified mRNAs, YTHDF2 accelerates the decay of m^{6}A-modified transcripts, and YTHDF3 affects both processes in synergy with YTHDF1 and 2. The existence of numerous m^{6}A readers co-regulating groups of m^{6}A-modified RNAs implies that, aside from the specialized function of each reader, the interaction and coordination among different readers also impact the final outcomes of m^{6}A regulation. In addition to their molecular functions, m^{6}A reader proteins may also affect more complex phenotypic events by recognizing relevant functional cohorts of m^{6}A-modified mRNAs and regulating their metabolism collectively, resulting in the control of complex cellular functions. Such controls may be required during the cellular transition between distinct states when cells rapidly refresh their state-specific transcriptome/proteome and re-establish a new identity. We chose zebrafish development as a model system to showcase this higher-level regulation by m^{6}A, focusing on the maternal-to-zygotic transition (MZT) stage during which maternal RNAs are rapidly degraded, hinting m^{6}A/Ythdf2 involvement. We found that m^{6}A marks up to one-third of these maternal RNAs and zebrafish Ythdf2 is responsible for the degradation of these m^{6}A-modified transcripts. Removal of Ythdf2 from zebrafish embryos impedes maternal RNA clearance and causing delays in the subsequent developmental program. Preliminary data in mouse also suggest vital roles of m^{6}A readers in the regulation of brain functions. Given these findings, we propose that reader-guided m^{6}A regulatory pathway acts as a general mechanism to initiate and facilitate the switching of cell states: clearing the genes products that govern the old cell state and promoting the formation of a new one. In conclusion, the influence of m^{6}A exists broadly in fundamental biological processes, manifested by the spectrum of m^{6}A readers with diverse functions and complex interplays.

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4 Visualizing translation by ensemble cryo-EM

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Translation of mRNA is a key step of gene expression and stress responses, and a therapeutic target. Translation is governed by complicated ribosome dynamics. To visualize the translational machinery in action, we determine high-resolution ensembles of structures by electron cryo-microscopy. I will discuss how this approach provides insights into the essential aspects of gene expression, such as translation initiation, translation fidelity and ribosome-induced stress signalling.

5 Compact, High-Accuracy Cas9 Orthologs and their Natural Inhibitors

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Clustered, regularly interspaced, short, palindromic repeats (CRISPR) loci, along with proteins encoded by CRISPR-associated (*cas*) genes, encode RNA-guided, adaptive immune systems that protect bacterial or archaeal hosts from viral genomes and other invasive nucleic acids. These systems are widespread and mechanistically diverse, and most of them use their CRISPR RNA (crRNA) guides to target DNA molecules for destruction. This RNA-guided DNA-targeting capability has been exploited for genome editing and other applications, spawning a revolution in the life sciences and biotechnology. The most commonly used CRISPR-Cas genome editing platforms are derived from the "type II" systems that employ the Cas9 protein as an effector. Different Cas9 proteins exhibit distinct properties such as size, protospacer adjacent motif (PAM) preferences for target site selection, editing efficiencies in eukaryotic cells, and on-target specificities. Cas9 orthologs that are sufficiently compact for all-in-one adeno-associated virus (AAV) delivery, and that have been validated for mammalian genome editing, include NmeCas9, SauCas9, CjeCas9 and GeoCas9. Each of these orthologs has a relatively long (~4-nucleotide) PAM that limits target site density. We have identified a compact Cas9 ortholog with a 2-nucleotide PAM, validated it for *in vivo* editing, and identified anti-CRISPR proteins that can inhibit its activities. Despite the high target-site density that results from its 2-nucleotide PAM (one potential target site every ~8 base pairs on average), this Cas9 is naturally hyper-accurate during mammalian genome editing applications. The results of our analyses on these and other aspects of CRISPR genome editing will be discussed.

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6 Misregulation of an activity-dependent splicing network impacts neuronal translation and underlies autism spectrum disorders

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Alternative splicing represents a key mechanism which can increase the complexity of the transcriptome and thereby expand the repertoire of protein interactions and functions. This is particularly evident in the mammalian nervous system. We are analyzing how alternative splicing regulation underlies nervous system development and autism spectrum disorder (ASD). Recently we have shown that a neuronal-specific alternative splicing network of microexons (short 3-27 nt exons) is misregulated in brains of a substantial proportion of ASD individuals. The same subset of patients showed correlated reductions in the expression of the neuronal microexon regulator SRRM4/nSR100 (Irimia et al., Cell, 2014). To investigate whether nSR100 misregulation is causally linked to autism, we generated mutant mice with reduced levels of this protein and its target splicing program. Remarkably, these mice display multiple hallmark features of autism, including altered social behaviors, synaptic density and signaling. Moreover, increased neuronal activity, which is often associated with autism, results in a rapid decrease in nSR100 and splicing of microexons that significantly overlap those misregulated in autistic brains. Collectively, our results provide evidence that misregulation of an nSR100-dependent splicing network controlled by changes in neuronal activity is causally linked to an important subset of autism cases (Quesnel-Vallieres et al., Mol. Cell, 2016).

To identify direct nSR100 target exons that contribute significantly to ASD associated behaviors, we have performed iCLIP-Seq experiments, which, amongst other targets, identified two conserved paralogue microexons in Eif4g1 and Eif4g3 translation initiation factors. Eif4g forms a scaffold linking the cap-binding protein Eif4e with other translation factors, and mutations that affect these interactions have been linked to ASD. Interestingly, proteomic analysis revealed that Eif4g microexons control the expression of synaptic receptors linked to the regulation of neuronal activity and transcriptome profiling further revealed that the mutant neurons mirror that of activated neurons. Remarkably, deletion of the Eif4g1 microexon in mice results in a mild autistic phenotype. These results place activity dependent splicing upstream of key translational regulatory events and our observations suggest mechanisms by which an nSR100-dependent microexon network might provide avenues to reverse both idiopathic ASD as well as syndromic ASD, such as Fragile X syndrome.

7 Prespliceosome structure provides insight into spliceosome assembly and regulation

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For pre-mRNA splicing, the intron 5'-splice site (5'SS) and the branch point sequence are held in the U1–U2 snRNP prespliceosome, which then associates with the U4/U6.U5 tri-snRNP to assemble the complete pre-catalytic spliceosome. While recent studies have revealed the structural basis of the branching and exon-ligation reactions, the structural basis of early spliceosome assembly events remains poorly understood. In my talk I will present the cryo-electron microscopy structure of the yeast *Saccharomyces cerevisiae* prespliceosome at near-atomic resolution. The structure reveals an induced stabilization of the 5'SS in the U1 snRNP, and provides insight into the roles of the human alternative splicing factors TIA-1 and LUC7-like. The U1 snRNP associates with the U2 snRNP through a stable contact with the U2 3' domain and a transient yeast-specific contact with the U2 SF3b-containing domain, leaving its tri-snRNP-binding interface fully exposed. The results suggest a mechanism for 5'SS transfer to the U6 ACAGAGA region within the assembled spliceosome and for its subsequent conversion to the activation-competent B complex spliceosome. Taken together, the data reveal the structure of an important regulatory splicing intermediate and provide a working model to investigate the early steps of spliceosome assembly.

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8 CryoEM structure of the yeast spliceosomal P complex at 3.3 Å resolution

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The spliceosome undergoes dramatic changes signified by the E, A, Pre-B, B, B^{act}, B*, C, C*, P, and ILS complexes in a splicing cycle. CryoEM structures of B, B^{act}, C, C*, and ILS revealed mechanisms of 5' ss recognition, branching, and intron release, but lacked information on 3' ss recognition, exon ligation and release, all related to the post-catalytic P complex. We have recently determined the cryoEM structure of the yeast P complex at 3.3Å resolution. Our atomic model of the P complex reveals that the 3' ss recognition is driven by the interaction between 3' and 5' ss, likely facilitated by a stem-like structure formed between the intronic regions close to the branch site and 3' ss. Our structure reveals that one or more new proteins become stably associated with the core components of the P complex around Prp8 and Prp22, securing the 3' exon and potentially regulating the activity of Prp22. The structure demonstrates that Prp22 binds 15-21 nucleotides downstream of the exon-exon junction, enabling it to pull the 3' intron-exon and ligated exon in a 3' to 5' direction to achieve 3' ss proofreading and exon release, respectively. Our further biochemical analyses revealed an unexpected function of the C-terminal tail of Prp22 that may be extended to other DEAH-box spliceosomal helicases. Thus, by providing insights into the molecular mechanism of 3' ss recognition, exon ligation, and the action of RNA helicase Prp22, the atomic model of the P complex fills a major gap in our understanding of the splicing cycle.

9 Structural basis for specific pre-mRNA splicing correction at the 5'-splice site induced by small molecules

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Pre-mRNA splicing is a major step of gene expression and misregulations have been associated with diseases. Thus, being able to rationally correct specific splicing defect represents a promising strategy to develop new therapeutic approaches. Spliceosome assembly initiates with the recognition of the exon-intron junction by U1snRNP and the structure of U1 snRNP in complex with the regular 5'-splice site showed that the U1-C zinc finger stabilizes the RNA duplex by contacting the RNA backbone in the minor groove (1). Therefore, 5'-splice site mismatches close to the U1-C interaction surface could change minor groove geometry or accessibility and decrease the spliceosome assembly efficiency. Recently, small molecules that induce SMN2 exon 7 inclusion have been discovered and pinpointed as potential drugs to cure spinal muscular atrophy (2,3). Genome wide transcriptome analysis coupled with in vitro RNA binding assays allowed us to propose that the splicing modifier specifically stabilizes U1snRNP on the weak SMN2 exon 7 5'-splice site (4). Indeed, using solution state NMR, we could show that the molecule potency correlates with its ability to bind the 5'-SS:U1 duplex just upstream the invariant GU dinucleotide in a pocket where an unpaired adenine was previously shown to be responsive for the weakness of this 5'-splice site. We recently solved the solution structures of the 5'-SS:U1 duplex bound to several splicing modifiers and revealed that the small molecules interact specifically with the unpaired adenine to pull it into the RNA helix base stack. One main consequence is that the unpaired adenine does not bury the minor groove anymore and the U1-C RNA binding gets favoured. Altogether, our results illustrate a new concept in targeted pre-mRNA splicing correction that we coined "5'-splice site mismatch repair". We believe that this concept should stimulate rational drug design and could dramatically change the current view of how pre-mRNA splicing defects can be corrected to cure RNA splicing induced diseases.

(1) Kondo et al., eLife 2015
(2) Naryshkin et al., Science 2014
(3) Palacino et al., Nature Chemical Biology 2015
(4) Sivaramakrishnan, McCarthy et al., Nature Communications 2017

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10 Quantitative activity profile and context dependence of all human 5′ splice sites

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Pre-mRNA splicing is an essential step for expression of most human genes. Mutations at the 5′ splice site (5′ss) frequently cause defective splicing and disease, due to interference with the initial recognition of the exon-intron boundary by U1 small nuclear ribonucleoprotein (snRNP), a component of the spliceosome. It is estimated that 14% of all disease-associated point mutations affect the splice sites, and that as many as 50% of all mutations alter splicing when accounting for mutations that affect enhancer and silencer elements as well. To enhance our understanding of 5′ss recognition and determine the characteristics of 5′ss that are prone to perturbation by point mutations, we use massively parallel analysis to quantify the activity of the 32,768 unique 9-nucleotide GU and GC candidate 5′ss sequences in three heterologous gene contexts (*BRCA2*, *SMN1*, and *IKBKAP*) and comprehensively characterize 5′ss recognition and context-dependence. Our results reveal that about 60-70% of the variation in 5′ss efficiency originates from the 5’ss sequence itself, and the remaining variability is attributable to the overall context, including 3′-splice-site strength and the presence of various exonic and/or intronic enhancers and/or silencers. We can predict the effects of many known pathogenic mutations that affect 5′ss in different genes. Our results provide evidence for a set of universal rules governing 5′ss recognition that are widely applicable to the major spliceosomal introns of any gene in the human transcriptome. The data generated provide a framework to assess which mutations are likely to be pathogenic in different contexts, and to streamline the development of splicing-corrective therapies.

11 Decoding extensive regulation of *RON* alternative splicing with a high-throughput mutagenesis screen

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Mutations causing aberrant splicing are frequently implicated in human diseases including cancer. Here, we establish a high-throughput screen of randomly mutated minigenes to decode the cis regulatory landscape that determines alternative splicing of the proto-oncogene *MST1R* (*RON*). Mathematical modelling of splicing kinetics enables us to identify more than 1,000 mutations affecting *RON* exon 11 skipping, which corresponds to the pathological isoform RONΔ165. Importantly, the effects correlate well with *RON* alternative splicing in cancer patients bearing the same mutations. Moreover, we highlight heterogeneous nuclear ribonucleoprotein H (hnRNP H) as a master regulator of *RON* splicing in healthy tissues and cancer. Using iCLIP and epistasis analysis, we pinpoint the functionally most relevant hnRNP H binding sites and demonstrate how cooperative hnRNP H binding facilitates a splicing switch of *RON* exon 11. Our results thereby offer new insights into splicing regulation and the functional impact of mutations in human disease.

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12 DDD40800- a novel modulator of pre-mRNA Splicing

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During the EU-funded Eurasnet project (see; www.eurasnet.info), we, together with the group of Reinhard Lührmann and the University of Dundee Drug Discovery Unit (DDU), used a high throughput *in vitro* splicing assay (adapted from Samatov et. al., 2012), to screen a curated library of >75,000 small, drug-like compounds. This identified several novel classes of small molecule splicing inhibitors, including madrasin (Pawellek et al., J.Biol. Chem. 2014). Another drug-like, small molecule identified in this screen was the compound 'DDD40800', which inhibits splicing *in vitro* by blocking one or more early steps of spliceosome formation. DDD40800 also disrupts nuclear Cajal bodies and results in altered patterns of alternative pre-mRNA splicing *in cellulo*. Thus, RNAseq experiments identified ~3,000 altered splicing events in either HeLa, or HEK293 cells, after treatment with DDD40800 for 24h. The major class of splicing events affected by DDD40800 was exon skipping, which accounted for ~50% of altered transcripts. GO term analysis showed an enrichment of transcripts in this data set that encode proteins involved in RNA processing, cell cycle and DNA damage and repair. FACS and immunofluorescence analysis showed that treatment of multiple human cell lines with DDD40800 inhibited cell cycle progression, with arrest in S/G2&M phase and led to the formation of abnormal mitotic cells in a time and dose-dependent manner. Thermal proteome profiling (TPP) identified a potential cellular target of DDD40800, which is a protein linked with both RNA processing and cancer mechanisms. Additional experiments are in progress to characterise in more detail how DDD40800 modulates pre-mRNA splicing and its potential application as an anti-cancer therapeutic.

13 DDX17 specifically, and independently of DDX5, controls the use of the HIV A4/5 splice acceptor cluster and is essential for efficient replication of the virus

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HIV splicing involves 5 splice donor and 8 splice acceptor sequences which, together with cryptic splice sites, generate over 100 mRNA species. 90% of both partially spliced and fully spliced transcripts utilise the intrinsically weak A4/A5 3' splice site cluster. We show that DDX17, but not its close paralog DDX5, specifically controls the usage of this splice acceptor group. In its absence production of the viral envelope protein and other regulatory and accessory proteins are grossly reduced whilst Vif, which uses the A1 splice acceptor is unaffected. This is associated with a profound decrease in viral export from the cell. Loss of Vpu expression causing upregulation of cellular Tetherin compounds the phenotype. The activity of DDX17 is RNA dependent and we identify RNA binding motifs essential for its role whilst the Walker A, Walker B (DEAD), Q motif and the glycine doublet motif are all dispensable. We show that DDX17 interacts with cellular splicing factors in a model consistent with it facilitating the interaction between SRSF1/SF2 and the heterodimeric auxiliary factor U2AF65/35.

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14 Smu1 and RED are required for the activation of spliceosomal B complexes assembled on short introns

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Transformation of the pre-catalytic, spliceosomal B complex into an activated (B^{act}) spliceosome, involves extensive RNA and protein rearrangements. The proteins RED and Smu1, which are absent in *S. cerevisiae*, join the spliceosome at the B complex stage and leave during/after activation, however their roles in splicing are poorly understood. siRNA knockdown of Smu1 and RED in HeLa cells led to a decrease in B^{act} complex formation and an accumulation of its precursor, the B complex, demonstrating that Smu1/RED are required for efficient spliceosome activation. Smu1/RED knockdown resulted in profound changes in alternative splicing patterns and in the retention of constitutively spliced introns, the vast majority of which were shorter than 100 nt. This block in the splicing of short introns could be recapitulated *in vitro*; spliceosomes assembled in Smu1/RED-depleted extract on MINX pre-mRNAs containing longer introns, i.e. 120-150 nts long, underwent activation, although at a significantly slower rate, while spliceosomes assembled on shorter introns, i.e. 80-90 nts long, were stalled at the B complex stage and splicing was inhibited. The distance separating the 5' splice site (5'SS) and branch site (BS), as opposed to intron length *per se*, was the deciding factor whether splicing was dependent on Smu1/RED; when this distance was below 55 nt, splicing was inhibited in their absence. Such a short distance likely leads to steric hinderance in the stalled DSmu1/RED B-complex, preventing its activation. Indeed, cleaving the short MINX80 intron between the 5'SS and BS, restored splicesome assembly and splicing. These results are consistent with the cryo-EM structure of the human B complex, which showed that the 5'SS and BS are separated by a minimum distance of ca 56 nt, and that Smu1 and RED form a critical bridge connecting the U2 snRNP to the tri-snRNP helicase Brr2. Furthermore, preliminary EM studies support the idea that in spliceosomes formed on short introns, the absence of the Smu1/RED bridge results in movement of the U2 snRNP away form Brr2, thereby preventing the B to B^{act} transition. Our study provides novel insights into how intron architecture impacts spliceosome assembly and splicing.

15 piRNA Biogenesis and Function

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piRNAs are germline-enriched small RNAs that control transposons to maintain germline genome integrity. To achieve this, piRNAs bind members of PIWI family of proteins upon being processed from piRNA precursors, which are RNA transcripts of intergenic piRNA clusters and active transposons. This pathway is fundamentally similar to siRNA/miRNA-dependent gene silencing in the sense that a small RNA guides its partner protein Argonaute to target gene transcripts via RNA-RNA base-pairings to repress them. However, uniqueness of the piRNA pathway has gradually emerged through recent investigation relying on genetics, biochemistry, bioinformatics and structural biology. At this meeting, I will describe our recent findings regarding the piRNA pathway using two cultured cell lines, *Drosophila* ovarian somatic cells (OSCs) and *Bombyx* BmN4 cells as experimental models.

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16 Loss of *Sfpq* causes long-gene transcriptopathy in the brain

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From an evolutionary perspective, the pre-mRNA transcripts of vertebrates are comparatively expanded, and in mammals, genes preferentially expressed in the brain have significantly longer introns. Longer genes present a novel problem with respect to fulfilment of gene-length transcription and evidence suggests that dysregulation of long genes is a mechanism underlying neurodegenerative and psychiatric disorders, like amyotrophic lateral sclerosis (ALS) or autism spectrum disorder (ASD). These observations have yielded the hypothesis that some neurodegenerative and psychiatric diseases are in fact "long gene diseases" or "long genopathies." Yet, it has remained unclear what mechanism specifically regulates long genes to ensure their long-distance transcription.

Here, we report the discovery that RNA-binding protein (RBP) Sfpq is a critical factor for maintaining transcriptional elongation of long genes. Loss of *Sfpq* specifically down-regulated long genes > 100 kbp, which we have termed long-gene transcriptopathy. We found that Sfpq co-transcriptionally binds to long introns and is required for sustaining long-gene transcription by RNA polymerase II through recruiting cyclin-dependent kinase 9 to the elongation complex. These findings revealed the novel function of RBP in regulating transcription through binding to pre-mRNAs. We will discuss that RBPs could have a more central role as coordinators of transcription and pre-mRNA processing in contrast to existing proposed peripheral roles of RBPs only in post-transcriptional mRNA regulation. We also argue that identified molecular machinery will provide insights into the association between long gene transcriptopathy and neuronal diseases.

17 ICP27-mediated suppression of mRNA 3' processing as a key host shut-off mechanism for Herpes Simplex Virus

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Herpes simplex virus 1 (HSV-1) is a highly contagious pathogen that causes a number of diseases ranging from painful skin lesions to keratitis and encephalitis. The gene expression program of HSV-1 has been extensively studied for the past several decades. Despite the intensive effort, however, it remains unclear how HSV-1 suppresses host gene expression to allow efficient viral replication.

It has recently been shown that HSV-1 infection induces widespread defect in host gene transcription termination while viral genes are unaffected. Genetic studies have demonstrated that ICP27, encoded by an essential immediate early gene, plays an essential role in the inhibition of host mRNA biogenesis. The underlying mechanism, however, remain poorly understood. To elucidate the underlying mechanism and the functional significance of this phenomenon, we have demonstrated that the HSV-1 immediate early factor ICP27 potently inhibit mRNA 3' processing and it does so by targeting the essential mRNA 3' processing factor, CPSF, and disrupting the assembly of mRNA 3' processing complex. Mutant HSV-1 with an ICP27 deletion displayed significantly less transcription termination defect. Interestingly, the mRNA 3' processing and transcription termination of viral genes are unaffected due to highly GC-rich sequences upstream of viral poly(A) sites. Finally we demonstrated that disrupting ICP27-CPSF interaction and alleviating ICP27-mediated inhibition of host mRNA 3' processing resulted in significantly lower viral replication.

Together our data suggest that ICP27-mediated inhibition of host mRNA 3' processing is a major mechanism for HSV-1-induced host gene shut-off. Our study also raises the possibility that the ICP27-CPSF interaction may serve as a novel therapeutic target for treating HSV-1 infection.

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18 m6A mRNA methylation controls the innate immune response to infection by targeting IFNB

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N6-methyladenosine (m6A) is the most common and abundant mRNA modification, that has been linked to many stages of RNA processing and fate. Recent studies revealed that proteins in the m6A pathway play significant roles in regulating the lifecycles of diverse viruses. Here we show that following viral infection or stimulation of cells with an inactivated virus, the deletion of m6A 'writer' protein METTL3 or 'reader' protein YTHDF2 result in increased induction of hundreds of interferon-stimulated genes (ISGs), which constitutes the first line of antiviral defense. Consequently, replication of the herpesvirus human cytomegalovirus (CMV) and influenza virus is markedly suppressed in an interferon (IFN)-signaling dependent manner. Consistent with these observations we reveal that the mRNAs of IFNB, the central cytokine that activates the type-I IFN response, are marked by m6A, exhibit slower mRNA decay and increased mRNA and protein expression in METTL3 and YTHDF2 deficient cells. Furthermore, we show that methylation of IFNB is conserved in mouse cells and that after infection with mouse CMV, METTL3, YTHDF1 and YTHDF3 depleted MEFs show enhanced expression of IFNB and ISGs. These findings highlight the role of m6A as negative regulator of type-I IFN response, by dictating the fast turnover of IFNB mRNA.

19 The ribonuclease Regnase-1 maintains iron homeostasis via the destabilization of iron-regulatory transcripts

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The coordinate regulation of iron homeostasis is largely dependent on the post-transcriptional control. Iron-regulatory proteins (IRP1,2), which stabilize transferrin receptor (TfR1) mRNA, are key players in the post-transcriptional regulation of iron metabolism. In addition to regulation by IRPs, it has long been thought that TfR1 mRNA is controlled by endogenous ribonucleases. However, the endonucleases responsible for the TfR1 mRNA decay have not yet been identified.

Our group previously identified an endoribonuclease, Regnase-1, which destabilizes a set of inflammation-related mRNAs, thereby suppressing aberrant immune activation. Interestingly, we also found that Regnase-1-deficient mice suffer from severe anemia, as well as inflammatory diseases. However, the mechanisms by which mice with Regnase-1 deficiency develop anemia have remained obscure.

In this study, we investigated the role of Regnase-1 in the control of iron homeostasis. We found that Regnase-1 directly destabilizes TfR1 mRNA via its endonuclease activity. Additionally, we demonstrated that Regnase-1 has the potential to counteract TfR1 mRNA stabilization by IRPs. Subsequently we next investigated the role of Regnase-1 in vivo and found that Regnase-1-deficient mice showed severe iron deficiency anemia. This anemia was partly restored by the intraperitoneal iron supplementation. Moreover, we revealed that mice lacking Regnase-1 in intestinal epithelial cells also showed a defect in iron metabolism. These findings suggest that intestinal Regnase-1 is critical for the maintenance of iron homeostasis. To identify Regnase-1 target mRNAs responsible for iron uptake, we conducted transcriptome analysis in the duodenum, where iron uptake takes place, and found that several iron-controlling genes, including PHD3, were up-regulated under Regnase-1 deficiency. The overexpression of Regnase-1 accelerated the decay of the PHD3 mRNA via its 3' untranslated region. Consistently, the expression of PHD3-regulated HIF-2α target genes was impaired in Regnase-1-deficient mice compared with iron-deficient control mice. Furthermore, the abrogation of PHD3 in Regnase-1-deficient mice ameliorated the iron deficiency anemia. Collectively, these results demonstrate that Regnase-1-mediated regulation of iron-related transcripts is essential for the maintenance of iron homeostasis.

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20 Living life on the edge: a high-resolution map of the mammalian RNA decay landscape

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RNA degradation regulates gene expression, removes RNA processing by-products, and eliminates aberrant transcripts. This complex system is difficult to study using classical genetics, so we developed a direct approach to address key questions such as which features target RNAs for degradation; when and where do different decay pathways operate in the cell; and how are they regulated? We applied the Crosslinking and Analysis of cDNAs (CRAC) technique to quantify transcriptome-wide, endogenous targets of core components (Mtr4, Xrn1 and Ski2) of the major RNA decay pathways in mouse embryonic stem cells. Our high-resolution map of the RNA decay landscape distinguishes degradation pathways acting throughout the RNA life cycle, establishes oligo(A)/oligo(U) tailing as prevalent, and reveals coupling with transcription and translation. For example, some lincRNA transcription terminates early coupled to nuclear exosome-mediated decay, whereas other non-coding RNAs are degraded in the cytoplasm, apparently after being read by the ribosome. Our data show that Xrn1 and Ski2 interact with ribosomes, and support the model whereby most mRNA half-lives are determined by Xrn1-mediated cytoplasmic 5'-to-3' decay facilitated by ribosome translocation, whereas cytoplasmic 3'-to-5' decay involving Ski2 responds almost exclusively to non-canonical translation events. These include ribosome stalling at specific codons or RNA structures, upstream ORF translation, or aberrant translation initiation. Cytoplasmic 3'-to-5' decay is also enlisted for specialised substrates including histone mRNAs and pre-miRNAs. We are now exploring how RNA decay pathways respond to cellular cues, by analysing cells treated with translation inhibitors or depleted of the RNA modification m6A. Furthermore, we are using our knowledge of how lincRNAs are degraded to predict their functions, which we test using a conditional, ribozyme-based RNA depletion system. Overall, our approach reveals how diverse decay pathways operate at every stage of the RNA life cycle to maintain a functional, adaptive transcriptome.

21 The exon junction complex undergoes a compositional switch that alters its nonsense-mediated mRNA decay activity and overall mRNP structure

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During pre-mRNA splicing the exon junction complex (EJC) assembles 24 nucleotides upstream of most exon-exon junctions and is a key constituent of spliced messenger RNA particles (mRNPs). The stable EJC core consists of EIF4A3, MAGOH and RBM8A (a.k.a. Y14), and provides an interaction platform for several peripheral proteins that direct mRNA packaging, export, localization, translation and nonsense-mediated mRNA decay (NMD). Spurred by our previous observations that CASC3, a protein widely presumed to be an EJC core factor, and several peripheral EJC proteins are sub-stoichiometric in EJCs purified from human cells, we hypothesized that EJC composition within mRNPs is likely to be variable. We have now discovered that RNPS1 and CASC3, two EJC proteins implicated in NMD, exist in two mutually exclusive EJCs in human and mouse cells. Strikingly, RNPS1 containing EJCs are enriched in SR and SR-like proteins and resemble previously reported megadalton sized RNPs. In contrast, CASC3 containing EJCs are completely devoid of SR and SR-like proteins and exist in low molecular weight complexes. Proteomic data suggests that RNPS1 EJCs assemble co-incident with co-transcriptional splicing whereas CASC3 EJCs arise post splicing. While both alternate complexes bind to similar sites transcriptome-wide, RNPS1 EJCs are predominant on nucleus-enriched RNAs whereas CASC3 EJCs are preferentially associated with RNAs that accumulate in cytoplasm. These data suggest that EJC composition switches from SR-rich RNPS1 EJCs to SR-devoid CASC3 EJCs as mRNPs move from nucleus to cytoplasm with a concomitant alteration in mRNP structure. Surprisingly, we find that RNPS1 depletion causes robust upregulation of several endogenous NMD-targeted mRNAs while CASC3 depletion leads to only modest or in some cases no increase in their levels. Conversely, overexpression of CASC3 slows down NMD of some endogenous RNAs and of a well-known NMD reporter. Overall, our data show that inside cells mRNPs undergo a switch in their EJC composition, which alters overall mRNP structure. This compositional switch likely leads to two distinct NMD branches - an early acting branch where RNPS1 EJCs, in cooperation with SR proteins, promote more efficient RNA turnover and a late-acting branch where CASC3 EJCs trigger a more modest NMD.

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22 Isoform-specific catalytic activity of the NMD RNA helicase UPF1: how alternative splicing introduces structural changes to differentially regulate an enzyme

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The RNA helicase UPF1 is a key component of the nonsense mediated mRNA decay (NMD) pathway. Previous X-ray crystal structures of UPF1, together with biochemical studies, elucidated the molecular mechanisms of its catalytic activity and regulation. In this study, we examine features of the UPF1 helicase core and identify a structural element that adopts different conformations in the various nucleotide- and RNA-bound states of UPF1. We demonstrate, using biochemical assays, that this structural element modulates UPF1 catalytic activity and thereby refer to it as the regulatory loop. Interestingly, there are two alternatively spliced isoforms of UPF1 in mammals which differ only in the lengths of their regulatory loops The loop in UPF1 isoform 1 ($UPF1_1$) is 11 residues longer than that of the more abundant isoform 2 ($UPF1_2$). Here we compare the catalytic activities of $UPF1_1$ and $UPF1_2$ using biochemical and single-molecule tools and find that the small insertion within the regulatory loop of $UPF1_1$ leads to an increase in its translocation and ATPase activities. To determine the mechanistic basis of this differential catalytic activity, we have determined the X-ray crystal structure of the helicase core of $UPF1_1$ in its apo-state. We observe that the regulatory loop adopts different conformations in the two isoforms of UPF1, thereby mediating differential effects on its catalytic activities. Our results point towards a novel mechanism of regulation of RNA helicases, wherein alternative splicing leads to subtle structural rearrangements within the protein that are critical to modulate enzyme movements and catalytic activity.

23 Unique repression domains in Pumilio accelerate destruction of target mRNAs

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Pumilio (Pum) is a sequence-specific RNA-binding protein that represses an extensive network of mRNAs to control embryogenesis, stem cell maintenance, fertility, and neurological functions in *Drosophila*. Moreover, Pum orthologs have roles in cancer, neurodegeneration, ataxia, and epilepsy. Given these crucial functions, we seek to identify the mechanism of mRNA regulation by Pum. We find that Pum accelerates degradation of target mRNAs, and this activity is primarily caused by three repression domains (RDs) in the protein's N-terminus. The RDs are unique to Pum and its orthologs and can function autonomously when directed to a reporter mRNA. Each Pum RD causes repression and mRNA degradation, and we found that their activity requires the Ccr4-Not (CNOT) deadenylase complex. The Pop2 deadenylase subunit of CNOT is crucial for Pum RD-mediated repression of a reporter mRNA, as is Pop2's catalytic activity, indicating that Pum RDs require the deadenylation activity of CNOT. Our biochemical data reveal that multiple regions of Pum recruit CNOT to target mRNAs, including the N-terminus containing the three RDs, and the C-terminal RNA-binding domain. Consistent with this model, the ability of Pum to accelerate decay of target mRNAs requires CNOT. Extending our analysis to human orthologs, we found that the CNOT complex is crucial for repression by PUM1 and PUM2. We also observe that the N-terminus of Pum contains an additional repressive activity that is independent of the CNOT complex and does not require the poly(A) tail. First, we interrogated a proposed model wherein Pum binds to the 5' cap to inhibit translation; however, mutation of the cap-binding residue had no effect on Pum activity. Next, we investigated the role of the 5' mRNA decay pathway and found that decapping factors participate in repression by Pum N-terminal RDs. Together, our data reveal that Pum utilizes multiple mRNA decay pathways to repress target mRNAs.

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24 How piRNAs make planarians regenerate

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Even tiny body fragments of planarian flatworms regenerate into a full worm over the course of a week. Studying these fantastic regenerative abilities on a molecular level therefore not only helps us to understand planarian regeneration, yet it also holds the potential of deepening our limited knowledge on the molecular determinants of regeneration in general. Intriguingly, more than 10 years ago planarian PIWI proteins and their bound piRNAs were found essential during this regenerative response - the molecular details of this dependence of regeneration on piRNAs is still not clear to date.

This is why we comprehensively studied both essential planarian PIWI proteins (termed SMEDWI-2 and SMEDWI-3) to decipher their precise molecular role during regeneration. First, we were able to establish efficient immunoprecipitation, ChIP-seq, ribodepletion and HITS-CLIP protocols for both proteins in planarians (To the best of our knowledge the latter two techniques have not been reported before in planarians). Using these molecular tools, we characterized planarian piRNAs in a detailed way and determined planarian piRNA clusters and their epigenetic marks genome-wide. Furthermore, using RNA-seq after ribodepletion, we demonstrate that SMEDWI-2 is a main epigenetic regulator of transposon activity in planarians. However, SMEDWI-2 also plays "ping-pong" with SMEDWI-3 to degrade already transcribed transposons in the planarian cytoplasm.

In contrast to SMEDWI-2, SMEDWI-3 does not possess a dual role in epigenetic and post-transcriptional silencing. Its role is restricted to the cytoplasm, where it is enriched in chromatoid bodies, the analog of the *Drosophila* nuage. Using a combination of HITS-CLIP and RNA-seq we demonstrate that SMEDWI-3 has a fascinating role in directing the degradation of specific planarian mRNAs. Even more interesting, this role seems to be dictated by the secondary structure of the targeted mRNAs.

In summary, our results show that piRNAs in planarians have a variety of functions in both epigenetic regulation of transposable elements and in mRNA degradation. This seems a wise decision given the enormous amount of piRNAs in planarian stem cells and explains why piRNAs are essential for planarian regeneration.

25 Structures of ykkC riboswitches bound to ppGpp and PRPP reveal a novel principle of ligand recognition

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Interactions between RNA and small molecules play important roles in regulating gene expression. Riboswitches, evolutionarily conserved non-coding RNA elements, have emerged as one of the most significant means of modulating gene expression in response to specific binding of cellular metabolites. Representatives of the common ykkC riboswitch class were originally shown to bind to the small molecule guanidine. This riboswitch class has recently been divided into several subclasses on the basis of small variations in sequence conservation and location near different genes. Two ykkC subclasses have been shown to sense two distinct phosphate-rich cellular metabolites. The first subclass binds to the second messenger guanosine tetraphosphate (ppGpp), an important bacterial alarmone implicated in the control of branched-chain amino acids and stringent stress response. The second subclass recognizes the small molecule phosphoribosyl pyrophosphate (PRPP) and controls the expression of genes responsible for de novo biosynthesis of purines.

We have determined the X-ray crystal structures of two distinct ykkC riboswitches bound to their cognate ligands ppGpp and PRPP at 2.2 and 2.6 Å resolution, respectively. Remarkably, these structures share a large conserved core with the guanidine riboswitch, despite a lack of similarity between riboswitch ligands. In contrast to the guanidine riboswitch, the ppGpp and PRPP riboswitches contain an additional helical element that closes the sensing domain and creates a central four-way junction connecting two composite helices. This additional element forms an intimate ligand binding channel for direct and Mg2+-mediated recognition of cognate metabolites that stabilize the helix and thereby control expression of adjacent genes. Our mutational and footprinting experiments highlight the importance of conserved nucleotides layering the channel for ligand binding.

In previously described riboswitches, recognition of distinct ligands by similar RNA structures was achieved by small changes within the ligand-binding pockets. Therefore, guanidine, ppGpp, and PRPP riboswitches demonstrate a novel principle of changing ligand specificity that involves a small structural element added to the same structural core. Our study raises a question on evolution of similar riboswitches that recognize principally different ligands and highlight the versatility of RNA structures in binding to distinct types of metabolites.

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26 Structures of riboswitch aptamers that recognize phosphoribosyl pyrophosphate (PRPP) and guanosine tetraphosphate (ppGpp) reveal ruggedness in the functional landscape of the *ykkC* RNA

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The *ykkC* RNA is a conserved RNA motif that acts as a riboswitch in bacteria. The main subclass binds guanidine and turns on the transcription of downstream genes related to relieving guanidine toxicity. Variant subclasses 2a and 2b of the *ykkC* RNA specifically recognize phosphoribosyl pyrophosphate (PRPP) and guanosine tetra/pentaphosphate ((p)ppGpp), which are highly dissimilar from guanidine. These two variant RNAs differ from the main guanidine-binding subclass by a handful of nucleotides as well as the addition of conserved 5′ and 3′ tails. Variant subclasses 2a and 2b differ from each other by only a few nucleotides and both recognize polyanionic ligands. We used X-ray crystallography to determine the co-crystal structures of the PRPP riboswitch aptamer from *Thermoanaerobacter mathranii* with PRPP. We also determined the structure of the G96A point mutant, which prefers ppGpp over PRPP with a dramatic 40,000-fold switch in specificity. The conserved 5′ and 3′ ends of the aptamer base pair to form a helix that coaxially stacks with P3 and is not present in the guanidine-I riboswitch aptamer. In the G96A mutant, the base of ppGpp occupies the same space as G96 does in the wild type, forming a Watson-Crick base pair to the conserved C75 residue. This disrupts the S-turn, which is a conserved structural feature of the *ykkC* RNA motif. The dramatic differences in ligand specificity within the *ykkC* structural motif demonstrate that this an example of an RNA fold with a rugged functional landscape. The ease with which the *ykkC* aptamer acquires new specificity represents a striking case of evolvability in RNA.

27 ciRS-7 exonic sequence is embedded in a long non-coding RNA locus

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ciRS-7 is an intensely studied, highly expressed and conserved circRNA. Essentially nothing is known about its biogenesis, including the location of its promoter. A prevailing assumption has been that ciRS-7 is an exceptional circRNA because it is transcribed from a locus lacking any mature linear RNA transcripts of the same sense. To study the biogenesis of ciRS-7, we developed an algorithm to define its promoter and predicted that the human ciRS-7 promoter coincides with that of the long non-coding RNA, LINC00632. We validated this prediction using multiple orthogonal experimental assays. We also used computational approaches and experimental validation to establish that ciRS-7 exonic sequence is embedded in linear transcripts that are flanked by cryptic exons in both human and mouse. Together, this experimental and computational evidence generates a new model for regulation of this locus: (a) ciRS-7 is like other circRNAs, as it is spliced into linear transcripts; (b) expression of ciRS-7 is primarily determined by the chromatin state of LINC00632 promoters; (c) transcription and splicing factors sufficient for ciRS-7 biogenesis are expressed in cells that lack detectable ciRS-7 expression.

These findings have significant implications for the study of the regulation and function of ciRS-7, and the analytic framework we developed to jointly analyze RNA-seq and ChIP-seq data reveal the potential for genome-wide discovery of important biological regulation missed in current reference annotations and have revealed insight into the biogenesis of ciRS-7.

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28 The small non-coding vault RNA1-1 regulates autophagy by direct interaction with the autophagy receptor p62

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Autophagy is a highly regulated process for recycling cellular components and degrading foreign particles in eukaryotic cells. p62/sequestosome-1 functions as a receptor for selective autophagy, bringing ubiquitinated cargos to enclosing autophagosomal membranes. The internal content of mature autophagosomes including p62 is eventually degraded upon fusion with lysosomes. We have discovered that the autophagy receptor p62 is an RNA-binding protein, prompting us to investigate the physiological significance of its RNA binding. We show that the small, non-coding vault RNA1-1 (vtRNA1-1) selectively binds p62. Vault RNAs are small non-coding RNA polymerase III transcripts, best known for their association with a giant cytosolic RNP termed the vault RNP, whose function has remained elusive so far. We confirmed the specificity of vtRNA1-1-p62 interaction in cellulo and in vitro, respectively, and identified the RNA interaction interface on p62. We reveal that depletion of vtRNA1-1 augments, whereas increased vtRNA1-1 expression restricts, autophagic flux in a p62-dependent manner. Autophagy induced by starvation reduces the levels of vtRNA1-1 independently of lysosomal activity or p62 degradation, respectively, and starvation results in a progressive decrease in the RNA binding of p62 as well as a decrease in its interaction with vtRNA1-1. Thus, vtRNA1-1 acts as a direct negative regulator of autophagy via p62. We characterized RNA-binding mutants of p62 and show that lack of vtRNA1-1 binding renders the mutants engaging more actively with the autophagy effector proteins LC3B and GABARAP than the wild-type counterpart. Collectively, we propose that vtRNA1-1 functions as an inhibitor of p62-mediated autophagy. Our data uncover that RNAs can act as riboregulators of biological processes by interacting with proteins, and assign a function to a vault RNA.

29 Dicer cleaves 5'-extended microRNA precursors originating from RNA Polymerase II transcription start sites

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MicroRNAs (miRNAs) are approximately 22 nucleotide (nt) long and play important roles in post-transcriptional regulation in both plants and animals. In animals, precursor (pre-) miRNAs are ~70 nt hairpins produced by Drosha cleavage of long primary miRNAs in the nucleus. Exportin-5 (XPO5) transports pre-miRNAs into the cytoplasm for Dicer processing. Alternatively, pre-miRNAs containing a 5′ 7-methylguanine (m^7G-) cap can be generated independently of Drosha and XPO5. Here we identify a novel class of m^7G-capped pre-miRNAs with 5′ extensions up to 39 nt long. The 5′-extended pre-miRNAs are transported by Exportin-1. Unexpectedly, a long 5′ extension does not block Dicer processing. Rather, Dicer directly cleaves 5′-extended pre-miRNAs by recognizing its 3′ end to produce mature 3p miRNA and extended 5p miRNA both *in vivo* and *in vitro*. The recognition of 5′-extended pre-miRNAs by the Dicer Platform-PAZ-Connector domain can be traced back to ancestral animal Dicers, suggesting that this previously unrecognized Dicer reaction mode is evolutionarily conserved. Our work reveals additional genetic sources for small regulatory RNAs and substantiates Dicer's essential role in RNAi-based gene regulation.

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30 Cardioprotective microRNA-574-Fam210a axis maintains mitochondrial translational homeostasis

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Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. A promising therapeutic strategy to improve treatment of heart failure (HF)-a primary CVD-focuses on mitochondria, which control cell growth and generate ATP for heart contractility. HF results from cardiomyocyte (CM) hypertrophy and apoptosis, combined with cardiac fibroblast (CF) proliferation and cardiac fibrosis; these are hallmarks of cardiac pathological remodeling, which is regulated by specific miRNAs and mitochondrial-encoded genes (MEGs). Many studies have examined mechanisms that coordinate mRNA transcription of nuclear-encoded mitochondrial genes (NEMGs) and MEGs. However, regulatory mechanisms of MEG mRNA translation and its coordination with NEMG mRNA translation in the heart remain virtually unexplored. Here, we have identified a microRNA-574-Fam210a axis that maintains the optimal translation of MEGs and mitochondrial homeostasis in both CM and CF, as a compensatory cardioprotective pathway. In contrast to most other single strand miRNAs, miR-574 produces two functional strands, miR-574-5p and miR-574-3p. The common target of both miRNAs, Fam210a, is identified by transcriptome profiling of hearts from wild-type and miR-574 null mice. At early stage of CH, miR-574-5p antagonizes Fam210a expression in CM to prevent excessive MEG expression, enhanced ROS production and impaired mitochondrial activity, thereby preventing CM hypertrophy and apoptosis. Moreover, hypertrophic stress activates Src kinase-mediated Tyr^{359} phosphorylation and cytoplasmic accumulation of hnRNP L in CM. P-hnRNP L captures miR-574-3p and promotes exosome-mediated release of miR-574-3p and reduces CF proliferation by targeting CF Fam210a. miR-574 null mice exhibited an advanced cardiac hypertrophy phenotype associated with increased fibrosis, compared to wild-type mice after isoproterenol injection and transverse aortic constriction surgery, uncovering miR-574-5p/3p as novel cardioprotective miRNAs. Our results define a conserved role of Fam210a in translational control of mitochondrial-encoded genes and in regulation of mitochondrial activities, which may be therapeutically targeted to limit pathological cardiac hypertrophy, fibrosis and ventricular remodeling.

31 LIN28 selectively modulates a subclass of let-7 microRNAs

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LIN28 is a bipartite RNA-binding protein that post-transcriptionally inhibits let-7 microRNAs to regulate development and influence disease states. However, the mechanisms of let-7 suppression remains poorly understood, because LIN28 recognition depends on coordinated targeting by both the zinc knuckle domain (ZKD) -which binds a GGAG-like element in the precursor-and the cold shock domain (CSD), whose binding sites have not been systematically characterized. By leveraging single-nucleotide-resolution mapping of LIN28 binding sites *in vivo*, we determined that the CSD recognizes a (U) GAU motif. This motif partitions the let-7 family into Class I precursors with both CSD and ZKD binding sites and Class II precursors with ZKD but no CSD binding sites. LIN28 *in vivo* recognition-and subsequent 3′ uridylation and degradation-of Class I precursors is more efficient, leading to their stronger suppression in LIN28-activated cells and cancers. Thus, CSD binding sites amplify the effects of the LIN28 activation with potential implication in development and cancer.

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32 Mechanism of internalization of extracellular vesicle (EV) derived single stranded microRNAs in mammalian cells

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ABSTRACT:

Animal cells release different types of vesicles into the extracellular milieu. These vesicles which either originate from endosomes or plasma membrane; have been recently found to be involved in intercellular communication. Depending upon the origin, these EVs are classified either as microvesicles, ectosomes, microparticles or exosomes among others. Recently it has been found that exosomes can transfer mRNAs, microRNAs along with other cargo proteins and molecules which can remain functional in the recipient cell and play a pivotal role in immunity and cancer progression.

Previously we have identified how the RNA binding protein HuR binds to miRNAs and ensures export of specific miRNAs from animal cells (Mukherjee et al. 2016). However, the mechanism of internalization of EV derived miRNAs into target cells to elicit a response remains unknown. To understand the mechanism of uptake of these microRNAs we explored the factors that allow them to remain functional in the recipient cell. We have found that exosomal miRNAs were transferred to recipient cells where they remained functional. The transferred miRNA was found to localize in the Endoplasmic Reticulum (ER) and get transferred to the Argonaute2 (Ago2) protein of the recipient cells. On blocking the different components of the endocytic pathway we observed a difference in the total transferred miRNA content and their functionality suggesting a direct role of endocytic pathway in intercellular recycling of miRNAs in mammalian cells. I will present the mechanistic detail of this unique process of exchange of epigenetic signals between mammalian cells.

Reference:

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33 Transgenerational inheritance masks a critical role for piRNA methylation in C. elegans

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In *C. elegans*, RNAi involves at least two distinct phases. First, primary small RNAs act as triggers to initiate the process. Second, the target mRNA is routed into a highly effective siRNA amplification circuit involving a protein complex that contains at its catalytic core an RNA-dependent RNA polymerase. There are numerous classes of small RNAs in C. elegans, only some of which function in the canonical RNAi pathway. We show that small RNAs partition between those that act as triggers for RNAi and those that don't based on 2'-O methylation at their 3' ends. Surprisingly, many miRNAs are also methylated. Whether or not a miRNA is methylated is entirely dependent on which Argonaute it binds, indicating that, as in Drosophila, small RNA methylation in C. elegans is dictated by Argonaute association. Finally, we show that methylation of piRNAs is essential for their function, however, its role is normally masked by the transgenerational epigenetic memory of piRNA activity.

34 The ribosome dependent function of the universally conserved translational ATPase YchF

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Recent structural and biochemical studies have demonstrated that *in vivo* ribosome-dependent protein synthesis in bacteria requires an increasing number of non-canonical translation factors. Many of them are universally conserved GTPases such as HflX (1) and LepA (2). Among these emerging translation factors the protein YchF is of particular interest. Its sequence is highly conserved and although annotated as a GTPase YchF binds and hydrolyses ATP more efficiently than GTP (3,4). We demonstrate that YchF is essential at low temperatures and provides a strong fitness advantage at 37°C. Together with its expression profile, this suggests a role of YchF during ribosome biogenesis. Here we report for the first time structural information revealing YchF bound to the bacterial ribosome in complex with a non-coding RNA (ncRNA). Overall the YchF-ncRNA complex resembles the shape of several canonical translation factors, thereby allowing YchF to mimic the interactions these translation factors make with the ribosome. We propose based on the data presented, that YchF and the ncRNA are involved in ribosome quality control to ensure proper function during protein synthesis. Furthermore, our detailed biochemical analysis reveals a previously unidentified enzymatic activity of YchF that is consistent with its role in ribosome quality control. The fitness advantage conferred by YchF and its potential role in ribosome biogenesis represent new point of regulation during ribosome-dependent protein synthesis in bacteria that has the potential to become a target of novel antibiotics.

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35 TRAPPing the RNA-associated proteome

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We recently reported that alterations in the targets for RNA surveillance factors help rapidly reshape the transcriptome following glucose withdrawal. It seemed very likely that changes in the interactions of other RNA binding proteins (RBPs) also participate in the response to glucose withdrawal and many other regulated cellular process. Most analyses use a candidate approach, but quantitative, unbiased analyses of changes in the RNA-interacting proteome was expected to yield fresh insights. RBP detection based on poly(A) selection has been very effective, but is limited to the analysis of eukaryotic mRNAs. In contrast, the vast majority of transcripts are non-polyadenylated; including the precursors and mature forms of the many stable RNAs, nascent transcripts, and all prokaryotic and plastid RNAs. We therefore developed a method to identify the total RNA binding proteome in an unbiased, scalable and cost-effective way. In TRAPP (total RNA-associated proteome purification), RNP complexes are UV-crosslinked in vivo, denatured and isolated through their RNA component using silica beads. Subsequently, crosslinked proteins are detected and quantified using SILAC-MS/MS. Using this method, with robust cut-offs for the degree of enrichment, we identified hundreds of novel RNA binding proteins in Saccharomyces cerevisiae, Escherichia coli, and human HEK293 cells. A number of the novel RNA-associated proteins show conserved RNA binding activity throughout evolution. We applied TRAPP to yeast cells exposed to various cell stresses, including glucose withdrawal, rapamycin treatment, acid stress and heat shock. In the minutes following each stress, we observed and quantified robust changes in RNA binding for numerous proteins, many of which were not previously implicated in the stress response program. We are currently determining the signaling pathways involved in these responses. For small numbers of specific factors, the stress dependent changes in RNA binding were highly dependent on the conserved protein phosphatase Glc7 (PP1), which itself shows robust RNA binding. In conclusion, the TRAPP protocol provides an efficient and unbiased approach to characterize and quantify changes in the global RNA-binding proteome.

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36 KIN-CLIP: transcriptome-wide kinetics for RNA-protein interactions in cells

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RNA-binding proteins (RBPs) often interact with many different RNAs at sometimes large numbers of binding sites. These interactions are dynamic. Association and dissociation rate constants by which an RBP interacts with each binding site thus determine global RNA binding patterns of the protein and ultimately its biological function. However, it has not been possible to measure kinetic parameters for protein binding at individual RNA sites in cells.

Here, we describe a transcriptome-wide approach to determine kinetic parameters for protein binding at individual RNA sites in cells. We combine time-resolved UV-crosslinking with a high intensity pulsed UV-laser, Immunoprecipitation, Next Generation Sequencing, and large scale kinetic modeling, to determine rate constants for association, dissociation, crosslinking as well as the fractional occupancy for thousands of binding sites for the mouse RBP Dazl in GC1 cells. This kinetic CLIP (KIN-CLIP) approach reveals that both, association and dissociation rate constants for Dazl vary by 2 to 3 orders of magnitude among different binding sites, thus providing Dazl with a large dynamic range for binding to various sites. Crosslinking rate constants differ by only about one order of magnitude over all binding sites.

Our data reveal that Dazl stays bound at its binding sites for only few seconds or less, and that discrimination between different binding sites occurs predominantly during the association step. The fractional occupancy for a majority of Dazl binding sites is smaller than 10%, suggesting that regulation of binding site accessibility plays a large role for Dazl-RNA binding in the cell. Finally, correlations of kinetic parameters with ribosome profiling data highlight connections between the Dazl binding kinetics and Dazl's impact on the metabolism of individual transcripts.

By bridging biochemical and transcriptomic approaches, the KIN-CLIP technique allows the measurement of previously inaccessible, quantitative, biochemical parameters for RNA-protein interactions in cells, which are critical for mechanistically driven models of global RNA-protein interactions.

37 Deconvolving the RNA life cycle from high-resolution time-resolved protein-RNA interaction data

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Cells are constantly subjected to changes in their environment and as a result need to rapidly alter their transcriptional and translational program in response to these environmental changes. RNA abundance is the result of a balance between RNA synthesis and degradation. Traditionally RNA degradation rates are modelled as exponential decay kinetics, however, considering the complexity of RNA decay in many organisms and the number of enzymes involved, it is logical to assume that the life cycle of many transcripts cannot be explained using only a single parameter. In addition, CLIP/CRAC experiments have shown that many RNA decay factors bind overlapping substrates, however, in many cases it remained unclear what their effective contribution is. We hypothesized that by measuring the *in vivo* RNA-binding dynamics of individual RNA decay factors during adaptive responses, we would be able to better understand how the fate of RNA is determined. To address this, we applied kinetic CRAC (χCRAC; van Nues et al 2017, Nature Comm.) in yeast to monitor RNA synthesis (RNA Pol II) and the RNA-binding dynamics of nuclear (Nab3) and cytoplasmic (Xrn1) RNA decay factors under normal and stress conditions. Our high-resolution time-resolved studies revealed major changes in protein-RNA interaction dynamics during glucose starvation and uncovered a novel role for Nab3 in controlling the expression kinetics of stress-responsive genes. Much to our surprise, modelling of the χCRAC data from the three analysed proteins with only three global parameters was sufficient to explain the dynamic behaviour of ~45% of the analysed transcripts during glucose starvation. Performing a local optimisation of each transcript separately, we found that the transcripts form highly discrete clusters where transcription, nuclear and cytoplasmic degradation have defined contributions. In conclusion, our analyses enabled us to, for the first time, quantify the impact of nuclear and cytoplasmic degradation factors on the fate of RNA *in vivo*. We envision that combining χCRAC on individual RNA decay factors with modelling will make it possible to dissect how RNA decay factors and other RNA-binding proteins control gene expression kinetics during adaptive responses.

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38 Spliceosome profiling visualizes operations of a dynamic RNP at nucleotide resolution

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Tools to understand how the spliceosome functions *in vivo* have lagged behind advances in its structural biology. Here, methods are described to globally profile spliceosome-bound pre-mRNA, intermediates and spliced mRNA at nucleotide resolution. These tools are applied three yeast species that span 600 million years of evolution. The sensitivity of the approach enables detection of novel cases of canonical as well as non-canonical events including interrupted, recursive and nested splicing. Application of statistical modeling uncovers independent roles for intron size, position and number in substrate progression through the two catalytic stages. These include species-specific inputs suggestive of spliceosome-transcriptome coevolution. Further investigations reveal ATP-dependent discard of numerous endogenous substrates after spliceosome assembly *in vivo* and connect this discard to intron retention, a form of splicing regulation. Spliceosome profiling is a quantitative, generalizable global technology to investigate an RNP central to eukaryotic gene expression.

39 Spliceosome profiling reveals mega-RNPs active in splicing in human cells

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Pre-mRNA splicing, critical for mRNA biogenesis in eukaryotes, is well-studied in vitro; however, the complete composition of spliceosomes and their transcriptome locations in vivo are not known. Here, to address these, we combined protein-RNA and protein-protein crosslinking-immunoprecipitations (XLIPs) of snRNPs and splicing factors (snRNPs/SFs), RNA-seq and mass spectrometry, providing transcriptome-wide profiling of spliceosomes in human cells. We describe complexes embedded in hnRNPs comprising most known spliceosome components, RNA-modifying enzymes, transcription regulators, chromatin modifiers and additional pre-mRNA processing factors. Spliceosomes crosslinked to both 5'- and 3'-splice sites of the spliced intron and >75 nucleotides of the flanking exons, and to corresponding splice-junctions. Active spliceosomes were detected at >120,000 locations, including >15,000 novel/unannotated sites unpredicted from steady-state RNA. The splicing output of each was determined by RNA pulse-labeling-RNA-seq. Our findings suggest that pre-mRNA splicing in vivo occurs in mega-RNPs, comprising >80 splicing and other components, and reveal unexpected insights into pre-mRNA processing.

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40 Transcriptome-wide identification and validation of AGO-RBP co-regulation on mRNA targets

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The 3′ UTR of mRNAs is the primary regulatory region that mediates post-transcriptional control by microRNAs and RNA-binding proteins (RBPs) in the cytoplasm. Aside from their individual effects, higher-order combinatorial interactions between RBPs on specific mRNAs have been proposed to underpin the regulatory network. To assess the extent of such co-regulatory control, we took a global experimental approach followed by targeted validation to examine interactions between Argonaute2 (AGO2) and the well-characterized RBPs HuR and Pumilio (PUM1 and PUM2). Transcriptome-wide changes in AGO2-mRNA binding upon RBP knockdown were quantified by CLIP-seq, and the presence of RBP binding on the same 3′ UTR corresponded with cooperative and antagonistic effects on AGO2 occupancy. In addition, PUM binding sites that overlap with AGO2 showed differential, weakened binding profiles upon abrogation of AGO2 association, indicative of cooperative interactions. Focusing on sites of AGO2-RBP colocalization, candidate screening in luciferase reporter assays was carried out using site mutant constructs and WT, DICER- and RBP-knockout cell lines to identify individual examples of HuR and PUM co-regulation with AGO2. Our data experimentally confirms the combinatorial regulatory model and presents a search and validation system for studying miRNA-RBP interactions. Overall, the approach underscores the importance of further elucidation of complex interactions between RBPs and their transcriptome-wide extent.

41 N6-methyladenosine-dependent regulation of the pre-replicated Chikungunya viral genome

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The battle between viral RNA and host factors begins the instant the viral genome enters a cell. Prior to transcriptional upregulation of interferons, cytokines, and antiviral genes, a cell must rely on mRNAs and proteins that already exist in its cytoplasmic arsenal. The virus also benefits from any mechanism it can utilize to co-opt host processes before the onslaught of new defenses from transcription alters the internal cellular environment. However, the molecular events that comprise these primary interactions between host protein and viral RNA are not well understood. We developed a novel method to identify proteins that directly bind to pre-replicated, primary, viral RNA genomes that we term VIR-CLASP for **Vir**al **C**ross-**L**inking **A**nd **S**olid-phase **P**urification. Our approach is amenable to essentially any RNA virus, and captures interactions that occur within minutes of viral entry. In this report, we used this approach to identify hundreds of host RBPs that interact with the primary Chikungunya virus (CHIKV) RNA genome, including the YTHDF family of N6-methyladenosine (m6A) binding proteins. We established that m6A-modifications are abundant on CHIKV genomic RNA. m6A is involved in regulating many aspects of cellular RNA metabolism, and can modulate the pathogenicity of several RNA viruses including Zika, HIV, and Influenza. However, the biological impact of interaction between m6A-binding proteins and primary viral genomes is not known. We discovered that the effect of m6A on CHIKV viral replication is subject to combinatorial regulation by YTHDFs: knockdown and over-expression studies revealed that YTHDF1 strongly restricts viral replication, while YTHDF2 and YTHDF3 have the opposite effect. Our data indicate that the YTHDF proteins have distinct regulatory effects on CHIKV replication and depending on their relative intrinsic cellular levels, can tip the balance of a successful infection. There are currently no direct therapeutic options available to those at risk for CHIKV infection and the debilitating, long-lasting joint pain that follow. VIR-CLASP aims to increase the known repertoire of drug targets for CHIKV, and other RNA viruses, in order to advance current efforts to design vaccines and anti-viral compounds.

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42 Inferring gene regulatory landscapes from single-cell RNA-seq data

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In recent years, the volume of single-cell RNA-seq data has been increasing at a staggering pace. Much of the excitement in the field derives from the fact that single-cell transcriptomic measurements hold the promise to systematically answer fundamental questions regarding the organization of multi-cellular organisms, specifically about the definition of cell types and their observed stability despite stochastic fluctuations, about cell fate decisions and routes to cell reprogramming.

However, approaching these fundamental questions is non-trivial due to several methodological challenges. The variation in observed gene expression across single-cells derives not only from changes in the regulatory state of individual cells, but also from Poisson-like sampling noise that derives from both the intrinsic noise inherent in gene expression itself as well as the sampling in the process of RNA capture and sequencing.

Here we present novel methodologies to address three important aspects of single cell RNA-seq analysis. First, we present a new Bayesian methodology that rigorously separates this sampling noise from true variations in the transcriptional activity across single cells.

Second, we map these transcriptional activities to a much lower dimensional space of `regulatory states' using an extension of our previously developed motif activity response analysis (MARA). MARA models the transcriptional activity of all promoters in terms of computationally predicted transcription factor binding sites and infers the activities of the regulators binding to these sites. In this way the gene expression state of each single cell is projected on a motif activity state representing the activities of transcriptional regulators.

Third, we show how the observed distribution of single-cells in motif activity space can be used to reconstruct the locations of 'cell type attractors', to identify which regulators are crucial for stabilizing different cell types, and to determine the minimal perturbations in motif activities that would transform on cell type into another. We apply our methods to various single-cell transcriptomic datasets, and show how our methodology both reproduces previously reported results and offers new insights.

43 Quantifying regulatory factors transcriptome-wide utilizing gene co-expression networks inferred from single-cell sequencing

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Gene expression covariances are used to infer putative gene function and regulation. Traditionally specific processes or regulators are analyzed through time-scale or dose-dependent experiments. Additionally meta-studies combining hundreds of bulk transcriptome data sets have revealed new co-expression networks. Recently, advances in single cell biology have created new opportunities for the study of gene covariances.

We performed single-cell RNA-sequencing on a highly homogeneous population of mouse embryonic stem cells allowing us to infer gene covariances in physiological conditions, transcriptome-wide, and—importantly—independent of dominant dynamic processes such as cell-cycle or development.

We assayed over 600 cells that had passed a strict quality filter. Over 19,000 genes were detected in total—9,000 of which showed variance levels exceeding the estimated technical background variation. The false discovery rate for significant covariances was estimated to be lower than 2%.

The co-expression network we retrieved consists of over 100,000 significant gene-pair covariances, is analytically robust, hierarchical, and recovers known regulatory features. Notably it provides novel and surprising insights into a multitude of transcriptional and post-transcriptional layers of regulation, their quantitative contributions, as well as their cooperation and cross-talk.

Our approach overcomes existing limitations in gene co-expression analysis and provides a framework for studies in different tissues.

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44 Direct RNA sequencing to detect modified nucleotides using Oxford Nanopore

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Direct RNA sequencing reads have the capability to provide exon connectivity, accurate measurement of gene fusion events, an estimate of poly-A tail length and the ability to directly detect RNA modifications. As an international consortium of Oxford Nanopore MinION and GridION users, we have generated a comprehensive dataset composed of 13M direct RNA and 24M cDNA sequences and alignments based on poly-A RNA isolated from the human GM12878 reference cell line. We have made this dataset publically available here: https://github.com/nanopore-wgs-consortium/NA12878/blob/master/RNA.md. From this dataset, we aim to reanalyze raw current data for discrimination of modified and canonical nucleotides using Nanopolish (https://github.com/jts/nanopolish).

In order to accurately detect modifications on native RNA molecules, we first need to empirically establish how different modifications in variable sequence contexts will modulate the nanopore current, as per our methodology to detect 5mC in DNA (Simpson et al 2017). This method requires generation and sequencing of training sets. To this end, we have generated training sets using multiple methods to characterize N6- methyladenosine in different sequence contexts, focusing our GM12878 validation on the METTL3 motif (GGm6ACU). First, we used in vitro transcription with mixes of modified and unmodified nucleotides to generate RNAs with differing levels of m6A. Next, we used commercial direct synthesis (Trilink) of RNA oligos with precisely synthesized placement of modified and unmodified METTL3 motifs, then ligated this to a "handle" RNA strand with a polyA tail to increase transcript length and enable sequencing. Using these approaches, we identified variable current signatures in regions with known modifications, and are currently expanding our training sets to increase the scope of nucleotide context and the ability to call multiple modifications simultaneously. We will present our current work on these training sets and their application to our GM12878 dataset.

45 Spatial reconstruction of single enterocytes uncovers broad zonation along the intestinal villus axis

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The intestinal epithelium is a highly structured tissue composed of repeating crypt-villus units. Enterocytes, which constitute the most abundant cell type, perform the diverse tasks of absorbing a wide range of nutrients while protecting the body from the harsh bacterial-rich environment. It is unknown if these tasks are equally performed by all enterocytes or whether they are spatially zonated along the villus axis. Here, we performed whole-transcriptome measurements of laser-capture-microdissected villus segments to extract a large panel of landmark genes, expressed in a zonated manner. We used these genes to localize single sequenced enterocytes along the villus axis, thus reconstructing a global spatial expression map. We found that most enterocyte genes were zonated. Enterocytes at villi bottoms expressed an anti-bacterial Reg gene program in a microbiome-dependent manner, potentially reducing the crypt pathogen exposure. Translation, splicing and respiration genes steadily decreased in expression towards the villi tops, whereas distinct mid-top villus zones sub-specialized in the absorption of carbohydrates, peptides and fat. Enterocytes at the villi tips exhibited a unique gene-expression signature consisting of Klf4, Egfr, Neat1, Malat1, cell adhesion and purine metabolism genes. Our study exposes broad spatial heterogeneity of enterocytes, which could be important for achieving their diverse tasks.

46 The NMD Factor UPF3B Shapes Olfactory Neurogenesis and the Olfactory Receptor Repertoire

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Nonsense-mediated RNA decay (NMD) is a highly conserved and selective RNA turnover pathway. A key factor in this pathway, UPF3B, is an RNA-binding adaptor protein critical for normal cognition in humans. To understand the underlying mechanism(s) by which UPF3B acts in the nervous system, we generated *Upf3b*-null mice. These NMD-deficient mice suffer from defects in fear-conditioned learning and pre-pulse inhibition, as well as exhibiting decreased dendritic spine maturation in specific regions of the brain. To decipher UPF3B's role in neurogenesis, we used the olfactory system as a model, as this system has several advantages, including its ability to undergo synchronized regeneration. Analysis of markers specific for different olfactory sensory neuron (OSN) stages indicated that *Upf3b*-null mice have defects in olfactory neurogenesis. To understand the precise nature of these defects, we used single-cell RNA sequencing (scRNAseq) analysis. This analysis identified new OSN subsets not previously recognized by the field and revealed specific defects in *Upf3b*-null mice at several stages of olfactory neurogenesis. We focused our analysis on horizontal basal cells (HBCs), which serve as stem cells in the olfactory epithelium (OE). scRNAseq analysis, coupled with flow cytometric analysis, indicated that *Upf3b* promotes the progression of one HBC subset into a more differentiated HBC subset. Regeneration experiments confirmed this role and also provided evidence for the role of *Upf3b* in other OE cell stages. Despite the importance of *Upf3b* in olfactory neurogenesis, we found that *Upf3b*-null mice had normal numbers of mature (m) OSNs, suggesting the existence of a protective homeostatic mechanism. However, *Upf3b* loss had a profound effect on the repertoire of olfactory receptors (ORs) expressed in mOSNs. This is of interest given that *Or* genes are controlled by an intricate mechanism that selects a single *Or* gene (from a large repository of *Or* genes) to be expressed in a given mOSN. scRNAseq analysis, coupled with RNAseq analysis of purified mOSNs, indicated that *Upf3b* promotes the selection of almost eighty *Or* genes for expression in mOSNs. Our study reveals that NMD factor UPF3B is critical for specific stages of olfactory neurogenesis and shapes the olfactory receptor repertoire.

47 RNA structure prediction with interaction constraints (RNA SPICs)

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The ability to form base pairs determines the structure and function of RNA. It also permits structural flexibility, dynamics and formation of alternative conformations. Over the last few years we have observed a proliferation of new genome-wide approaches to map cellular dsRNA-protein and RNA-RNA interactions (CLASH, RPL, PARIS, LIGR-seq, SPLASH). Sequencing data from this type of experiments can be processed by the hyb program to produce a list of RNA-RNA interactions. These data emphasise not only the level of probability of RNA to be base-paired but also identify the partner of this interaction and provide information on alternative interactions. Structures of large RNAs, like viral RNA or pre-ribosomal RNA, are especially challenging to predict.

We used large data sets on ribosome assembly, mature ribosome and viral RNA to analyse RNA structures within different cellular compartments and time stages. Analysis of highly scored hybrids allowed us to extract specific structural RNA constraints. We are proposing a new computational approach called RNA SPICs (RNA Structure Prediction with Interaction Constraints). RNA SPICs splits large RNA molecules into fragments, folds these fragments using subsets of structural constraints and scores the folded structures according to their experimental support and folding energy. With this approach we are able to provide wider and more dynamic view of RNA folding, alternative conformations and interactions taking place during ribosome assembly, translation and viral infection.

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48 Rapid transcriptome-wide search for functional RNAs *in vivo* via structural data signatures

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Establishing a link between RNA structure and function remains a great challenge in RNA biology. The emergence of high-throughput structure profiling experiments is revolutionizing our ability to decipher structure at transcriptome-wide scale and *in vivo*. However, principled approaches for extracting information on functional structural elements, like riboswitches, thermosensors, and riboSNitches, directly from these datasets are severely lacking. Currently, biologists resort to methods tailored to specific biological questions or to thermodynamic modeling, which is primarily meaningful for RNAs folded in well-controlled *in vitro* conditions and also scales poorly to transcriptome-wide applications.

In this context, we developed patteRNA, a fast machine-learning algorithm to identify structural elements in large-scale structure profiling data. Briefly, our method learns the properties of RNA secondary structures and the statistical characteristics of each dataset without the need for reference structures (unsupervised learning). Our trained model then translates structure profiles into probabilities of nucleotide states (paired/unpaired bases) that are subsequently used to score for the presence of a target motif, as scanned across the entire dataset. We show that patteRNA is applicable to diverse profiling techniques, such as DMS-Seq, SHAPE-Seq, SHAPE-MaP, and PARS. In practice, patteRNA processes several transcriptome-wide datasets in a matter of hours, a task that currently requires several months with state-of-the-art thermodynamics-based algorithms.

To evaluate the accuracy of our algorithm, we use an *in vitro* SHAPE dataset of RNAs with known secondary structures and demonstrate motifs detection with accuracy comparable to existing algorithms. Next, we highlight its usefulness in automating data-directed structure modeling from cotranscriptional SHAPE-Seq data to quantitatively elucidate riboswitch folding pathways. Finally, we show that patteRNA mines accurate and biologically meaningful riboSNitch candidates in transcriptome-wide PARS data, thereby demonstrating its promise to accelerate our understanding of RNA structure-function relationships.

49 Light-Activated Probing of RNA Structure Inside Cells

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RNA molecules hold many mysteries in its biological function and regulation. These molecules contain many indispensable structural features that can dictate RNA functions; thus, it is imperative to develop methods to link RNA structure and function. Current approaches to RNA structure *in vitro* use a wide array of chemical probes which can be coupled using reverse transcription to generate a structural readout. However, analysis of RNA structure inside cells is limited, as only a handful of these probes can be utilized in cells. We present a novel approach, Light Activated Structural Examination of RNA (LASER), to look at solvent accessibility of purine nucleobases inside cells. This technique has the ability to identify structural changes of a RNA riboswitch independent from other probing methods *in vitro* using a handheld UV lamp. LASER is also successful in detecting solvent exposed regions of RNA in complex cellular environments. We expect our method can serve as an addition to the current arsenal for monitoring RNA structure inside cells.

50 Elucidating Signal Transduction Pathways in RNA Mediated Gene Regulation

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The rise of antibiotic resistance calls for immediate focus on identifying novel drug targets. RNAs are integral to cellular function, and a subset, called riboswitches, represent a distinct class of biomacromolecules that have already been validated as drug targets. Riboswitches take part in gene regulation in direct response to specific small-molecule effectors' levels. They usually reside in the 5′-leader sequence of bacterial mRNAs and exhibit a bipartite organisation. An aptamer domain senses the effector, while an expression platform regulates a gene or operon. Studies on riboswitches have mostly focused on their ligand binding abilities *in vitro*, outpacing our understanding of the underlying mechanisms that link effector-binding to gene-regulation.

To convincingly relate RNA structure to function, we analysed the $preQ_1$-II (class-2) riboswitch: a well-characterised HL_{out} pseudoknot that recognises the metabolite pre-queuosine$_1$ ($preQ_1$). A novel RNA-modifier called NAI was used to perform *in cell* (ic)SHAPE to compare flexibility changes of individual nucleotides in response to $preQ_1$. When mapped onto our crystal structure, our data showed excellent support of the gene-OFF conformational state. We developed a reporter assay in which GFPuv is controlled by a $preQ_1$-II riboswitch to study its gene regulatory function. Added effector showed a 10-fold repression of GFPuv expression (EC_{50}=19.5±1.1 nM), consistent with binding studies done by Isothermal Titration Calorimetry (ITC) (K_D=17.9±0.6 nM). The functional relevance of *in vitro* observations thus established, we sought to identify molecular interactions that connect $preQ_1$ binding to gene-regulation. We used our reporter assay along with site-directed mutagenesis to study specific nucleobase interactions hypothesised to be on molecular signal-transduction pathways. Repression analyses were conducted on >10 mutants flanking the $preQ_1$-binding pocket and extending into the expression platform. Subsequently, we performed ITC on the mutants to compare affinity to GFPuv-repression.

Our findings indicate the need for strong base-pairing in the SDS-antiSDS region and maintenance of long-range base-triples for effective switching. The results also suggest that the A-minor bases flanking the binding pocket, and helix P4 of the pseudoknot, play an unexpected role in gene regulation. Finally, we have identified mutants showing 3 log-units of difference between K_D and EC_{50}, indicating decoupling of binding and gene expression.

51 Shift of Monomer-Dimer Equilibrium Reveals An Irreversible RNA Structural Rearrangement

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Our long term goal is to develop a physicochemical understanding of rugged folding energy landscape of RNA. An RNA molecule can function as a molecular switch or play more than one biological function by adopting alternative structures. Thermodynamically, this structural heterogeneity can be simplified as an energy landscape in which two (meta)stable states are separated by a high kinetic barrier. An obvious question thus arises: how does an RNA transit from one structure to another? In HIV, dimerization initiation site (DIS) RNA forms different structures at different stages of viral life cycle, serving as an indispensable linchpin for dimerization of viral genomes, recombination, packaging, and maturation. Two DIS hairpins form a loop-loop interaction known as kissing complex (KC) in cytosol, whereas in mature viral particles, the DIS RNAs fold into an extended duplex (ED). The KC-to-ED conversion requires facilitation by viral nucleocapsid protein, an RNA chaperone protein. However, the mechanism underlying this important structural rearrangement remains unknown, largely because the two conformations are difficult to be distinguished. To disentangle such structural heterogeneity, we took advantage of different thermal stability of the two dimers. A temperature controlled mass spectrometer (MS) was developed to interrogate the DIS monomer-dimer equilibrium in a temperature dependent fashion. As temperature is raised, a simple structure is expected to become less stable. The DIS KC dimers, however, displayed a reentrant melting curve, in which dimer abundance increased with temperature between 45 oC and 65 oC. This counterintuitive heat-induced stabilization was proved to be the irreversible KC-to-ED transition. Furthermore, we used the MS melting method to isolate different roles of individual structural domains in the structural rearrangement. Instrument and methodology developments presented here open a new way to dissect structural heterogeneity of RNA and to monitor structural interconversions in real time.

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52 Enzymatic or *in vivo* installation of propargyl groups in combination with click chemistry enables enrichment and detection of methyltransferase target sites in RNA

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m^{6}A is the most abundant internal modification in eukaryotic mRNA. It is introduced by METTL3-METTL14 and tunes mRNA metabolism, impacting cell differentiation and development. Precise transcriptome-wide assignment of m^{6}A sites is of utmost importance. However, m^{6}A does not interfere with Watson-Crick base pairing making polymerase-based detection challenging. We developed a chemical-biology approach for the precise mapping of methyltransferase (MTase) target sites based on the introduction of a bioorthogonal propargyl group *in vitro* and in cells. We show that propargyl can be introduced enzymatically by wild-type METTL3-METTL14. Reverse transcription terminated up to 65 % at m^{6}A sites after bioconjugation and purification, hence enabling detection of METTL3-METTL14 target sites by next generation sequencing. Importantly, we implemented metabolic propargyl labeling of RNA MTase target sites *in vivo* based on propargyl-L-selenohomocysteine and validated different types of known rRNA methylation sites.

53 Novel molecular tools for targeted labeling of RNA

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Biophysical and mechanistic investigation of RNA function requires site-specific incorporation of spectroscopic and chemical probes, which is difficult to achieve using current technologies. We devised two AdoMet-dependent methyltransferase-based systems for targeted covalent labeling of RNA. First, we have *in vitro* reconstituted a functional box C/D small ribonucleoprotein RNA 2'-*O*-methyltransferase from the thermophilic archaeon *P. abyssi* and demonstrated its ability to transfer a prop-2-ynyl group from a synthetic cofactor analog to a series of preselected sites in model tRNA and pre-mRNA molecules. The target selection was programmed by changing a dodecanucleotide guide sequence in a 64-nt C/D guide RNA, which for the first time permitted synthetically tunable sequence-specific internal labeling of RNA with single-nucleotide precision. We have also employed the HEN1 RNA 2′-*O*-methyltransferase from *A. thaliana* to perform the transfer of extended propargylic moieties from a range of synthetic AdoMet cofactor analogs to 3'-terminal nucleotides in duplex miRNAs or siRNAs or in an individual miRNA strand paired with a complementary synthetic DNA probe that itself can carry sophisticated user-defined chemical moieties. We demonstrate that the transferred alkyne or azide groups can be further appended with fluorophore or biotin reporters using a bioorthogonal 1,3-cycloaddition (click) reaction, permitting selective optical detection or physical enrichment of the target RNA molecules. The obtained results broaden our understanding of the substrate specificity of RNA methyltransferases and pave the way to numerous applications of these enabling tools in RNomics, synthetic biology, and nanomedicine.

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54 Orientation-dependent FRET using rigid fluorogenic RNA aptamers

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The angle dependence of fluorescence resonance energy transfer (FRET) has many useful applications for the study of conformationally active RNA structures. Angle-dependent FRET has been difficult to study as it is hard to ensure that the donor and acceptor dipoles of the fluorescent system precisely track the orientation of the structure under study. Chemically coupling fluorophore reporters directly to nucleic acid partially solves this problem, but the fluorophores are not fully oriented (Iqbal et al. 2008). Guided by crystal structures of RNA Spinach (Huang et al. 2014; Warner et al. 2014)and Mango I (Trachman et al. 2017)and the orthogonal fluorophore binding potential of these two aptamers (Jeng et al. 2016), it was recently demonstrated that a fluorogenic aptamer-based FRET signal can be modulated by a small molecule, or invader nucleic acid strands (Jepsen et al. 2018). Structural data, however, suggest the fluorophore binding core of Mango I is flexibly connected to external sequence (Trachman et al. 2017)presumably precluding precise angular measurements.

Recently we developed three new RNA Mango aptamers (Autour et al. 2018). Like Mango I, all of these aptamers connect to external RNA helices. Using Broccoli/DFHBI as a donor and the far red-shifted Mango III/TO3-Biotin complex as an acceptor, we measured FRET efficiency using an RNA duplex of variable length between the two aptamers. FRET was dependent on the length of the joining RNA duplex, and oscillated in intensity precisely with the predicted twist of the duplex. In contrast, replacing Mango III with Mango I resulted in a FRET signal that was substantially rotationally averaged. This finding is consistent with a recent crystal structure of Mango III (Trachman et al. Ferré-D'Amaré, in preparation) that indicates that the fluorophore binding domain of Mango III is rigidly connected to its arbitrary closing helix. As aptamer-tagged RNA constructs can be transcribed in living cells, and since the fluorogenic dyes used are cell permeable and non-toxic, we believe that rigid fluorogenic aptamers offer the prospect of building reliable biological FRET reporter systems.

55 Detection of Ligand-Induced Conformational Changes in Oligonucleotides by Second-Harmonic Generation

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RNA and DNA structural elements play an important role in a variety of biological processes, which make them an appealing target for drug discovery. However, traditional protein higher order structural techniques often face challenges when applied to RNA and DNA molecules. Furthermore, these structural techniques are laborious and not amenable to the throughput needed in drug discovery. Moreover, binding techniques capable of higher throughput often do not resolve functional structural elements. In this study, second-harmonic generation (SHG) technology[1]was adapted to study RNA and DNA oligonucleotide conformational changes associated with ligand binding. The technique was applied to three distinct RNA/DNA structural classes, including RNA hairpins, G-quartets, and riboswitches, all of which are known to undergo conformational changes upon binding either protein or small molecule ligands.[2,3]In all three cases, SHG was able to resolve conformational changes in these oligonucleotides sensitively and specifically, in solution and in real time, using nanogram amounts of material. Furthermore, these changes were distinct from conformational changes associated with known nonspecific binders This work demonstrates the broad potential of SHG for studying oligonucleotides and their conformational changes upon interaction with ligands. As SHG offers a powerful, high-throughput screening approach, our results here also open an important new avenue for identifying novel chemical probes or sequence-targeted drugs that disrupt or modulate DNA or RNA structure and function.

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56 OTTER, a new method for measuring absolute quantity of tRNAs

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Translation is not only constitutive mechanism to make polypeptides but an elaborated platform for regulation and quality control. Thus, cytosolic environments for translation have been accumulating more interest in these years, and one of such parameters to be investigated is a tRNA repertoire. Indeed, recent reports in human suggest that individual tRNA species are under regulation according to physiological and developmental conditions. Although microarray and RNA-seq are excellent methods to explore repertoires of mRNA and some classes of non-coding RNAs, they are not perfect choices to analyze tRNA quantity mainly because of heavy modifications of tRNA nucleotides. Here, we introduce a novel method, OTTER (*o*ligonucletide-directed *t*hree-prime *t*erminal *e*xtension of *R*NA), where the 3'-terminus of a specific tRNA species is labeled with a fluorescent nucleotide by hybridization of template oligo DNA to tRNA's 3'-terminal region and extension of the tRNA molecule by DNA polymerase. By this method, we measured absolute quantities of tRNA isoacceptors in the yeast *Saccharomyces cerevisiae* and found the following facts. 1) A quantity of a tRNA isoacceptor is well correlated with a number of synonymous genes encoding the tRNA than requirement of the tRNA estimated from total codon usage of the yeast genome. 2) When looking at individual tRNAs, variation of tRNA quantity per tRNA gene has tendency to become smaller when the number of synonymous genes for the tRNA increases. 3) tRNA amounts increased according to progression of growth phases when cultured in rich media while such increase is not prominent in synthetic media. 4) Rations between the tRNA quantity in the log phase and that in the stationary phase vary among tRNA isoacceptors, suggesting that quantities of tRNA species are regulated individually in part. We will discuss these results with expected tRNA dynamism according to physiological changes in our presentation.

57 *De novo* computational RNA modeling into cryoEM maps of large ribonucleoprotein complexes

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RNA-protein assemblies carry out many critical biological functions including translation, RNA splicing, and telomere extension. Increasingly, cryo-electron microscopy (cryoEM) is used to solve the structures of these complexes, but nearly all maps solved with this method have regions in which the local resolution does not permit manual coordinate tracing. Because RNA coordinates typically cannot be solved through crystal structures of separate components, they are frequently omitted from final models despite their biological importance. To address these omissions, we have developed a new framework for De novo Ribonucleoprotein modeling in Real-space through Assembly of Fragments Together with Electron density in Rosetta (DRRAFTER). We show that DRRAFTER recovers near-native models for a diverse benchmark set of small RNA-protein complexes, as well as for large RNA-protein machines, including the spliceosome, mitochondrial ribosome, and CRISPR-Cas9-sgRNA complexes where both high and low resolution maps enable rigorous tests. Blind tests on yeast U1 snRNP and spliceosomal P complex maps demonstrate that the method can successfully build RNA coordinates in real-world modeling scenarios. Additionally, to aid in final model interpretation, we present a method for reliable *in situ* estimation of DRRAFTER model accuracy. Finally, we apply this method to recently solved maps of telomerase, the HIV-1 reverse transcriptase initiation complex, and the packaged MS2 genome, demonstrating that DRRAFTER can be used to accelerate accurate model building in challenging cases.

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58 Structure of the Ago2:miR-122:HCV complex

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microRNAs (miRNAs) are short non-coding RNAs that act in complex with Argonaute (Ago) proteins to direct RNA silencing. They form a regulatory network orchestrating cellular growth, differentiation, and homeostatic maintenance[1]. Many viral interactions with miRNAs are known, which modulate[2]or redirect Ago:miRNA complexes[3], or even repurpose them, a trait present in multiple RNA viruses of the Flaviviridae family[4], with the most studied interaction being between the hepatitis C virus (HCV) and the liver miRNA miR-122[5].

HCV causes acute liver infection that usually progresses to chronic, leading to long-term liver damage, and affects about 170 million patients worldwide[5]. A tandem binding site at the very 5'-end of the viral RNA genome recruits two Ago2:miR-122 complexes to protect HCV from the cellular antiviral response[5].

Here we present the crystal structure of the ternary complex of Ago2:miR-122 with the 5'-terminal binding site of the HCV genome at 3.2 Å resolution. It reveals how the viral RNA forces Ago2 to adopt an open conformation that accommodates secondary structure in the viral 5' UTR and masks the 5'-end of the viral genome from cellular surveillance machinery.

This is the first structure of Ago in complex with an RNA that is of viral origin, and any target that contains secondary structure. It may reveal new therapeutic strategies against HCV, and suggests how Ago may be able to interact with more complicated targets than is currently appreciated.

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59 Cryo-EM structure of human Dicer and its complexes with a pre-miRNA substrate

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Human Dicer (hDicer) is a multi-domain protein belonging to the RNase III family. It plays pivotal roles in small RNA biogenesis during the RNA interference (RNAi) pathway by processing a diverse range of double-stranded RNA (dsRNA) precursors to generate ~22-nt microRNA (miRNA) or small interfering RNA (siRNA) products for sequence-directed gene silencing. In this work, we solved the cryo-EM structure of hDicer in complex with its cofactor protein TRBP and revealed the precise spatial arrangement of hDicer's multiple domains. We further solved structures of the hDicer-TRBP complex bound with pre-let-7 RNA in two distinct conformations. In combination with biochemical analysis, these structures reveal a property of the hDicer-TRBP complex to promote the stability of pre-miRNA's stem duplex in a pre-dicing state. These results provide new insights into the mechanism of RNA processing by hDicer and illustrate the regulatory role of hDicer's N-terminal helicase domain.

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60 Repetitive DNA reeling by the Cascade-Cas3 complex in nucleotide unwinding steps

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CRISPR-Cas provides RNA-guided adaptive immunity against invading genetic elements. Interference in type-I systems relies on the RNA-guided Cascade complex for target DNA recognition and the Cas3 helicase/nuclease protein for target degradation. Even though the biochemistry of CRISPR interference has been largely covered, the biophysics of DNA unwinding and coupling of the helicase and nuclease domains of Cas3 remains elusive. Here we employed single-molecule FRET to probe the helicase activity with high spatiotemporal resolution. We show that Cas3 remains tightly associated with the target-bound Cascade complex while reeling in the target DNA using a spring-loaded mechanism. This spring-loaded reeling occurs in distinct bursts of three base pairs, that each underlie three successive 1-nt unwinding events. Reeling is highly repetitive, allowing Cas3 to repeatedly present its inefficient nuclease domain with ssDNA substrate. Our study reveals that the discontinuous helicase properties of Cas3 and its tight interaction with Cascade ensure controlled degradation of target DNA only.

61 Cas4-dependent prespacer processing ensures high-fidelity programming of CRISPR arrays

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CRISPR-Cas systems provide bacteria and archaea with RNA-guided adaptive immunity against infection. The basis of this immunity is the integration of invasive DNA sequences as short spacers within the host CRISPR array. This array is transcribed and processed into short CRISPR RNAs, which guide effector Cas proteins to target and destroy DNA sequences complementary to the guide spacer sequence. CRISPR RNA-based targeting also requires the recognition of a protospacer adjacent motif (PAM) that can be found immediately next to target sequence. In order to achieve efficient defense against invaders, functional spacers from foreign DNA must be captured from sites with correct PAM sequences and processed precisely prior to integration into the CRISPR array. Cas1 and Cas2 proteins are universally conserved in all CRISPR–Cas types and Cas1–Cas2 complex formation is required for integration. Other core Cas proteins, such as Cas4, are widespread in CRISPR-Cas systems and are thought to participate in this step, although their function remains unknown. Here we show that *Bacillus halodurans* type I-C Cas4 is required for efficient prespacer processing prior to Cas1-Cas2 mediated integration. Cas4 interacts tightly with the Cas1 integrase, forming a heterohexameric complex containing two Cas1 dimers and two Cas4 subunits. In the presence of Cas1 and Cas2, Cas4 processes double-stranded substrates with long 3′-overhangs through site-specific endonucleolytic cleavage. Cas4 recognizes PAM sequences within the prespacer and prevents integration of unprocessed prespacers, ensuring that only functional spacers will be integrated into the CRISPR array. Our results reveal the critical role of Cas4 in maintaining fidelity during CRISPR adaptation, providing a structural and mechanistic model for prespacer processing and integration.

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62 Recruitment of a Type III-A CRISPR-Cas Csm complex to the transcription elongation complex by recognition of the nascent RNA transcript

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CRISPR-Cas systems are RNA-guided prokaryotic immune systems that protect against invasive genetic elements, such as phages and plasmids. Type III CRISPR-Cas systems belong to the most common class of CRISPR-Cas systems in bacteria and archaea (Class 1) and may also be the most evolutionarily ancient system. They use a multisubunit effector complex, Csm (Type III-A) or Cmr (Type III-B), composed of several Cas proteins and a CRISPR RNA (crRNA) to target complementary sequences in transcriptionally active DNA and single-stranded RNA. How exactly Type III complexes recognize and cleave their targets is not well understood. Here, we show that the *Thermus thermophilus* Csm is recruited to a transcription elongation complex (TEC) via recognition of a complementary RNA transcript. Electron microscopy structures of the Csm-TEC complex show that Csm does not interact directly with the RNA polymerase, but is instead flexibly tethered by the RNA to the transcription bubble. We also show that TEC-bound Csm is active for cleavage of single-stranded DNA, which could be transiently exposed during transcription. We propose that tethering of Csm to the TEC would increase the effective local concentration of Csm near the DNA target to enable efficient and specific degradation of transcriptionally active DNA. Our findings also provide an explanation for how Type III systems became widespread in bacteria and archaea, as targeting of the effector complex to a transcriptionally active DNA target would not require recognition of any specific RNA polymerase, but instead rely on crRNA:RNA base-pairing.

63 Co-assembly of liquid and gel-like phases in an RNA granule

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RNA granules are RNA/protein condensates proposed to form in cells by liquid-liquid phase separation. The *C. elegans* P granules are RNA granules that exhibit liquid-like behaviors. Using *in vivo* and *ex vivo* methods, we find that P granules contain at least two phases: a scaffold phase required for granule assembly and a client phase that is recruited to the scaffold. The scaffold phase is assembled by intrinsically-disordered proteins that form a gel-like matrix that recruits the liquid client phase. Our observations suggest that P granules assembly requires stabilization of a liquid phase by a gel-like scaffold.

64 Genome-wide determination of 1st and 2nd step rates of pre-mRNA splicing in vivo using rapid metabolic RNA labeling time courses coupled with a novel targeted sequencing approach

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Next-generation sequencing (NGS) technologies have had a profound effect on our understanding of pre-mRNA splicing: implementation of NGS via RNA sequencing (RNA-Seq) has enabled the unambiguous detection of vast numbers of novel splice isoforms generated within a cell. Nevertheless, it is less widely appreciated that the depth of sequencing necessary to quantitatively detect many splicing isoforms is significantly higher than most RNA-Seq experiments generate. Moreover, methods implemented to date have lacked the capability to distinguish between pre-mRNA intermediates generated during the splicing process. Here we present the development and implementation of a novel sequencing method designed to harness the quantitative power of sequencing while focusing it on user-selected splice junctions of interest. We demonstrate the ability of this approach to dramatically enrich the fraction of reads in a given sequencing experiment that are informative about splicing status, and in doing so enable a significant increase in the precision with which changes in splicing can be detected, while maintaining high accuracy and decreasing experimental costs. We demonstrate the relative ease with which this approach can be adopted to diverse systems, facilitating a wide variety of experiments designed to understand the mechanistic underpinnings of splicing regulation in different organisms. Importantly, we demonstrate the capacity of this approach to distinguish between pre-mRNA molecules that have or have not completed the first chemical step of splicing, to our knowledge marking the first time these intermediates have been identified in vivo on a global scale. By coupling this targeted sequencing approach to a rapid metabolic RNA labeling time course, we have measured the genome wide rates of the 1st and 2nd steps of pre-mRNA spicing, providing unprecedented insights into the genome-wide processing of mRNAs.

65 Structural characterization of the UsnRNP assembly machinery and its regulation by post-translational modifications

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The assembly of macromolecular RNA-protein complexes (RNPs) often depends on trans-acting factors in vivo, as their unassembled protein-subunits are prone to aggregation or engage in non-cognate interactions. The formation of the common Sm/LSm core of spliceosomal and histone-mRNA processing UsnRNPs serves as well-studied paradigm for assisted assembly of RNPs. The assembly factors of this process are united in PRMT5- and SMN-complexes, which suppress Sm/LSm protein aggregation and facilitate their faithful binding onto cognate UsnRNA targets. Assembly is initiated by the PRMT5-complex subunit pICln, which pre-arranges Sm/LSm proteins into spatial positions occupied in the mature U snRNP. The SMN complex subsequently takes over these Sm/LSm units, displaces pICln and unites them with U snRNA. The SMN complex of higher eukaryotes is a macromolecular machine consisting of nine proteins (termed SMN, Gemins 2-8 and Unrip). We report on the biochemical reconstitution of the SMN complex and its detailed structural analysis by means of X-ray crystallography and Cryo-EM. We present atomic structure models of subcomplexes and elements of the SMN complex enabling insight into the architecture of the entire assembly machinery. The activity of the SMN complex in UsnRNP assembly is likely regulated by post-translational modifications. To identify the corresponding modifying enzymes, we have systematically screened human kinases that target the assembly machinery. We identified mTOR and S6 kinase as potential positive, as well as transcription-regulating, cyclin dependent kinases (CDKs) as possible negative regulators of SMN function in human cells. We will report on the analysis of the identified signalling pathways, their impact of the composition and phosphorylation state of the SMN complex and their relevance in the regulation of U snRNP biogenesis.

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66 U6 snRNA m^{6}A stabilizes 5' splice site recognition

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In eukaryotes, N^6-methyladenosine (m^{6}A) is an abundant modification in mRNAs and lncRNAs. m^{6}A is also present in U6 snRNA introduced by METTL16 methyltransferase, although its molecular function and physiological role remain to be elucidated. Through RNA-seq analysis of fission yeast METTL16 ortholog mtl16 knockout strain, we found a subset of introns retained in mRNAs, indicating that loss of m^{6}A in U6 snRNA results in splicing defect. The affected introns were mainly those bearing an adenosine at the 4th position from the 5' splice site, which is faced against m^{6}A of U6 snRNA recognizing 5' splice site in the spliceosome. In addition, the retained introns tend to bear sequences weakly recognized by U5 snRNA in the 3' end region of the 5' exons. These findings suggest a novel function of m^{6}A involved in the splicing machinery, assisting the binding with U6 snRNA when an intron bears A at the 4th position, and has weak binding affinity to U5 snRNA.

67 Global Interplay of A-to-I RNA editing and pre-mRNA Splicing

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Adenosine deamination-type editing and pre-mRNA splicing are tightly interlinked processes. Adenosine deaminases acting on RNA (ADARs) recognize double stranded structures, typically formed between adjacent complementary sequences within one RNA. Frequently, editing events that lead to the recoding of mRNAs are defined by base pairing beween the *exonic* editing site with an adjacent *intronic* editing complementary sequence (ECS). Consequently, editing needs to occur before the ECS is removed by splicing, implying that the speed of splicing affects the extent of editing. Conversely, it has been show that inhibition of editing can interfere with splicing. Lastly, nuclear and cytoplasmic editing levels can vary, indicating selective processing and / or export of mRNAs, depending on their editing status.

Using inhibitors of splicing and genetic mouse models in which either one of the two editing enzymes ADAR1 or ADAR2 are deleted, we determine the interplay of pre-mRNA splicing and RNA editing. Our studies show an unexpected high level of intronic RNA editing events, exceeding the previously known number of editing events in the mouse. We show further, that inhibition of pre-mRNA splicing increases the rate of RNA editing, primarily in introns and UTRs. Lastly, we can show that inhibition of editing by ADAR1 can strongly affect alternative splicing while ADAR2 only has a minor impact on splicing. Currently, we are determining whether tissue-specific splice rates can affect editing rates in vivo and explain the observed tissue specific variation in editing patterns despite relatively constant levels of the editing enzymes.

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68 Conserved interaction between yeast Hsh155 and Cus2 enforces ATP-dependent prespliceosome assembly by antagonizing Prp5 ATPase activity

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During pre-mRNA splicing, the action of ATP-dependent RNA helicase proteins is required for efficient or correct progression of the spliceosome. The first ATP-dependent step in yeast extracts is the stable association of the U2 snRNP with the intron branchpoint (BP) to form the prespliceosome (PSP). For this to occur, a region of U2 snRNA must be folded into the stem IIa structure rather than the IIc structure. Cus2 (human homolog: Tat-SF1), identified through genetic suppression of U2 mutations, promotes formation of stem IIa prior to the ATP-dependent stabilization step and antagonizes the ATP-dependent activity of Prp5: Deletion of *CUS2* suppresses lethal mutations in the ATP-binding site of Prp5, and PSP formation does not require ATP when Cus2 is absent in vitro. Here, we present new biochemical and genetic evidence showing that Cus2 binds to U2 snRNP protein Hsh155 (human homolog: SF3B1) through its U2AF homology motif (UHM) and a conserved U2AF ligand motif (ULM) found on Hsh155. Subtle alteration of the UHM of Cus2 or the ULM of Hsh155 abolishes the interaction. A peptide derived from the Hsh155 ULM binds the Cus2 UHM with a Kd ~20 nM. Disruption of Hsh155 or Cus2 at either binding site permits PSP formation to proceed in the absence of ATP, similar to depletion of Cus2. The same mutation suppresses the lethality of a Prp5 ATP-binding mutant, showing that the ATP-dependent activity of Prp5 is no longer required when Cus2 and Hsh155 do not interact. In the yeast B spliceosome models, the Hsh155 region that binds Cus2 is not resolved, but is positioned near the U2/BP helix between U2 stem loop IIa and HEAT-repeats of Hsh155 that interact with Prp5. Our data support a model in which the ATP-dependent activity of Prp5 displaces Cus2 from Hsh155 to promote the stable binding of the U2 snRNP to the intron BP, possibly through unwinding the branchpoint stem loop of U2 and helping establish extended base pairing between U2 and the intron. The relationship between each of these events and whether this reaction governs the fidelity of branchpoint selection or simply its efficiency remains to be seen.

69 High-throughput suppressor analysis identifies molecular surfaces and contacts that likely control activation of the B complex spliceosome

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Cryo-EM structures of several different yeast and human spliceosomal complexes have been determined over the last few years, providing near-atomic resolution views of the network of dynamic protein-protein, protein-RNA, and RNA-RNA interactions that occur during assembly, activation, catalysis, and disassembly. These models also suggest pathways for the transduction of allosteric signals that link spliceosome activation with splice site recognition, thereby enforcing splicing fidelity. For example, it is likely that base-pairing of the 5' splice site with U6 snRNA elicits the expulsion of U4 snRNA from the spliceosome so that U6 can form the catalytic core with U2 snRNA. Genetic suppression studies provide a means for validating these signaling pathways in living yeast cells, but have been hampered by the expensive and time-consuming task of identifying suppressor mutations in whole-genome selections. Here we report a high-throughput and economical method for identifying suppressor mutations, which exploits a custom, next-generation sequencing panel that targets 112 known or likely splicing factors.

We tested this approach by selecting spontaneous suppressors of a cold-sensitive mutation in yeast U4 snRNA, U4-cs1, that blocks activation of the spliceosomal B complex by competing with U6/5' splice site pairing. Activation of the B complex requires stimulation of the U5 snRNP ATPase Brr2 to unwind U4 snRNA from U6 snRNA. Starting with 61 U4-cs1-suppressor strains, we identified 27 unique mutations in five different proteins, including 11 in Prp8: six new mutations and five obtained previously in a gene-targeted selection. Four U4-cs1-suppressor substitutions were obtained in Brr2 and cluster in a 75-residue stretch of the 2163-residue protein. These substitutions delineate a discrete spot on the N-terminal PWI domain that may mediate a negative regulatory interaction. The U4-cs1-suppressor mutations we obtained in other splicing factors suggest additional mechanisms for regulation of the B-to-B^{act} transition. Our approach of mapping suppressor mutations to extant spliceosome structures will be useful for predicting the path of allosteric signals through the interaction network of the dynamic spliceosome as it proceeds through the splicing reaction.

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70 Both U4 and U6 snRNAs promote Brr2 dependent U4/U6 unwinding

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Splicing is catalyzed by the small nuclear RNA (snRNA) U6 that, prior to catalysis, is in an inactive conformation base-paired with U4 snRNA. Therefore, spliceosome activation requires unwinding of this base-paired U4/U6, which is comprised of stem I and stem II. U4/U6 unwinding requires the DExH-box ATPase Brr2, and ultimately results in the irreversible release of U4; however, a deep understanding of the molecular mechanisms underlying U4/U6 unwinding is lacking. In budding yeast, we used an unwinding deficient, cold-sensitive *BRR2* mutation to screen for genetic interaction with U4 and U6 snRNA. This *BRR2* mutation results in the degenerative eye disease retinitis pigmentosa in humans. We identified suppressors and enhancers in U6 at positions that in the B-complex can be part of an extended U6-5'SS interaction, suggesting that this region of U6 activates Brr2 as a consequence of 5'SS recognition. In U4 stem I, we identified suppressors of the *brr2* mutant implying destabilization of stem I by Brr2, consistent with in vivo crosslinking data. Unexpectedly, however, many other mutations in stem I exacerbated the *brr2* mutant. These mutations disrupt an intramolecular stem loop in U4 that is mutually exclusive with U4/U6 stem I, providing evidence that this intramolecular stem loop promotes unwinding by preventing re-annealing of U4/U6 stem I. Mutations that disrupt stem II also suppress the *brr2* mutant, supporting a role for Brr2 in destabilizing the entire duplex. Lastly, mutations in stem II that increase complementarity in a competing intramolecular stem loop also suppressed the *brr2* mutant, suggesting that the entire unwinding process is reversible, which could be important for proofreading. Overall, these data indicate that U4 and U6 are not passive substrates during unwinding but active participants in spliceosome activation.

71 Structural accommodations accompanying splicing of a group II intron RNP

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Group II introns, proposed progenitors of nuclear spliceosomal introns and retrotransposons, are ribozymes capable of target DNA invasion. In the cell, group II intron RNAs form ribonucleoprotein (RNP) complexes with an intron-encoded protein (IEP), which is essential to folding, splicing and retromobility of the intron. Although the structure of the endogenously expressed *Lactococcus lactis* Ll.LtrB group II intron RNP in the post-catalytic form have been reported, structural and mechanistic information regarding the precursor and the splicing process of the RNA-protein complex begs interrogation. Here we report that the intron RNP in the pre-catalytic state forms a dimer that undergoes reversible temperature-dependent monomerization, while the post-catalytic RNP remains a monomer. The SHAPE (Selective 2'-Hydroxyl Acylation analyzed by Primer Extension) profile of the RNP in the post-catalytic state not only corresponds to the recently reported cryo-electron microscopy (cryo-EM) structure of the same RNP, but also provides additional insights into RNA dynamics as well as RNA-RNA and RNA-protein interactions. By comparing the SHAPE profiles of the excised intron RNP with mutant RNPs in the precursor state, confined SHAPE profile differences were observed in transition between the two states, indicative of rearrangements at the active site as well as disengagement at the functional RNA-protein interface in transition between the two states. In addition, exon-binding sequences 1 and 2 (EBS1 and EBS2) in the intron RNA, which form essential base pairs with corresponding sequences in the 5' exon and that of the target DNA, show increased flexibility after splicing. In contrast, stability of major tertiary and protein interactions maintains the scaffold of the RNA through the splicing transition, while the active site is realigned in preparation for retromobility.

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72 Structural Insights into Thermostable Group II Intron Reverse Transcriptases (TGIRTs) and their Functions in RNA Splicing, Intron Retromobility, and RNA-seq

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Group II intron-encoded reverse transcriptases (RTs) sit at the intersection of molecular diagnostics, genome research, and the evolution of eukaryotes. These ancient enzymes function in RNA splicing and intron mobility ("retrohoming") and are evolutionary precursors of all modern RTs, as well as spliceosomal protein Prp8. We have focused on thermostable group II intron RTs (TGIRTs) from bacterial thermophiles, both for their potential utility for biochemical and structural studies of RNA splicing and intron mobility and for their biotechnological applications, particularly RNA-seq. The latter benefits from the high fidelity, strong strand-displacement activity, and high processivity of group II intron RTs, as well their proficient template-switching activity, which facilitates RNA-seq adapter addition. Recently we determined a 3.0-Å crystal structure of a full-length TGIRT (GsI-IIC RT encoded by a prolific group IIC intron in *Geobacillus stearothermophilus*) in complex with template-primer substrate (Stamos et al., Mol. Cell 68, 926-939, 2017). The structure revealed a remarkably close evolutionary relationship between group II intron RTs and RNA-dependent RNA polymerases, as well as a host of structural features not seen previously in RTs that may contribute to the beneficial biochemical properties of group II intron RTs. Ongoing structure-function investigations of the unique structural motifs of TGIRTs identified key features affecting template-switching, processivity, strand-displacement activity, and other biochemical properties. Regions required for RNA splicing and DNA target site recognition are located on the outer surface of the protein and do not overlap regions involved in template-primer binding. By using a combination of modeling, *in vitro* mutagenesis, and biochemical and genetic assays of RNA splicing and intron mobility, we show that a basic cleft formed between the thumb and DNA-binding domains plays a critical role in retrohoming by recognizing a 5'-exon DNA hairpin, typically from a bacterial transcription terminator upstream of intron insertion sites. The DNA target site recognition mechanism enables the intron to proliferate to high copy number in its host genome by recognizing and inserting downstream of multiple transcription terminators.

73 A Dual-Activity Topoisomerase Interacts with RNAi Machinery to Promote Heterochromatin Formation and Transcriptional Silencing

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Topoisomerases solve topological problems generated during DNA metabolism. However, whether and how these enzymes participate in RNA metabolism remains unclear. We and others have previously shown that Type IA topoisomerases from all domains of life often possess dual topoisomerase activities for both DNA and RNA. In animals, one of the two Type IA topoisomerases, Top3b, contains an RNA-binding domain, possesses RNA topoisomerase activity, binds mRNAs, interacts with an RNA-binding protein (FMRP), to regulate translation of mRNAs important for neurodevelopment, autism and schizophrenia. The RNA-binding domain is required for targeting top3b to mRNAs, as well as for promoting neurodevelopment. Here we show that in Drosophila, Top3b biochemically and genetically interacts with the RNAi-induced silencing complex (RISC), which contains FMRP, Argonaute 2 (Ago2), p68 RNA helicase (p68), and Vig. Top3b mutant flies display phenotypes similar to those of the RISC mutants, including defective heterochromatin formation and transcriptional silencing in the Position Effect Variegation (PEV) assay. Notably, this phenotype was largely suppressed in the double mutants between Top3b and Ago2, p68, and the siRNA biogenesis nuclease, Dicer-2, indicating that Top3b works coordinately with the siRNA machinery to facilitate heterochromatic gene silencing. Consistent with this notion, both Top3b and Ago2 single mutant flies exhibit reduced levels of heterochromatin markers at sub-telomeric and pericentric heterochromatin regions, whereas this reduction was largely suppressed in their double mutant. Furthermore, expression of several genes and transposable elements within the affected heterochromatin regions was increased in the Top3b mutant. Our data suggest that Top3b works with the FMRP-associated siRNA machinery to promote heterochromatin formation and transcriptional silencing. We propose a model that the topoisomerase-helicase pair of Top3b-p68 may work coordinately to resolve complex RNA structures similarly as that of Top3a-BLM in resolving complex DNA structures.

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74 SWI/SNF-regulated alternative splicing produces metabolic-state specific protein isoforms, dynamically controlling Coenzyme Q_6 biosynthesis and expression of stress-response genes

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Despite its relatively streamlined genome, there are many important examples of regulated RNA splicing in Saccharomyces cerevisiae. Here, we report a role for the chromatin remodeler SWI/SNF in respiration, partially via the regulation of splicing. We find that a nutrient-dependent decrease in Snf2 leads to an increase in splicing of the *PTC7* transcript. The spliced *PTC7* transcript encodes a mitochondrial phosphatase regulator ($Ptc7_S$) of biosynthesis of coenzyme Q_6 (ubiquinone or CoQ_6), a mitochondrial redox-active lipid essential for electron and proton transport in respiration. The increase in *PTC7* splicing occurs at least in part due to down-regulation of ribosomal protein gene expression, leading to the redistribution of spliceosomes from this abundant class of intron-containing RNAs to otherwise poorly spliced transcripts. Increased splicing of *PTC7* increases CoQ_6 levels. The *PTC7* intron is particularly intriguing because it lacks a premature termination codon and is translated in-frame. The protein encoded by the unspliced isoform of *PTC7* ($Ptc7_{NS}$) localizes to the nuclear membrane, and actively represses CoQ6 biosynthesis. Further, we discovered that $Ptc7_{NS}$ plays a novel role in the downregulation of a number of stress-responsive transcripts under ambient environmental conditions, likely by regulating the phosphorylation state of stress-specific transcription factors. Taken together, these findings establish the SWI/SNF complex as a regulator of the transition of yeast from fermentative to respiratory modes of metabolism, partly by regulating the splicing of *PTC7* and the relative abundances of the two Ptc7 isoforms. This work also reveals novel regulation of expression of stress-response genes via a nuclear membrane bound phosphatase.

75 The Conserved RNA Binding Protein ZFR Represses Innate Immune Signaling by Controlling Splicing and Decay of Histone Variant macroH2A mRNAs

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Control of type-I interferon production is crucial to combat bacterial and viral infection while preventing deleterious inflammatory responses. Pathways promoting rapid transcriptional induction of type-I interferons have been intensively studied, but the contribution of post-transcriptional regulatory mechanisms to innate immune signaling is poorly understood. Here, we show that the human zinc finger RNA-binding protein (ZFR) represses the interferon response by regulating alternative splicing and decay of histone variant macroH2A1/H2AFY mRNAs.

In normal cells, two functionally distinct macroH2A1 isoforms are expressed via alternative splicing of mutually exclusive exons. If ZFR is depleted, both mutually exclusive exons are skipped, generating an aberrant spliced product containing a premature stop codon. We show that the isoform generated by this unusual alternative splicing event is efficiently degraded by nonsense-mediated mRNA decay, effectively abolishing macroH2A1 expression in cells lacking ZFR. Depletion of either ZFR or macroH2A1 causes hyper-induction of interferon β (IFN β) and downstream antiviral proteins in response to treatment with dsRNA or lipopolysaccharides. Accounting for the aberrant type I interferon responses observed upon ZFR depletion, we find that macroH2A1 protein directly binds and represses the IFN β promoter.

Underlining the physiological importance of this mechanism, our data suggest that ZFR and NMD are used to control macroH2A expression during macrophage development. We find that ZFR is differentially expressed from alternative promoters in monocytes and macrophages. Monocytes express a truncated form of the protein lacking zinc-finger motifs, while macrophages express a full-length isoform capable of modulating alternative splicing. Correspondingly, macroH2A1 protein levels are low in monocytes and elevated in macrophages. Together, our findings show that induction of ZFR in developing macrophages helps to guard against aberrant activation of the type-I interferon response and reveal a network of mRNA processing and decay events that shapes the transcriptional response to infection.

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76 The spliceosomal component Sf3b1 protects embryonic neurons from R-loop mediated DNA damage

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Mutations in spliceosomal components are prevalent in cancer and neurodegenerative diseases, but how defects in these essential factors contribute to disease is unclear. Emerging data has demonstrated that disruption of spliceosomal components can lead to genomic instability through accumulation of R-loops, nucleic acid structures consisting of RNA:DNA hybrids and ssDNA. We utilized a zebrafish loss-of-function mutant in the spliceosomal component Sf3b1 (Splicing factor 3b, subunit1) to interrogate tissue-specific regulation of R-loops and genome stability. We observed significantly elevated R-loop accumulation and double strand DNA breaks in embryonic neurons of *sf3b1* mutants compared to their wild-type siblings as measure by single-cell immunofluorescence quantitation of RNA:DNA hybrids and γH2AX, respectively. The response to DNA damage ultimately results in various cellular fates, including cell cycle arrest and apoptosis. Compared to wild-type cells, *sf3b1* mutant cells displayed a G0/G1 phase arrest, as assayed by quantifying DNA content (DAPI) and DNA replication via labeling with the thymidine analogue, 5-Ethynyl-2′-deoxyuridine (EdU). Apoptosis, as measure by active caspase-3, was increased selectively in the neural tissue of *sf3b1* mutants. We showed that depletion of R-loops by conditional overexpression of RNASEH1, an enzyme that degrades RNA:DNA hybrids, alleviated cell cycle arrest, apoptosis and DNA damage in *sf3b1* mutants. R-loop accumulation is known to trigger distinct DNA damage responses, mediated by Ataxia-telengiasta-mutated (ATM) and Ataxia-Telangiectasia and Rad3-related (ATR), signaling. ATM and ATR kinases each initiate specific downstream signaling cascades, resulting in distinct cellular consequences, including cell cycle checkpoints. Our cell cycle arrest data is in line with an ATM-triggered G1-to-S phase cell cycle checkpoint. To confirm ATM pathway activation, we found a significant increase in phosphorylated Chk2, a direct downstream substrate of the ATM kinase. Taken together, these results signify Sf3b1 regulated R-loops trigger an ATM-mediated DNA damage response resulting in cell cycle arrest and neuronal apoptosis. Our data indicate R-loop accumulation and DNA damage are repressed by Sf3b1 in a cell-type selective manner, which could be exploited therapeutically for spliceosomal-defective diseases.

77 The regulation of mRNA nuclear export and translation by RanBP2-dependent sumoylation

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Mutations in RanBP2/Nup358, a protein found on the cytoplasmic face of the nuclear pore, cause acute necrotizing encephalopathy 1 (ANE1). In response to influenza infection, individuals with these dominant mutations have a 40% chance of experiencing a massive secretion of cytokines, known as a "cytokine storm". The resulting elevated levels of cytokines cause seizures, encephalopathy, coma and a high rate of mortality. Previously we showed that RanBP2 directly interacts with, and promotes the translation of, many mRNAs that code for secretory and membrane-bound proteins (Mahadevan et al., PLoS Bio 2013). We now have found that RanBP2 supresses the translation of the interleukin 6 (IL6) mRNA, which encodes a key cytokine associated with ANE1. There are at least three elements in the 3'UTR of IL6 mRNA that mediate the regulation of RanBP2. Two of these elements inhibit the nuclear export of IL6 mRNA in a RanBP2-dependent manner, while the third element supresses translation. Using CRISPR-Cas9 we have engineered cells that have versions of RanBP2 that lack E3 SUMO-ligase activity. These cells no longer downregulate IL6 expression. We propose a model where RanBP2 sorts mRNAs into distinct regulatory groups as they emerge from the nucleus. RanBP2 accomplishes this by altering how each of these mRNAs is packaged into a mRNP complex, likely through sumoylation. RanBP2 also likely regulates the recycling of mRNP components that accompany exported mRNAs out to the cytoplasm and must be returned to the nucleoplasm. Finally, our work suggests that RanBP2 regulation of the IL6 mRNA is likely disabled by ANE1 mutations.

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78 tRNA synthetases as novel mRNA binding proteins: target mRNAs, mode of recognition and regulatory role

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RNA-binding proteins coordinate every step in the life of an mRNA molecule. Recent proteomic studies identified novel mRNA-binding proteins, many of which are well-studied proteins of whom mRNA binding activity appears as a new feature. A recurring group in these studies is the amino acyl tRNA synthetases (aaRS) family; an extensively studied family for its critical role in charging tRNAs with their cognate amino acids. We wish to determine the mRNA targets of this group, their RNA recognition site and the regulatory role of this association. RNA Immunoprecipitation followed by deep sequencing (RIP-Seq) identified the repertoire of mRNAs associated with several yeast aaRSs. All exhibited a strong tendency to bind their own mRNA, as well as various additional mRNAs. Further analysis of Histidine tRNA Synthetase (HisRS) revealed that its anticodon binding domain is important for mRNA association, suggesting recognition through anticodon-like structures. To explore this, we devised a method to map mRNA regions that are in direct association with an aaRS, and found that a region bound by HisRS contains a predicted anticodon-like structure. Single mutation within this anticodon-like triplet significantly reduced the association between the mRNA and HisRS. Finally, we found that cognate tRNA overexpression can compete with the association between HisRS and its target mRNA. Overall, this study provides understanding regarding the specificity and mechanisms of mRNA association by aaRS. Importantly, it suggests a novel layer of coordination between tRNA and mRNA processes.

79 The structure of yeast tRNA ligase reveals a competition between non-conventional mRNA splicing and RNA decay

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Yeast tRNA ligase (Trl1) is an essential trifunctional enzyme consisting of a cyclic phosphodiesterase, a polynucleotide kinase and an ATP-dependent RNA ligase. It catalyzes exon-exon ligation of tRNA half-molecules during tRNA splicing. Trl1 is also required for *HAC1* mRNA splicing during the unfolded protein response (UPR). The UPR is an intracellular signaling network that monitors and regulates the protein folding capacity of the endoplasmic reticulum (ER). Upon sensing protein folding perturbations in the ER, the kinase/endonuclease Ire1 initiates the UPR signal by a unique mechanism: In a non-conventional, cytoplasmic splicing reaction, Ire1 removes an intron from *HAC1* mRNA followed by exon-exon ligation by Trl1, allowing the production of the Hac1p transcription factor that drives the transcriptional response. How the splicing reaction is orchestrated with fidelity to ensure stress signaling remains an outstanding question.

Here, we report the crystal structure of the RNA ligase domain of *Chaetomium thermophilum* Trl1 at 1.9 Å resolution. The molecular architecture of the active site reveals the principles of RNA substrate recognition. A Trl1 mutant variant that uncouples both functional outputs in vivo allowed us to identify a competition between RNA ligation and degradation during *HAC1* mRNA splicing in the cell. Incompletely processed *HAC1* mRNA is rapidly degraded by Xrn1 and the Ski/exosome complex. Our results show the functional importance of mRNA decay pathways as well as ribosome-associated quality control in maintaining the fidelity of this non-conventional splicing reaction.

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80 Formation of tRNA wobble inosine in humans is perturbed by a primeval mutation linked to intellectual disability

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The formation of inosine at the wobble position of eukaryotic tRNAs is an essential modification catalyzed by the ADAT2/ADAT3 complex. In humans, a valine to methionine mutation (V144M) in ADAT3 that originated ~1,600 years ago is the most common cause of autosomal-recessive intellectual disability (ID) in Arabia. Here, we show that ADAT3-V144M exhibits perturbations in subcellular localization and has increased propensity to form aggregates associated with cytoplasmic chaperonins. While ADAT2 co-expression can suppress the aggregation of ADAT3-V144M, the ADAT2/3 complexes assembled with ADAT3-V144M exhibit defects in adenosine deaminase activity. Moreover, extracts from cell lines derived from ID-affected individuals expressing only ADAT3-V144M display a reduction in tRNA deaminase activity. Notably, we find that the same cell lines from ID-affected individuals exhibit decreased wobble inosine in certain tRNAs. These results identify a role for ADAT2-dependent localization and folding of ADAT3 in wobble inosine modification that is crucial for the developing human brain.

81 PRMT7-dependent arginine methylation regulates the stability and function of RNA-binding proteins

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Protein aRginine MethylTransferases (PRMTs) catalyse arginine methylation in various cellular processes. The chromatin modifier PRMT7 is the only Type III PRMT found in higher eukaryotes and a restricted number of unicellular eukaryotes. Leishmania major PRMT7 is a cytoplasmic protein implicit in pathogenesis with unknown substrates. Using comparative methyl-SILAC proteomics for the first time in protozoa, we identified 40 putative monomethylation target proteins, including 17 RNA-binding proteins (RBPs) hypomethylated in PRMT7-null mutants. Isolated peptides confirm a significant RGG-motif enrichment. In vitro, PRMT7 can modify RBPs Alba3 and RBP16 as direct substrates. In vivo PRMT7 knockout reduces both RBP16 protein half-life and Alba3 mRNA-binding capacity. RNA immunoprecipitation (RIP) analyses demonstrate PRMT7-dependent methylation promotes Alba3 association with target transcripts and consequent stability of delta-amastin surface antigen. These results highlight a novel role for PRMT7-mediated arginine methylation of RBP substrates, suggesting a post-translationally-directed regulatory pathway controlling both gene expression and virulence in Leishmania. This work introduces Leishmania PRMTs as epigenetic regulators of mRNA metabolism with novel mechanistic insight into the functional manipulation of RBPs by methylation.

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82 Diverse roles of RNA helicases in driving structural transitions and compositional changes in pre-ribosomal complexes

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RNA-protein complexes (RNPs) play key roles at all stages of gene expression and in various other pathways of RNA metabolism. Production of eukaryotic ribosomal subunits is a highly dynamic process involving numerous structural rearrangements of the ribosomal RNAs (rRNAs) and the hierarchical recruitment of approximately 80 ribosomal proteins. Such remodelling events not only establish the architecture present in mature complexes, but also serve as key checkpoints, ensuring the fidelity of ribosome assembly. RNA helicases are important regulators of such structural transitions and multiple helicases have been implicated in ribosome synthesis. However, the molecular functions and sites of action of many of these enzymes during subunit assembly have remained elusive. Using *in vivo* crosslinking and analysis of cDNA (CRAC), we have identified the pre-ribosomal binding sites of the three RNA helicases Has1, Mak5 and Spb4. By elucidating the precise targets of these enzymes, we uncover direct roles for Has1 in triggering dissociation of a cluster of early pre-60S biogenesis factors from domain I of the 25S rRNA and mediating release of the U14 snoRNP, a critical event during early 40S maturation. Furthermore, remodelling of helix 39 of the 25S rRNA sequences by Mak5 enables recruitment of the ribosomal protein Rpl10, which is necessary for subunit joining and ribosome function. Finally, our data show that Spb4 binds to a molecular hinge at the base of ES27, which forms a flexible arm that anchors the export factor Arx1 to pre-60S complexes. We show that Spb4 facilitates the pre-ribosomal recruitment of Arx1, implying that helicase-mediated remodelling of this region contributes to establishing the export competence of pre-60S particles. Taken together, our data provide important new insights into the driving forces behind key remodelling events during ribosomal subunit assembly.

83 Ribosome Biogenesis Coming Into Focus: Remodeling Events Driving Middle Stages of Large Subunit Assembly in Yeast

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Ribosome biogenesis requires a dynamic series of remodeling steps in which protein-protein, protein-RNA, and RNA-RNA interactions are established and reconfigured. Assembly must be efficient to conserve cellular resources and rapidly respond to cells' needs, and accurate to avoid making error-prone ribosomes. The many steps of subunit assembly in the yeast *Saccharomyces cerevisiae* are made more efficient and more accurate by the activities of more than 200 assembly factors, which are conserved across eukaryotes. To enable an in-depth study of the mechanisms driving ribosome assembly *in vivo*, we are focusing on "middle" stages of assembly of the yeast large ribosomal subunit: just prior to, during, and immediately after the exit of large ribosomal subunit precursors from the nucleolus into the nucleoplasm. In particular, we want to understand assembly of functional centers of the large subunit: the peptidyltransferase center (PTC), where peptide bonds are formed, the GTPase activating center (GAC), where translation factor GTPases bind to ribosomes and enable protein synthesis, and the polypeptide exit tunnel (PET), through which all nascent polypeptides travel to emerge from ribosomes.

We have carried out a detailed analysis of the effects of depleting assembly factors and ribosomal proteins required for these middle stages of large subunit biogenesis, and interpreted these results using recent cryo-EM structures of assembling 60S subunits. We are now focusing on the roles of the RNA helicases Drs1 and Has1 and the GTPase Nog1 in these steps. Our working hypothesis is that Drs1 and Has1 use ATP binding and hydrolysis to trigger remodeling events in the nucleolus required for construction of functional centers, removal of the ITS2 spacer RNA, and exit of pre-ribosomes from the nucleolus. We think that Nog1 uses GTP binding and hydrolysis to enable assembly of the GAC and PTC, and inserts its C-terminal tail into the PET to enable or inspect assembly of this tunnel.

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84 Ltv1 facilitates and monitors assembly of the 40S ribosome beak structure during ribosome biogenesis

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Ribosomes are crucial to producing a stable and functional proteome. The proper assembly of ribosomes from 4 rRNAs and 80 ribosomal proteins (RPs) is an enormous combinatorial problem, whose critical importance is evident in the numerous diseases caused by haploinsufficiency of RPs. How the cell resolves this complex assembly with high fidelity and accuracy, ensuring the proper placement of each RP, remains poorly understood. Interestingly, recent work has shown that ribosomes from cancer cells often have a limited complement of individual proteins, which is associated with negative patient outcomes. Here we present genetic, biochemical and structural data in yeast that Ltv1 facilitates and monitors the correct incorporation of Rps3, Rps10, Rps29 and Asc1/RACK1 into the head of 40S ribosomes. We show that Ltv1-deficient cells have defects in translational fidelity, which provide a survival benefit under stress conditions. Moreover, we found that breast cancer cells have reduced levels of Ltv1, leading to the production of ribosomes, which also lack Rps3 and RACK1, and which have similar defects on translation fidelity. This data describes a cellular mechanism to ensure that RPs are correctly incorporated, and also explains how cancer cells exploit this mechanism to produce multiple ribosome subpopulations with differing functionalities, that can promote survival under the stress conditions that cancer cells experience.

85 5' UTR RNA Elements Confer Translational Specificity to Shape Vertebrate Embryonic Development

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A central question in biology is how information encoded in the genome is translated into body form. Our lab has previously identified RNA motifs in Homeobox (Hox) 5' UTRs that in conjunction with more specialized ribosomes direct tissue-specific translational control. In particular, a subset of Hox mRNAs contain Internal Ribosome Entry Site (IRES)-like elements that control Hox spatiotemporal protein expression as well as a novel Translation Inhibitory Element (TIE) at the 5' cap that suppresses cap-dependent translation. We seek to understand the *cis*-acting RNA-protein complexes that associate with these 5' UTR RNA motifs and how they act to promote ribosome binding. We applied RNP affinity purification from mouse embryos and high-throughput quantitative mass spectrometry analysis of Hox IRES-protein complexes. This led to the identification of an only 35 nt long stem-loop of the Hoxa9 IRES structure that is remarkably sufficient for recruitment of the 40S ribosomal subunit. Next, we used RNA structural mutagenesis and RNA structure-function analysis to show that this short stem-loop in presence of a leader sequence can initiate translation of a reporter mRNA which requires a sequence motif in the stem-loop for this activity. To further characterize how this element interacts with the ribosome, we employed cryo-EM analysis and identified an 18S rRNA expansion segment (ES) in the human 40S subunit that directly binds to the Hoxa9 full-length IRES RNA, as well as the 35 nt stem-loop RNA alone. During eukaryotic evolution from yeast to mammals, this ES, with a yet unknown function, increased in size. While yeast ribosomes are not able to bind to the Hoxa9 IRES structure or stem-loop RNA, we engineered chimeric ribosomes by "humanizing" yeast 18S rRNA exclusively in the distal part of this specific ES. We show that such humanized ribosomes are sufficient to specifically reconstitute Hoxa9 IRES binding to the ribosome, but not other Hoxa IRES elements or the HCV IRES. These findings indicate a defined interplay of mRNA elements with the core ribosome for translation regulation and reveals a role for the evolution of the translation machinery itself, particularly its rRNA content, in guiding transcript-specific translation control in higher eukaryotes.

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86 Global Identification of Cap-Independent Translation Initiation Sites in Human Transcriptome

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Translation of most eukaryotic mRNA is dependent on 5'-cap structure. However, some mRNAs can initiate translation in a cap-independent fashion using IRES. Circular RNA (circRNA) is an RNA generated through back-splicing. Recently we found circRNA can function as mRNA to produce protein from IRES through cap-independent translation. Since circRNAs do not have 5' or 3' end, naturally they present a good system to measure IRES activity. Here, we developed a new circRNA-based system for transcriptome-wide screen of endogenous IRESs. Using a circRNA reporter encoding GFP, we firstly generated a library of short fragments (100-300 nt) from normalized human cDNA before the start codon, and used this library to produce millions of stably transfected clones. Subsequently we used FACS to collect green cells, purified total RNA and conducted amplicon-seq to identify sequences that drive circRNA translation. Using this system, we identified >10,000 endogenous IRES-like sequences from 6,000 genes in human transcriptome. These sites are located throughout the mRNA (including 5'-UTR, 3'-UTR, and coding region) with sequences features distinct from canonical IRES, and thus were defined as Cap-Independent Translation Initiation sites (CITI). Computational analysis reveals that CITIs at 5' and 3'-UTR have different enriched motifs, indicating they may promote mRNA translation via different mechanisms. CITIs are also enriched in genes encoding ribosomal components and translation initiation factors, implying a self-regulating circuit. Further analyses suggested that the CITIs at 5'-UTR have high tendency to base-pair with the 18s rRNA, whereas CITIs in 3'-UTR can promote translation in a context independent fashion. We found that CITIs enriched in genes with a possible downstream ORF, and can promote translation of the dORF and the upstream main ORF in a cap-independent fashion. Taking together, our data reveals a large amount of endogenous cap-independent translation in human transcriptome, suggesting a mechanism to diversity proteome through alternative translation. The proteins produced from alternative translation probably play diversity roles in responding to different stress conditions. These findings may also help us to better understand the relationship between translation regulation and diseases.

87 Translation elongation dynamics by ensemble cryo-EM

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During translation elongation, amino-acyl-tRNA is delivered to the ribosome as a ternary complex with EF-Tu and GTP. Translation accuracy is achieved by two stages of selection to match the tRNA anticodon and the mRNA codon, separated by GTP hydrolysis. We recently described an ensemble of cryo-EM structures of the first stage, the initial selection of tRNA (Loveland et al. Nature. 2017). We found that EF-Tu first binds to the 30S shoulder domain and away from the GTPase activating sarcin-ricin loop. Next, the decoding-center residue G530 stabilizes the matched codon:anticodon helix and facilitates movement of the shoulder domain of the 30S subunit. This "domain closure" brings EF-Tu to the sarcin-ricin loop for GTPase activation. By contrast, tRNA accommodation, which follows GTP hydrolysis and is critical for translation fidelity and peptidyl transfer, has not been structurally characterized. We will present our current work to visualize the structural dynamics of the latter steps of tRNA acceptance.

88 mRNA modifications alter translation elongation and fidelity

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Chemical modifications of RNAs have long been appreciated as key modulators of non-coding RNA structure and function in cells. However, it has only recently become apparent that such modifications are also widely distributed in the coding sequences of mRNAs, which direct protein synthesis. It is broadly believed that the modification of mRNAs may serve as a gene regulation mechanism because the enzymatic incorporation of mRNA modifications has the potential to modulate mRNA stability, protein-recruitment, and translation in a programmed manner. We tested how two of the most prevalent modifications present in mRNA coding sequences, pseudouridine (Ψ) and N6-methyladenosine (m^6A), impact protein synthesis using a fully-reconstituted *E. coli* translation system. Our work reveals that replacing a single nucleotide with Ψ or m^6A in an mRNA codon perturbs how the ribosome decodes an mRNA. For example, the presence of Ψ not only reduces the rate of amino acid incorporation, but also promotes the synthesis of multiple peptide products from a single mRNA sequence due to ribosome miscoding. Given the emerging importance of mRNA modifications we also explored the possibility of uncovering new modifications. We used an ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method to identify ten previously unknown modifications present in mRNAs of yeast cells. Moreover, we measured the levels of 46 modifications in parallel and found eleven mRNA modifications whose levels change in response to various cellular stresses. Together, our studies provide support for the provocative hypothesis that chemical modifications in mRNA could potentially provide a distinct way for cells to quickly and directly regulate and alter protein production.

89 Regulated translation elongation as a mechanism of differential protein synthesis during stress

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Protein translation is regulated at the stages of initiation, elongation, and termination. A common response, essential to cellular survival during adverse environmental conditions, is the suppression of overall translation during stress. Translation initiation has traditionally been thought of as the key control step of translation across a variety of stresses. Surprisingly, we find that during glucose starvation in yeast, differential translation elongation is a crucial mechanism that allows preferential translation of select genes during stress. For a number of growth mRNAs that are present pre-stress, we find similar levels of ribosomes present as well-translated stress genes, but minimal productive protein production from these ribosomes. While these growth mRNAs have similar overall ribosome occupancy compared to these well-translated stress mRNAs, their distribution of ribosomes is skewed towards the 3' end of the ORF. Secondly, we have found that a subset of transcriptionally upregulated mRNAs have high rates of translation initiation, but poor translation elongation, and therefore low protein production. Though these stress-induced mRNAs have low translation rates during glucose starvation, they are primed for translation upon carbon source readdition. We are currently exploring the role that various factors have in regulating translation elongation during glucose starvation. We are also trying to computationally model ribosome distribution across transcripts to infer dynamic ribosome movements from static ribosome profiling measurements during stress. Finally, we speculate that regulating translation through elongation may allow cells to more dynamically control protein synthesis during fluctuating environmental conditions rather than solely using differential initiation.

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90 Structure of a pre-handover mammalian ribosomal SRP•SRP receptor targeting complex

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Signal recognition particle (SRP) is a ribonucleoprotein complex that co-translationally targets membrane and secretory proteins to the endoplasmic reticulum (ER). SRP recognizes the ribosome synthesizing a signal sequence and delivers it to the SRP receptor (SR) on the ER membrane. Transfer of the signal sequence to the translocon is coupled to the GTP-dependent conformational change in the SRP and SR. Finally, SRP and SR hydrolyze the GTP and dissociate from the ribosome. Currently, we have the most complete mechanistic understanding of the membrane targeting process by SRP and SR for bacterial system. On the other hand, eukaryotic SRP and SR have eukaryotic-specific components, whose functions remain unclear. Here, we present the cryo-EM structure of the mammalian translating ribosome in complex with SRP and SR in a conformation preceding signal sequence handover, primed for the interactions with the translocon. This structure visualizes all eukaryotic-specific SRP and SR components and reveals their roles in stabilizing this conformation by forming a large protein assembly at the distal site of SRP RNA. Furthermore, we observe interactions between eukaryotic-specific components of SRP proteins with the GTPase active site of SRP•SR. We provide biochemical evidence that these eukaryotic-specific components of SRP and SR involve the regulation of GTP hydrolysis of SRP•SR.

91 mRNA secondary structures allosterically bias ribosomes into a kinetically altered slower pathway

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During protein synthesis, the ribosome translocates along messenger RNA (mRNA) in one-codon steps, catalyzed by the GTPase activity of elongation factor EF-G. mRNA secondary structures represent mechanical barriers that must be opened prior to translocation since the mRNA entry pore on the ribosome can only accommodate single-stranded RNA. Several positively charged amino acid side chains located at the entry pore facilitate this opening. Whether the ribosome disrupts the secondary structure at the mRNA entry site prior to or concomitantly with translocation, remains unclear. Moreover, the mechanism by which mRNA secondary structures regulate the rate of translation is not known.

Here, using high-resolution optical tweezers with single molecule fluorescence capability, we monitor in parallel hairpin opening (tweezers signal) and EF-G binding (fluorescence signal). We find that EF-G arrival precedes the opening of the mRNA hairpin, while EF-G release occurs after the hairpin is opened, providing direct evidence that the unwinding action of the ribosome is concomitant with translocation. Surprisingly, a strong mRNA hairpin barrier does not decrease the rate of EF-G catalyzed unwinding, nor results in futile catalysis events of EF-G; rather, it biases ribosomes *prior to EF-G binding* into an alternative 10-fold slower pathway that results in a dramatic reduction of the global translation rate in bulk.

Furthermore, the co-temporal detection of tweezers and fluorescence channels reveals that occasionally the hairpin is opened in two successive sub-codon steps while EF-G remains bound to the ribosome. Current models propose that translocation is driven by the rotation of the small subunit head domain. These observations suggest that in the presence of the barrier, this rotation might occur in two steps that can be resolved only when they happen slowly. Our results establish a strict coordination between the helicase activity and translocation, and find that the presence of an RNA barrier induces an allosteric change in the ribosome that dramatically reduces the average translation rate.

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92 Accurate design of translational output by a neural network model of ribosome distribution

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Synonymous codon choice can have dramatic effects on ribosome speed, RNA stability, and protein expression. Ribosome profiling experiments have underscored that ribosomes do not move uniformly along mRNAs, exposing a need for models of coding sequences that capture the full range of empirically observed variation. We present a method, Iχnos, that models this variation in translation elongation using a feedforward neural network to predict the translation elongation rate at each codon as a function of its sequence neighborhood. Our approach revealed sequence features affecting translation elongation and quantified the impact of large technical biases in ribosome profiling. We applied our model to design synonymous variants of a fluorescent protein spanning the range of possible translation speeds predicted with our model. We found that levels of the fluorescent protein in yeast closely tracked the predicted translation speeds across their full range. We therefore demonstrate that our model captures information determining translation dynamics in vivo, that we can harness this information to design coding sequences, and that control of translation elongation alone is sufficient to produce large, quantitative differences in protein output.

93 Monitoring protein synthesis in time and space with ribosome profiling

Jonathan Weissman
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I will talk about recent applications of ribosome profiling including: the identification of novel protein coding regions, demonstration of the principle of proportional synthesis of subunits in multiprotein complexes and monitoring localized protein translation.

94 Full-length characterization of transcript isoforms to investigate cancer-associated mutations

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Mutations in the splicing factor SF3B1 in various cancers have been associated with characteristic alterations in splicing. SF3B1 is one of the most frequently mutated genes in chronic lymphocytic leukemia and is associated with poor patient prognosis. While alternative splicing patterns caused by mutations in SF3B1 on a junction-level have been observed, these patterns have not been systematically examined on an isoform-level. As such, we have resequenced cDNA from CLL samples with and without K700E mutation in SF3B1, as well as a normal B cell with nanopore sequencing technology. By converting changes in current caused by blockage of DNA threading through a nanopore into sequence, nanopore sequencing can sequence complete molecules of DNA that exceed 200 kb in length. We have developed a novel workflow to perform isoform-level differential splicing analyses leveraging the full-length transcript data that nanopore affords. We report results from nanopore sequencing data that are concordant with known SF3B1 biology from short read sequencing as well as altered splicing patterns more confidently observed using long reads. Splicing analysis of nanopore reads between the SF3B1WT and SF3B1K700E identifies alternative upstream 3' splice sites caused by SF3B1K700E . In addition, we observe a relative decrease in the expression of genes with intron retention events in CLL samples compared to B cell. We also find an enrichment of intron retention events in SF3B1WT relative to SF3B1K700E and no enrichment between CLL SF3B1MT and B cell, suggesting an aberrant intron retention landscape in CLL samples with unmutated SF3B1. Gene ontology analyses reveal enrichment in antigen processing terms in genes associated with intron retention events, and we postulate that many of these are detained introns of transcripts that are kept in the nucleus. With full-length cDNA sequence, we are also able to better detect premature termination codons and estimate the proportion of transcripts subject to nonsense-mediated mRNA decay. As nanopore sequencing has yet to become a routine tool for characterization of the transcriptome, our work demonstrates the utility of nanopore sequencing for cancer and splicing research.

95 Deciphering the splicing code of coordinated RNA binding proteins PSI and hrp48 in sculpting the Drosophila transcriptome

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Alternative pre-mRNA splicing (AS) is a major gene regulatory mechanism in eukaryotes that greatly expands proteomic diversity and serves as a crucial determinant for cell fate and identity. AS is under stringent regulation by a constellation of *trans*-acting RNA-binding proteins that interact with *cis*-elements embedded in pre-mRNA sequences, forming the "splicing code". Although the splicing code of many individual RNA-binding proteins has been revealed, it remains elusive how multiple splicing regulators act coordinately to define tissue- and developmental stage-specific cellular AS profiles. Previously, we found that two Drosophila splicing regulators, PSI and hrp48, co-regulate the germ-line specific AS of the P-element transposon pre-mRNA. Here, we investigated the global cooperative regulation of PSI and hrp48 in sculpting the AS of the Drosophila transcriptome. Transcriptome-wide differential AS analysis revealed that PSI and hrp48 co-regulate the AS of ~650 target gene transcripts in Drosophila S2 cells, and exhibit three distinct modes of control: 1) an additive mode—the effect of PSI and hrp48 on the target transcripts is enhanced by each other; 2) a synergistic mode - PSI and hrp48 act coordinately to affect the AS of distinct target transcripts that is not achieved by either protein alone; and 3) a competitive mode – PSI and hrp48 exhibit opposite effects on the AS of the target transcripts with one of the two proteins holding a dominant regulatory role. *In vivo* iCLIP RNA binding assays of the PSI and hrp48 proteins identified thousands of binding sites for each protein, respectively. Interestingly, ~60% of co-regulated AS target transcripts of the two proteins showed strong co-localized PSI and hrp48 binding patterns in the vicinity of the alternatively spliced regions, especially adjacent to alternatively spliced 5' splice sites. This work reveals a complex and interwoven regulation of PSI and hrp48 in affecting alternative 5' splice site decisions and AS profiles in the Drosophila transcriptome. These results provide new insights into the splicing code by multiple coordinated AS regulatory proteins.

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96 Structural and functional mechanism of alternative splicing regulation of the immune cell protease MALT1

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Activation of CD4+ T-cells by concerted ligation of the T-cell receptor (TCR) and the CD28 co-receptor drives adaptive immune responses. The MALT1 paracaspase encoded by the MALT1 gene plays a key role in the cellular signaling pathways that promote T-cell activation. The MALT1 pre-mRNA was recently shown to express two isoforms, MALT1A and MALT1B, which include or exclude exon 7, respectively. TCR engagement induces alternative splicing and thereby an increase of MALT1A expression in activated CD4+ T-cells, which ultimately augments optimal T-cell activation. The splicing factors hnRNP U and hnRNP L were identified to exert antagonistic roles in MALT1 alternative splicing. Whereas hnRNP U suppresses inclusion of exon 7, hnRNP L promotes inclusion.

Here, we study structural and functional mechanisms of alternative splicing regulation of MALT1 exon7 by hnRNP U and hnRNP L. By correlating *in vitro* binding studies using gel shift assays, ITC and NMR with cellular splicing data using an exon7 minigene, we have mapped several intronic hnRNP U binding sites 3' and 5' to MALT1 exon 7. Furthermore, competition-binding assays with hnRNP U and hnRNP L reveal that these proteins compete for MALT1 pre-mRNA, consistent with their antagonistic roles in T-cell activation. Using SHAPE chemical probing, we determined the secondary structure of a functional MALT1 minigene and mapped hnRNP U (and hnRNP L) binding sites. We have identified secondary structured motifs in the RNA that have physiological relevance in T-cells, as confirmed by mutational analysis. Moreover, we have mapped regions in hnRNP U that bind to *cis*-regulatory motifs in MALT1 pre-mRNA and promote exon7 skipping and are currently studying the underlying structural mechanisms using NMR and X-ray crystallography. We propose a model where hnRNP U controls MALT1 exon 7 splicing by binding to the pre-mRNA. Thus, our data indicate that optimal T-cell stimulation is orchestrated by differential activity of splicing factors in activated T-cells.

97 RBM5 and RBM10 interact with components of the 17S U2 snRNP

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RBM5, 6 and 10 are a family of RNA-binding proteins with a common domain organization that appear to function as tumor suppressors since they are found to be mutated in lung cancers and to regulate exons involved in tumor progression. The best characterized family member, RBM5, was found to interact with splicing factors, including the U1 and U5 snRNP-specific proteins SNRNP70, PRP8, and SNRNP200, as well as PRPF19, and DHX15. The OCRE domain of RBM5 was found to bind the Sm core protein SNRPB. These studies used nuclear extracts or cell lysates that may not reflect the interactions of this splicing regulator when it is engaged with pre-mRNA.

Much of pre-mRNA splicing occurs while the primary transcript is associated with chromatin. To assess how interactions of spliceosome components and splicing regulators might differ in this cellular compartment from the soluble nucleoplasm, we examined proteins pelleting with chromatin and other high molecular weight nuclear material in mouse brain. We found that RBM 5/6/10 exist in large complexes resistant to RNase treatment that are not seen in the nucleoplasm.

To isolate the RBM5/6/10 complexes from this fraction, we generated isogenic 293Flp-In cell lines conditionally expressing Flag-tagged versions of each protein. We extracted proteins from the nucleoplasm and the high molecular weight nuclear pellet using DNase and/or RNase. We found that RBM5 and RBM10 copurify with components of the 17S U2 snRNP including SF3a and SF3b, U2SURP/SR140, DHX15/PRPF43, and RBM17/SPF45. Other previously identified RBM5 interactors copurified only in trace amounts from these extracts.

Surprisingly, we found RBM5 efficiently coprecipitated with the U2 snRNA. RNase treatment was required to extract this novel RBM5/U2 snRNP from the high molecular weight material, but the U2 snRNA remained partially protected in the RBM5 but not the RBM10 complex. The RBM6 coprecipitated a set of proteins different from that of RBM5/10, and did not appear to interact with spliceosomal snRNAs.

These data demonstrate the existence of novel protein and RNP complexes containing the RBM5/6/10 family of splicing regulators. The interaction of these proteins with the U2 snRNP provides new clues to how it can alter exon inclusion.

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98 Cdc2-like kinases 1/4 (CLK1/4) act as cellular thermometer to control body temperature dependent rhythmic alternative splicing in mammals

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We recently reported that body temperature cycles control a rhythmic alternative splicing program in mammals (Preußner et al. 2017). Body temperature cycles drive rhythmic SR protein phosphorylation resulting in a concerted splicing switch in a large group of functionally related genes. However, the temperature sensing mechanism upstream of SR protein phosphorylation remains elusive.

In the present study we have identified the cellular thermometer that is sensitive enough to use subtle changes in body temperature as input to produce altered alternative splicing as output.

The phosphorylation state of SR proteins is controlled by an interplay of kinases and phosphatases, where SR proteins are hyperphosphorylated in colder temperatures and vice versa in heat. Overexpression and knockdown experiments suggested a prominent role of Cdc2-like kinases 1 and 4 (CLK1/4), known SR protein specific kinases, in regulating temperature dependent SR protein phosphorylation, which led us to investigate the role of these kinases in more detail.

Using recombinant CLK1/4 proteins in in vitro kinase assays revealed extremely sensitive temperature-dependent CLK1/4 activity. Consistent with higher SR-protein phosphorylation at lower temperatures, CLK1/4 autophosphorylation as well as phosphorylation of an RS-repeat was more efficient at lower temperatures. Remarkably, we observe a strong linear increase of auto- and substrate phosphorylation within a temperature range of 2°C between 38°C and 36°C, reflecting the physiological circadian body temperature range in mammals. To further validate the temperature sensing function of CLK1/4 we generated HEK293 knock out cell lines using CRISPR/Cas9. Simulated body temperature cycles revealed strongly reduced temperature-regulated alternative splicing, confirming that CLK1/4 act as cellular thermometer connecting body temperature with alternative splicing. The extreme sensitivity of CLK1/4 to changes in the physiologically relevant temperature range and its surprising increase in activity at lower temperatures provide a mechanism for time of the day dependent splicing regulation in mammals.

Our current work is focused on investigating the structural basis and the evolutionary conservation of CLK1/4 temperature sensitivity.

99 Plant hnRNPs participate in light-regulated alternative splicing

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Alternative splicing (AS) is a widespread mechanism in eukaryotes that generate 2 or more mRNAs from the same pre-mRNA by using different splice sites. Splice site selection is largely influenced by splicing regulators like serine/arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs). Splicing regulators recognize regulatory *cis* elements to recruit spliceosomal components and initiate splicing reactions. Binding affinities to regulatory *cis* elements, differential expression and post-translational modification of splicing regulators together diversify AS patterns and tremendously increase transcriptome complexity and proteome diversity. Although the molecular mechanism of AS has been extensively studied in plants, whether and how it is modulated is still little elucidated.

We have recently performed mRNA sequencing to analyze transcriptome changes during light exposure in *Physcomitrella patens* and *Arabidopsis thaliana*. Our genome-wide analyses have showed that light induces intensive AS. Intron retention is rapidly induced by light but misregulated in knockout mutants of red/far-red light sensing phytochromes, suggesting the involvement of photoreceptors in splicing regulation. In *Physcomitrella*, we found phytochrome 4 displays a red light-dependent interaction with two hnRNPs *in vitro* and *in vivo*. Phenotypes of over-expression and knockout lines also revealed the involvement of hnRNPs in photomorphological control. Moreover, over-expressing and knocking out the two hnRNPs differ AS pattern globally, confirming their involvement in splicing regulation. We therefore propose that when plants expose to light, AS is rapidly fine-tuned to modulate certain metabolic processes and regulate gene expression. Phytochromes directly participate in regulation of AS through interacting with hnRNPs to control splicing activity.

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100 Splice-Switching Antisense Oligonucleotides to Correct CLN3 Gene Expression in Juvenile Batten Disease

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Splice-switching antisense oligonucleotides (SSOs) have emerged as effective tools for modulating pre-mRNA splicing, as well as powerful therapeutics for the treatment of disease. We have devised a splice-switching approach to investigate a potential therapeutic approach to treat Juvenile Batten disease also known as juvenile neuronal lipofuscinoses (JNCL). JNCL is a rare and fatal neurodegenerative disorder that presents with vision loss and seizures between 4-10 years of age and is quickly followed by loss of motor function, dementia, and premature death in the teens to twenties. JNCL is a lysosomal storage disease caused by mutations of CLN3, most commonly a 1.02 kb deletion encompassing exon 7 and 8 (CLN3Δex78). This deletion disrupts the mRNA open reading frame and creates a premature termination codon, which results in the production of a truncated, non-functional protein. There are currently no effective treatments for JNCL. We have designed SSOs that induce splicing changes that correct the CLN3Δex78 reading frame to restore partial protein function with the goal of improving disease related deficits. We have tested and identified SSOs that correct the CLN3Δex78 reading frame by skipping one of two different exons. Injection of these SSOs into the brain of neonatal mice with the common JNCL mutation efficiently targets splicing and improves motor deficits in these Batten mice. Together our results suggest that reading frame correction may be a potential therapeutic for juvenile Batten disease.

101 *HRAS* exon 2 is a vulnerable exon which can be targeted by SSOs to abolish RAS activity in cancer cells

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Human RAS genes (*HRAS*, *KRAS* and *NRAS*) encode small GTPases functioning as molecular switches, which controls signaling pathways crucial for proliferation, growth and survival of the cells. Whereas aberrant regulation of RAS in somatic cells usually leads to cancer, germline mutations lead to severe congenital syndromes. Approximately 30% of all cancers harbor activating RAS mutations affecting the codons for glycine 12 or 13 of exon 2, causing translation of a constitutively active RAS protein. We have recently reported a c.35_36GC>TG dinucleotide variation (p.Gly12Val) that abolishes an exon splicing enhancer (ESE) and generates an exon splicing silencer (ESS), causing skipping of HRAS exon 2, thereby attenuating the phenotype in a patient with Costello Syndrome. In the present study we employ *HRAS*, *KRAS* and *NRAS* minigenes and swapping of the 3'splice site to investigate the strength of exon 2 in all three RAS genes. We report that HRAS has the weakest exon 2, due to an intrinsically weak 3' splice site, in particular due to the presence of a GGG triplet which functions as an intronic hnRNPF/H binding splicing silencer. We performed a Splice Shifting Oligonucleotide (SSOs) walk covering the entire HRAS exon 2 and employed *HRAS* minigenes with serial deletions to delimitate new potential splicing regulatory elements (SREs). Using this approach we identified a new SRE region and confirmed the importance of the previously identified ESE located in codon 12 and 13. Based on the localization of the essential SREs, we have designed new SSOs, which successfully mediate exon 2 skipping in T24 bladder cancer cells harboring the p.G12V mutation. The SSO induced exclusion of HRAS exon 2 disrupts HRAS protein function and causes a decrease in proliferation and/or cell death.

To obtain more efficient exon skipping, we further improved the SSOs by attaching a nucleotide tail containing ESS motifs, shown to inhibit *HRAS* exon 2 inclusion. This ESS motif recruits the splicing inhibitory factors hnRNP F/H thereby further increasing the level of exon 2 skipping. We hope that our SSO based approach can be further developed into a future potential therapy.

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102 Proteome-wide and quantitative identification of RNA-dependent protein complexes – a novel concept to discover unexpected functions of RNAs

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Functional non-coding RNAs (ncRNAs) are often identified by their regulation or impact on cellular phenotypes. However, deciphering associated molecular mechanisms and interaction partners remains the biggest challenge in ncRNA research. In turn, RNA-binding proteins (RBPs) have been catalogued in large-scale studies by RNA pulldowns with modest overlap, raising questions about their specificity.

Here, we introduce the concept of RNA-dependence to overcome these challenges. We define a protein as RNA-dependent if its interactome (hence likely its function) depends on RNA without necessarily directly binding to RNA. Experimentally, RNA-dependent proteins can be identified by their apparent molecular weight in presence or absence of RNA. This concept offers important benefits:

1) RNA-dependent proteins can be identified specifically, proteome-wide and unbiasedly without specific enrichment strategies.
2) Novel RNA functions can be discovered from RNA-dependent proteins not previously linked to RNA.
3) Our approach yields quantitative data establishing which fraction of every protein is in an RNA-dependent complex.
4) Our screening approach can aid in the reconstruction of protein complexes based on co-segregation.

We identified proteins whose molecular interactions depend on RNA by density gradient fractionation in presence or absence of RNA and proteome-wide quantitative mass spectrometry. The screen was performed in triplicate of 25 protein fractions each in the presence or absence of RNase. Statistical analysis identified 1784 significantly RNA-dependent human proteins. 651 of these proteins were never found as RBP before. Vice versa, 1125 proteins previously claimed as RBPs showed no signs of RNA-dependence, highlighting the need for specificity. This powerful resource will be available at www.RdPC.dkfz.de.

Taking advantage of the quantitative nature of our approach and depending on the RNA-dependent fraction of protein, we classified each protein as partially, transiently or fully RNA-dependent. A notable example of the latter is the transcription factor CTCF, which was known to bind to RNA next to DNA. Surprisingly, all CTCF appeared RNA-dependent and removal of RNA led to a loss of CTCF interaction with chromatin.

In summary, we introduce the concept of RNA-dependence to discover novel RNA functions and provide a proteome-wide, specific and quantitative identification of proteins and complexes whose interactions depend on RNA.

103 Using customizable Pentatricopeptide Repeat (PPR) proteins as affinity tags to purify specific RNPs for proteomic analysis

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Pentatricopeptide repeat (PPR) proteins are a large family of helical-repeat RNA binding proteins that influence gene expression in mitochondria and chloroplasts by binding specific RNA sequences. Similarly to PUF proteins, each repeat binds a single nucleotide, and nucleotide specificity is determined by the identities of two amino acids in each repeat, which make hydrogen bonds with the Watson-Crick face of the cognate nucleotide. Unlike PUF proteins, however, the number of PPR motifs in native PPR proteins is variable, ranging from two to approximately 30; thus, the PPR scaffold may allow for greater flexibility in tailoring sequence specificity and affinity for specific purposes. Synthetic consensus PPR motifs have been designed by other groups based on the population of PPR motifs found in *A. thaliana*, and were shown to bind *in vitro* with some specificity for cognate RNA sequences, as predicted by the amino acid "code". We have built on these advances by (i) comprehensively analyzing the RNA binding specificity landscape of synthetic PPR tracts using a bind-n-seq approach; and (ii) using these principles to design proteins for specific *in vivo* applications. In one application, we designed synthetic PPRs to bind the 3' UTR of the chloroplast *psbA* mRNA, an mRNA that exhibits interesting translational control in response to shifting light conditions. We are using the engineered proteins as affinity tags to pull down *psbA* RNPs, as a resource for targeted proteomic analysis to identify potential translational regulators. We have determined that these synthetic PPRs bind specifically to the 3' UTR of *psbA in vivo* and that they coimmunoprecipitate with a known translation regulator of *psbA*. Mass spectrometry analysis of these coimmunoprecipitates is now in progress.

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104 An enhanced hybridization capture approach reveals different modes of lncRNA spreading on chromatin

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Chromatin modifying complexes spread across thousands of nucleotides to regulate gene expression by catalyzing reactions such as the modification of histone tails. Some of these complexes associate with lncRNAs, including those that regulate dosage compensation in mammals and flies where lncRNAs drive changes in gene expression across an entire chromosome. In male flies, the roX2 lncRNA assembles with proteins into the Male-Specific Lethal (MSL) complex that binds to high-affinity sites (HAS) on X chromosome. From these sites, the MSL complex spreads and upregulates the neighboring active genes enriched in H3K36me3 mark. Previous reports have suggested that HAS are positioned near chromatin TAD boundaries that might help to localize the MSL complex to these sites. However, the mechanism that enables efficient spreading to distal regions and the dynamics of this process are still poorly understood. This is partly because the specificity of ChIP and hybridization capture approaches (CHART and ChIRP) are not sufficient to detect the relatively low levels of MSL complex at these regions. Here we report an improved hybridization capture approach that combines enhanced nucleotide chemistry with DNA nanotechnology and provides vastly improved specificity of lncRNA enrichment. In our hands, >80% of the roX2 signal originated from chromosome X compared to only ~30% in ChIP/CHART/ChIRP. This improvement in signal enabled accurate detection of the roX2 spreading pattern, supporting models that demonstrated HAS are near TAD boundaries, but also revealing that MSL complex spreads to genes distant from HAS that are marked by insulator elements. These results suggest a model for how 3D chromatin structure leads to MSL spreading in chromatin. Using this improved hybridization capture approach, we observed surprisingly rapid changes in MSL complex spreading and distribution under acute stress conditions, revealing unexpected regulation of the localization and spreading of this lncRNA complex.

105 Biophysical and biological implications of a quantitative and comprehensive model for RNA binding by human Pumilio proteins

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Advances in high-throughput sequencing technologies have led to an avalanche of information about molecular interactions in gene regulation; it is now routine to generate target sets and motifs for RNAs bound by RNA-binding proteins. Nevertheless, motif and related representations of binding landscapes have known limitations, as they do not capture the full range of targets and possible binding modes and can overestimate interconnectivity of RNA/protein interaction networks. The next generation of models require quantitative thermodynamics and kinetics, as such information is needed to progressively build and test predictive models for the vast numbers of cellular interactions. We have used the RNA-MaP platform, which allows direct, parallel equilibrium binding measurements of 10^4–10^5 unique RNAs, in conjunction with a rationally designed RNA library, to obtain a complete and predictive thermodynamic model for RNA recognition by the human Pumilio proteins, PUM1 and PUM2. Despite the modular construction of Pumilio proteins and the prior findings of linear motifs, our >10,000 independent affinity measurements revealed widespread nonlinear binding sites and instances of positional coupling. Our model accounts for PUM1/2 binding well within 2-fold over >4 logs of affinity, reveals identical binding specificities for PUM1 and PUM2, and demonstrates that the altered affinities of a reengineered PUM1 for one-third of tested RNAs can be traced to a change in a single energy term in our binding model. Application of our quantitative in vitro binding model to published in vivo crosslinking data is consistent with thermodynamically-driven RNA occupancy, providing a key early step in developing quantitative models of cellular Pumilio/RNA interactions. Nevertheless, further controls and experiments are needed to ensure accuracy of crosslinking-based occupancies and to probe potential variations imposed by cellular factors. Most importantly, our broadly applicable approach allows systematic and stepwise testing of these and other models using a high-throughput, quantitative feedback loop of in vitro and cellular experiments.

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106 Modeling and assessing the CRISPR-Cas9 off-targeting potential by nucleic acid duplex interactions

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The bacterial CRISPR-Cas9 system has become a very popular tool for genomic editing. With its increasing use off-target cleavage has also received increased attention. Although this problem is recognized and several experimental methods have been proposed to detect off-targets, computational strategies seem to be more feasible. Current computational methods predict off-targets for all genomic locations matching the complement of the spacer in the guide RNA (gRNA) with up to a maximum number of allowed mismatches, by assigning a score for each such potential gRNA off-target position. Although these methods include machine learning strategies, surprisingly none of them take directly advantage of the full nucleotide duplex energies including those covering RNA-DNA interactions. Here, we present a model for CRISPR-Cas9 taking a range of nucleotide interactions into account, including approximated free energy parameters for RNA-DNA interactions, the possible intramolecular interactions of the gRNA, and the opening energy of the DNA-DNA region bound to the spacer of the gRNA. We score each potential off-target and we show that this score much more strongly correlates (r=0.43) with experimental data for CRISPR-Cas9 off-target activity than the scoring by other methods. This off-target score, CRISPRoff, is further benchmarked on available experimental data and we obtain a higher performance than the existing methods; for example at a false positive rate of 0.1, our true positive rate of 0.94 is significantly higher than that of any other method. In complement to scoring each of the potential off-target sites, we furthermore compute a score for the individual gRNA's off-target potential on a genome-wide scale. This specificity score, CRISPRspec, is calculated as the ratio of the Boltzmann-weighted ensemble of all off-targets to the Boltzmann-weighted ensemble of all off-targets and the on-target location. We find consistency with published experimental data, where a high CRISPRspec score is in agreement with low read coverage of the off-target regions and a low CRISPRspec score is in agreement with high read coverage. In conclusion, our nucleotide duplex energy based model for CRISPR-Cas9 off-targeting provides substantially higher correlation to the data and at the same time yields the state-of-the-art prediction of off-targets.

107 Heavily and Fully Modified RNAs Guide Efficient Cas9-Mediated Genome Editing

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RNA-based therapeutics provide an exciting new path towards the treatment of genetically defined disorders. RNA-based drugs have progressed into late-stage clinical trials and some have been approved for treatment of human diseases. The success of these drugs is largely a result of advances in the chemical modifications of oligonucleotides. These modifications not only decrease their innate immunogenicity but are also essential for potency and nuclease stability *in vivo*, and facilitate the use of conjugated chemical groups that tune delivery and uptake properties in specific tissues. In addition to the benefits seen in siRNA and ASO based therapeutics, such chemical modifications can also be applied to crRNA and tracrRNA for CRISPR-Cas9, an RNA-guided microbial effector complex that cleaves DNA. This natural bacterial process can be exploited to target mutations in the human genome that lead to disease. In fact, several groups have used chemical modifications to improve the specificity and efficacy of crRNA and tracrRNA. However, there are no published studies that have extensively explored the tolerability of chemical modifications at all positions of crRNA and tracrRNA. Here, we have succeeded in synthesizing several heavily modified versions of crRNA and tracrRNA that are more efficacious than their unmodified counterparts. In addition, we also describe fully chemically modified crRNA and tracrRNA (with no 2'-OH groups remaining) that are functional in human cells. Because stability *in vivo* is one of the biggest hurdles to oligonucleotide therapeutic development, these designs demonstrate a significant breakthrough for Cas9-based therapeutics. We anticipate that our designs will improve the use of Cas9 via multiple delivery routes for *in vivo* and *ex vivo* purposes.

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108 Argonaute-based programmable RNase as a tool for cleavage of highly-structured RNA

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The recent identification and development of RNA-guided enzymes for programmable cleavage of target nucleic acids offers exciting possibilities for both therapeutic and biotechnological applications. However, critical challenges such as expensive guide RNAs and inability to predict the efficiency of target recognition, especially for highly-structured RNAs, remain to be addressed. Here, we introduce a programmable RNA restriction enzyme, based on a budding yeast Argonaute (AGO), programmed with cost-effective 23-nucleotide (nt) single-stranded DNAs as guides. DNA guides offer the advantage that diverse sequences can be easily designed and purchased, enabling high-throughput screening to identify optimal recognition sites in the target RNA. Using this DNA-induced slicing complex (DISC) programmed with guide DNAs designed to span the sequence, accessible sites were identified in the 352-nt human immunodeficiency virus type 1 5′-untranslated region. This assay was coupled with primer extension and capillary electrophoresis to detect all DISC-accessible sites simultaneously in a single reaction. Comparison between DISC cleavage and RNase H cleavage reveals that DISC not only cleaves solvent-exposed sites, but also sites that become more accessible upon AGO binding. This study demonstrates the advantages of the DISC system for programmable cleavage and investigation of highly-structured, functional RNAs.

109 How mRNA wraps the 30S at the beginning of translation

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"Well begun is half done." In the cell, controls of translation initiation are important for protein expression and cell function. Structure data have revealed the prokaryotic ribosomal small subunit, 30S, binds to Shine-Dalgarno sequence of the mRNA which then wraps around the 30S to form a pre-initiation complex (pre-IC) in the presence of initiator tRNA. However, detailed mechanism of such dynamic binding process is unresolved. Here, we used single molecular Förster Resonance Energy Transfer (FRET) and optical tweezers to observe the interaction in real time. First, mRNAs were tagged with Cy3 and Cy5 at two ends through complementary strands. When incubated with the 30S, the unstructured mRNA showed static FRET values that were similar to the fully assembled IC, whereas FRET values of structured mRNA had much faster and larger dynamics. If using mRNAs with weaker Shine-Dalgarno sequences, we found they had much less binding events. Binding of the initiator tRNA resulted in static FRET values and unfolding of downstream structures. For all above conditions, addition of initiation factors, IF1 and especially IF3, stabilized the binding of mRNA to the 30S. In addition, different structures such as simple duplex and complex structures showed in different 30S binding patterns and different chances for pre-IC formation. In conclusion, we found the mRNA should be single stranded for better initiation. If there exists a structure downstream the initiation site, the mRNA wraps and unwraps the 30S quickly until the initiator tRNA comes. Binding of initiator tRNA opens downstream hairpin and holds the single-stranded mRNA stably; this implies the mRNA entry site is closed. Finally, we demonstrated the initiation factors do help the mRNA wrapping to the 30S to a state similar to fully initiated IC. After summing up all above evidences, we could decipher the mechanism that how mRNA wraps the ribosome at the very beginning of translation.

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110 Probing Mechanisms of RNA Chaperones with a Bacterial Three-Hybrid Assay

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Non-coding RNAs regulate gene expression in every domain of life. In bacteria, non-coding small RNAs (sRNAs) help cells respond to stress and are often assisted by protein chaperones. These sRNAs base pair with target mRNAs to alter their translation and stability. Hfq is a well characterized RNA chaperone protein that, through RNA interactions on its hexameric structure, supports the function of many sRNAs. Hfq proteins have been identified in ~50% of sequenced bacteria; the fact that many bacteria that display sRNA-mediated gene regulation lack an Hfq homolog suggests they may possess additional RNA chaperones. In order to facilitate the discovery and characterization of RNA chaperone proteins, we have recently developed a bacterial three-hybrid (B3H) assay that detects the binding of an RNA and protein inside of living bacterial cells. This assay, conceptually analogous to yeast three-hybrid assays, couples the transcription of a genetic reporter to the interaction of a DNA bound "bait" RNA (*e.g.* an sRNA) and an RNAP-fused "prey" protein (*e.g.* an RNA chaperone protein such as Hfq). Successful interaction between RNA and protein stabilizes RNA polymerase at a test promoter and activates reporter gene expression.

The B3H assay was initially established with sRNAs and Hfq from *E. coli*. Current efforts are focused on extending the assay to less well characterized RNA-binding proteins and optimizing the signal of the B3H assay so that weaker RNA-protein interactions can be reliably detected. We have been able to detect sRNA-protein interactions of Hfq homologs from bacteria beyond *E. coli*, and are using the B3H assay to probe species-specific RNA recognition by Hfq. In addition, The B3H assay detects the interaction of the structurally distinct ProQ protein with several RNA partners. ProQ has recently been shown to bind to dozens of *E. coli* sRNAs and mRNAs and may act as a widespread regulator of bacterial gene expression. We are using the B3H assay to probe the molecular mechanisms of ProQ's interaction with sRNAs, mapping the interaction sites that contribute to binding and regulation. With an optimized B3H assay, we aim to discover new sRNA-binding proteins from diverse bacteria and uncover their regulatory roles.

111 When antisense makes sense: exploring the role of RNA polymerase-binding RNA aptamers in control of bacterial antisense transcription

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The pervasive activity of the *Escherichia coli* RNA polymerase (RNAP) leads to transcription not only of genes but also of yet non-annotated genomic regions. Such is the case of the antisense strand, which has been shown to be pervasively transcribed. Antisense transcription can impact on the expression of sense genes by several mechanisms, either transcriptionally (e.g. transcriptional interference, TI) or post-transcriptionally (e.g. ribosome occlusion, RNAse III degradation). Therefore, the RNAP activity on the antisense strand needs to be controlled to guarantee proper gene expression. *E. coli* has evolved two main ways of modulating antisense transcription: inhibiting transcription initiation by H-NS and promoting premature transcription termination by Rho. In addition to these mechanisms, we have explored the role of a novel class of RNA regulatory elements in modulating the RNAP activity on the antisense strand. We have identified a new class of RNA regulatory elements that modulate transcription in cis, either by leading to premature termination or antitermination. RNAP-binding RNA aptamers (RAPs) are relatively short RNA sequences (30-100 nts) binding to the RNAP with high affinity. There are approximately 15,000 RAPs 'encoded' in the *E. coli* genome and the majority (~60%) is found antisense to annotated genes (asRAPs). Thanks to a massive screening of thousands of these elements, we were able to identify hundreds of active asRAPs located at genomic positions where they could modulate antisense transcription, either inhibiting or promoting it. Using a reporter assay, we have demonstrated that they can indeed regulate TI happening from two convergent promoters. They are able to terminate transcription of the antisense strand, reducing TI and increasing the expression level of genes expressed from the sense promoter. We are currently exploring their impact on the sense expression at their particular genomic locations. Taking into account their large number, their location and their effect on transcription, we suggest that asRAPs are widespread modulators of transcription interference.

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112 Site-specific two-color labeling of long RNAs for single-molecule FRET

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Fluorescent labeling of long RNA molecules in a site-specific yet generally applicable manner is integral to many spectroscopic applications. By introducing a pair of organic fluorophores at strategic sites within the RNA, one can monitor conformational dynamics via FRET. Here, we propose a novel, covalent labeling method that is position-selective and scalable to long, intricately folded RNAs [1]. In this modular approach, a custom-designed DNA probe hybridizes to a target RNA and guides an *in situ* activated functional group to react with a sterically close adenine or cytosine residue [2]. The resulting etheno adduct carries an alkyne handle which can be coupled subsequently to an azide functionalized fluorophore. Concomitantly to this RNA-templated transfer, the 3'-terminal ribose of the target RNA is oxidized, offering room for a second dye to be incorporated. The bio-orthogonal chemistry of these two functional moieties makes the bioconjugation proceed without cross-reactivity.

We validate our labeling protocol using a B12-riboswitch of 240 nucleotides in length. In order to fluorescently tag an adenine buried within a duplex region, we temporarily and locally disrupt the secondary structure of the RNA using a pair of helper DNA strands that anneal up- and downstream of the modification site. The precision of the base modification is further demonstrated by selectively targeting one out of two adjacent adenines in a loop region. The integrity of the fluorophores and their motional flexibility within the RNA environment is evaluated by their fluorescence lifetime and dynamic anisotropy [1,3]. Native folding and function of the riboswitch in response to its metabolite is confirmed on the single-molecule level. We use FRET as a sensor to probe the conformational equilibrium of the riboswitch that is tuned through binding of coenzyme B12 [1]. Overall, our labeling strategy overcomes size and site constraints that have hampered routine production of labeled RNA beyond 200 nucleotides in length.

[1] M. Zhao, *et al.*, Nucleic Acids Res. 2018, 46, e13.
[2] D. Egloff, *et al.*, ACS Chem. Biol. 2016, 11, 2558-2567.
[3] F.D. Steffen, *et al.*, Phys. Chem. Chem. Phys. 2016, 18, 29045-29055.

113 Kinetic Selection of Spliceosome Substrates by the Yeast U1 snRNP

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One of the earliest steps in spliceosome assembly is the association of the U1 small nuclear ribonucleoprotein (snRNP) complex with the 5′ splice site (5′SS) through U1 snRNA-RNA base pairing, U1 protein-RNA interactions, and protein-protein interactions with other splicing factors. Many RNP complexes, including U1, utilize an RNA (e.g., miRNA, gRNA) as a "guide" to convey a high level of specificity for targets via Watson-Crick base pairing. The 5′ end of U1 snRNA is perfectly conserved between *S. cerevisiae* and humans in both sequence and post-transcriptional modifications, yet 5′SS sequences are degenerate and often include mismatches with U1 snRNA.

Using colocalization single molecule spectroscopy (CoSMoS), we investigated the influence of base pairing on RNA binding kinetics using both an RNA-only system that mimics the U1 snRNA and purified yeast U1 snRNP. We find that the U1 snRNA mimic forms a complex with RNAs, whose dissociation rate decreases as duplex strength increases. In contrast, purified U1 snRNP binds these same RNAs with multi-exponential dissociation kinetics, consistent with a multi-step mechanism for 5′SS recognition. Above a certain threshold, U1 binding lifetimes become independent of duplex strength. Instead, increased duplex strength leads to increased abundance of longer-lived complexes without changing their lifetime. Long-lived complexes require formation of duplexes ≥7 bp and are strongly dependent on pairing with the G(+1) and U(+2) nucleotides of the 5′SS. U1 binding is strongly biased towards substrates that meet the functional requirements for splicing catalysis. These results also likely explain the requirement for additional, trans-acting splicing factors (e.g., E complex formation) for stable U1 association on pre-mRNAs containing weak 5'SS. Overall, these kinetic properties are likely critical for allowing U1 to rapidly identify diverse 5′SS and to prevent U1 from becoming trapped on RNAs with high base pairing potential.

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114 Direct measurements of protein synthesis kinetics in live cells using fluorescently labeled tRNAs

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Our ability to directly relate results from biochemical experiments in the test tube to the dynamic processes occurring in living cells is very limited. We have recently developed new experimental and analytical tools to directly study the kinetics of fast biochemical reactions in live cells. In a pilot study, dye-labeled tRNAs were electroporated into *E. coli* cells, and tracked using super-resolved single-molecule microscopy[1]. Trajectories were analyzed by machine-learning algorithms, and we were able to monitor the dwell times of the tRNAs in different diffusional states. We find dwell times of $tRNA^{Phe}$ on ribosomes in perfect agreement with previous indirect measurements of translation rates. In experiments with $tRNA^{fMet}$, we find surprisingly short ribosome bound dwell times, suggesting that once fMet-$tRNA^{fMet}$ has bound to the small ribosomal subunit, initiation of translation is fast and does not limit the overall rate of protein synthesis.

Our new experimental and analytical tools are very general and should be readily applicable to any RNA of interest. We are now expanding our studies in several directions.

[1] Volkov et al, submitted

115 Real-time quantification of single RNA frameshifting dynamics in living cells

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We have developed technology to image single RNA translation dynamics in living cells. Using high-affinity antibody-based probes, multimerized epitope tags, and single molecule microscopy, we are able to visualize and quantify the emergence of nascent protein chains from single pre-marked RNA. In this talk, I'll describe this technology as well as a new multi-frame tag that extends the technology to enable real-time quantification of single RNA translation kinetics in any two of the three possible open reading frames. As a first application of the multi-frame tag, we use it to dissect the kinetics of the HIV-1 frameshift sequence. Whereas previous bulk assays have shown this sequence leads to ~10% frameshifted product, it was not clear if all RNA frameshift with ~10% efficiency or if instead ~10% of RNA frameshift with ~100% efficiency. Interestingly, our live-cell data suggest the latter scenario, where a small subset of genetically identical RNA frameshift with high efficiency. The origin of this heterogeneity is not yet clear, but experiments are beginning to implicate a ribosomal pause that leads to a higher than normal density of ribosomes near the frameshift sequence on frameshifting RNA.

116 Intracellular single particle tracking of miRNA induced silencing complexes and mRNAs reveals sub-stoichiometric, transient binding and induced target aggregation

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MicroRNAs (miRNAs) are small non-coding RNAs that guide the RNA induced silencing complex (RISC) to messenger RNAs (mRNAs), resulting in repression of translation. miRNA-loaded RISC is known to bind mRNAs at target regions bearing short, sequence complementary miRNA recognition elements (MRE). The degree of translation repression of target mRNAs is known to be proportional to the number of MREs present in the 3' untranslated region (UTR), however, the nature and immediate molecular consequences of miRISC-mRNA binding inside the cell are poorly understood. Here, we utilized a two-color version of intracellular single molecule high-resolution localization and counting (iSHiRLoC; Pitchiaya et al. 2012, 2017; Custer et al. 2017) to simultaneously track fluorescently labeled single miRISC and mRNA particles in live cells. These particles exhibit repeated, transient (sub-second timescale) interactions independent of the presence of any MREs. This observation supports the notion that miRISC finds an MRE on an mRNA in the crowded molecular environment of the cell largely by 3D search. Increasing the number of MREs in the 3' UTR increases the frequency and duration of these miRISC-mRNA interactions, resulting in stable binding but at sub-stoichiometric levels. Finally, miRISC binding induces mRNA targets with a large (up to 11) number of MREs to aggregate, consistent with the paradigm of intracellular phase separation of multivalent interactors. Taken together, our intracellular single molecule observations reveal features of early steps in RNA silencing.

117 Real-time single-molecule imaging of riboswitch dynamics during transcription elongation

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Cotranscriptional RNA folding is crucial for the timely and selective control of biological processes. Recently, single-molecule Fluorescence Resonance Energy Transfer (smFRET) has been unique in monitoring RNA structural dynamics using strategically positioned fluorescent dyes. However, since bacterial and eukaryotic RNA polymerases (RNAP) do not incorporate fluorescent nucleotides during transcription elongation, smFRET analysis of RNA structures within such transcriptional complexes has been impossible. Here, we describe an approach allowing smFRET studies of nascent transcripts within either stalled or actively transcribing Escherichia coli elongation complexes. We successfully used this approach to structurally characterize nascent transcripts of the thiamin pyrophosphate (TPP)-sensing tbpA riboswitch. We found that riboswitch TPP sensing is efficiently performed within a narrow transcriptional window, where the RNAP assists metabolite binding by directing nascent transcript folding. Furthermore, real-time monitoring of single elongating complexes revealed a conditions-specific cotranscriptional folding sequence. We also found a small fraction of records showing TPP-unresponsive nascent transcripts that are trapped between two structures, consistent with cotranscriptional riboswitch misfolding. Our approach could be used to study in real time RNA structures in various native prokaryotic or eukaryotic transcription machineries, thus allowing to obtain previously inaccessible biological data.

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118 An RNA Binding Protein that Directs Substrate Binding of an Adenosine-to-Inosine RNA Editing Enzyme *in vivo* and Expands the Number of Editing Sites in the Transcriptome

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Adenosine-to-inosine RNA editing alters generates the transcriptomic diversity required for normal development and proper neuronal function of all animals. Consistent with this important function, high-throughput sequencing studies have identified thousands of edited transcripts in animal transcriptomes. However, how ADARs, the enzymes that catalyze this reaction, determine which adenosines within a transcript to edit and the extent of editing are not understood. Herein, we identify a regulatory mechanism that enhances the ability of *Caenorhabditis elegans* editing enzyme, ADR-2, to both bind specific target mRNAs and edit. Our biochemical studies indicate that ADR-2 has a low affinity for RNA. However, ADR-2 physically interacts with ADR-1, a deaminase-deficient member of the ADAR family, that has a 100-fold higher affinity for RNA than ADR-2. Editing assays reveal that both RNA binding by ADR-1 and the protein-protein interaction between ADR-1 and ADR-2 promotes RNA editing efficiency. Unbiased analysis of ADR-2 binding across the transcriptome identified approximately 950 targets, of which 86% are known edited transcripts. Furthermore, ADR-2 binding analysis in the absence of *adr-1* indicates that ADR-1 is required for ADR-2 to stably interact with a majority of target mRNAs *in vivo*. We conclude that the interaction of ADR-1 with ADR-2 serves as an important mechanism for regulating the ability of ADR-2 to both bind target mRNAs and edit. These results raise the possibility that dimerization between ADAR family members, and perhaps protein-protein interactions of ADARs with other dsRNA binding proteins, provides the A-to-I editing enzymes with a mechanism to recognize specific targets over the thousands of RNAs present in the cell.

119 BioID identifies novel regulators of ADARs and A-to-I RNA editing

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Adenosine-to-Inosine (A-to-I) RNA editing is catalyzed by ADAR enzymes that deaminate adenosine to inosine, which is recognized as guanosine by the cellular machinery. Millions of RNA editing sites have been identified, and the editing levels of many of these sites are spatiotemporally regulated. Intriguingly, the ADAR enzymes are expressed throughout development in all tissues and their level of expression does not fully explain the editing frequency of large numbers of sites *in vivo*, suggesting that there are additional layers of regulation mediating the frequency of editing at specific sites, in specific tissues, and at specific developmental stages; however, few putative tissue-specific regulators of editing are known. In this study, we perform BioID (enzyme mediated, proximity dependent biotinylation and pulldown) followed by mass-spectrometry to identify both previously known and novel *trans* regulators of ADAR1 and ADAR2 in HeLa and M17 neuroblastoma cells. We validate and characterize a subset of the novel ADAR-interacting proteins as global or site-specific RNA editing regulators using datasets from the ENCODE project. We also identify a class of ADAR-interacting proteins that all contain DZF dimerization domains, ILF3, ILF2, STRBP, and ZFR. We show these proteins interact with ADAR1 and/or ADAR2 and modulate RNA editing levels. We further demonstrate that ILF3 is a negative regulator of editing that preferentially binds RNA near editing sites to alter editing levels. In addition, we connect ILF3's role as a regulator of editing to its role in circular RNA biogenesis by showing that its overexpression preferentially increases circular RNA expression near sites with altered editing. This work produces an expansive list of novel *trans* regulators of ADAR proteins that provides new insight into the mechanisms by which RNA binding proteins in particular act to regulate RNA editing levels.

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120 Regulation of A-to-I RNA Editome by ADARs-interacting Partner Death Associated Protein 3 (DAP3) in Human Esophageal Cancer

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Post-transcriptional modifications such as RNA editing generate transcriptome diversity and play important roles in human cancer initiation and progression. Current studies mostly focused on the impact of dysregulated ADARs expression and activity on adenosine-to-inosine (A-to-I) RNA editome in human cancers, but it is unclear how other regulatory mechanisms and non-ADAR regulators govern the editing machinery. It remains a field of further investigation to identify ADARs binding factors involved in the modulation of the editing machinery and study their functions in regulating the RNA editing profiles in cancer pathogenesis. In this study, we examined the role of Death Associated Protein 3 (DAP3), a binding partner of ADARs, in the modulation of the RNA editing process. Using a high-throughput RNA sequencing (RNA-Seq) method, the transcriptome-wide analysis of A-to-I RNA editing events regulated by DAP3 in human esophageal squamous cell carcinoma (ESCC) cells was conducted. Our study reveals that DAP3 exerts its suppressive function on A-to-I editing via a direct disruption of ADAR2 binding to its target double-stranded RNAs (dsRNAs). Furthermore, in vitro and in vivo tumorigenicity studies suggest that DAP3 functions as an oncogene in ESCC. The oncogenic effect of DAP3 overexpression could be partially counteracted by ADAR2 expression. Moreover, we identified a recoding event in PDZD7 gene regulated by DAP3 that contributed to the oncogenic role of DAP3. Altogether, our study highlights another layer of complexity in the modulation of RNA editome by ADARs binding factor DAP3 and suggests its potential as a novel target for cancer therapy.

121 Uridylation by TUT4/7 restricts retrotransposition of human LINE-1

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Mobile genetic elements comprise nearly 45% of the human genome. Among these elements, LINE-1s are the only active autonomous elements. LINE-1s mobilize during gametogenesis and early embryo development but such mobilization can produce potentially mutagenic insertions. Therefore, through evolution retrotransposon mobility has been suppressed. Although epigenetic silencing by the piwi-interacting (piRNA) pathway has been studied in detail, understanding of post-transcriptional mechanisms involved in LINE-1 mobility restriction is limited.

TUT4 and TUT7 (TUTases) are multi-domain enzymes that add non-templated uridine residues to 3' ends of RNAs of various classes to affect their stability. In a proteomic screen, we curiously identified the helicase MOV10, a known retrotransposition restriction factor, as a relevant interactor with TUTases. Using well-established retrotransposition assays, biochemical in vitro reconstitution and transcriptomics we show that MOV10 cooperates with TUTases in uridylating the 3' end of active LINE-1 mRNAs. This uridylation drastically reduced LINE-1 retrotransposition without strong effect on RNA stability. Notably, we show pervasive uridylation of endogenous LINE-1 mRNAs in various human cell lines and mouse testis, indicating that this mechanism is relevant at an organismal level.

In sum, our results provide the first mechanistic model for LINE-1 restriction on a post-transcriptional level and suggest the main physiological role for TUT4/7-mediated uridylation.

122 Withdrawn

123 Transcriptome-wide identification of substrates of radical SAM RNA methylating enzymes

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Although present in both bacteria and eukaryotes, the large family of radical SAM RNA methylating enzymes is largely uncharacterized. Escherichia coli RlmN, the founding member of the family, methylates an adenosine in 23S rRNA and several tRNAs to yield 2-methyladenosine (m2A). However, varied RNA substrate specificity among RlmN enzymes, combined with the ability of certain family members to generate 8-methyladenosine (m8A), makes functional predictions across this family challenging. Here, we present a method for unbiased substrate identification that exploits highly efficient, mechanism-based crosslinking between the enzyme and its RNA substrates. Additionally, by determining that the thermostable group II intron reverse transcriptase introduces mismatches at the site of the crosslink, we have identified the precise positions of RNA modification using mismatch profiling. These results illustrate the capability of our method to define the substrate scope and determine modification sites of the largely uncharacterized radical SAM RNA methylating enzyme family.

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124 Polypyrimidine Tract Sequence Determines Splicing Efficiencies of Long Non-Coding RNAs

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Most long non-coding RNAs (lncRNAs) undergo the same maturation steps as protein-coding mRNAs including capping, splicing, and polyadenylation. Using bioinformatics as well as direct experimental analysis we showed that human long intergenic non-coding RNAs (lincRNA) are less efficiently spliced than protein-coding genes (PCGs). Mutagenesis of a model lincRNA revealed that intronic sequences are primarily responsible for inefficient splicing. Analysis of RNA-Seq data from five different human cell lines further showed a positive correlation between the thymidine content of the polypyrimidine tract (PPT) and the splicing efficiency of lincRNAs but not of PCGs. The role of thymidines in the PPT in determining lincRNA splicing efficiency was confirmed for several lincRNAs through mutagenesis. To monitor interactions of splicing factors with lincRNAs, we applied iCLIP and observed a significant preference of SR proteins (SRSF2, SRSF5, and SRSF6) in binding to PCGs compared to lincRNAs. Together, we speculate that lincRNAs lack the cooperative network of interaction that navigates the splicing machinery to PCGs and therefore thymidine-rich PPT sequences are necessary for efficient splicing.

125 An important class of intron retention events in human erythroblasts is regulated by cryptic exons proposed to function as splicing decoys

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During terminal erythropoiesis, the splicing machinery in differentiating erythroblasts executes a robust intron retention (IR) program that impacts expression of hundreds of genes. We used computational and experimental approaches to study IR mechanisms for a class of large (>1kb) introns exemplified by intron 4 (i4) in the SF3B1 splicing factor gene. Transcripts retaining i4 are dynamically regulated during erythroid differentiation and comprise approximately 50% of total SF3B1 RNA in late erythroblasts. I4 is ~1.8kb in length and contains highly conserved regions that are nearly identical from mammals to amphibians, and even (in two cases) to some species of fish. RNA-seq analysis of nonsense-mediated decay (NMD)-inhibited cells revealed splice junctions that connect constitutive exons 4 and 5 to several cryptic cassette exons encoded in the conserved regions of i4. Experimental studies with minigene splicing reporters showed that these cassette exons promote IR, since deletion of the exons or mutation of their splice sites reduced IR, and insertion of these cassettes into an otherwise efficiently spliced intron (<1% retention) induced substantial retention. Genome-wide analysis of splice junction reads demonstrated that cryptic cassette exons are 3-4-fold more common in large retained introns (>1kb) than in efficiently spliced introns of similar length, consistent with a major role in IR. Such cassettes were much less frequent in retained introns <1kb in length, suggesting that IR mechanisms may differ for small introns. Functional assays showed that heterologous cassettes from several other genes could promote retention of i4 in the SF3B1 splicing reporter. Although most of these cryptic cassettes were inefficiently spliced as independent exons, they nevertheless exhibited substantial binding to U2AF1 and U2AF2, as expected if they utilize spliceosome components to engage intron-terminal splice sites. We propose that these exons function as decoys, competing with cross-intron interactions required for intron excision, and thus enforcing retention of the intron. The overall abundance and expression pattern of these decoys suggest that decoy-mediated IR drives a major component of the erythroblast IR program.

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126 Widespread accumulation of ribosome-associated isolated 3' UTRs in specific neuronal cell populations of the aging brain

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Particular brain regions and cell populations exhibit increased susceptibility to the stresses of aging. Here, we catalogue the age- and brain region-specific accumulation of ribosome-associated 3' UTR RNAs, absent the 5' UTR and open reading frame. Our study reveals that this phenomenon impacts hundreds of genes in aged D1 spiny projection neurons of the mouse striatum datasets and also occurs in the aging human brain. Quantification of isolated 3' UTRs across thousands of RNA-seq datasets shows that accumulation of these species is tightly correlated with mitochondrial gene expression and oxidative stress. We find that reduced activity of the oxidation-sensitive Fe-S cluster ribosome recycling factor ABCE1 induces accumulation of 3' UTRs and present a model in which ribosome stalling and mRNA cleavage by the No-Go decay pathway yields isolated 3' UTR RNAs protected by ribosomes. We go on to perform ribosome profiling in the aging mouse brain and show that that cerebellum and cortex exhibit vastly different age associated changes in translational fidelity and 3' UTR ribosome accumulation. Isolated 3' UTR accumulation is a hallmark of brain aging, likely reflecting regional differences in metabolism and oxidative stress.

127 NMD-Degradome Sequencing Reveals Ribosome-Bound Decay Intermediates Tailored with Nontemplated Nucleotide Heterogeneity at Their 3'-Ends

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Nonsense-mediated mRNA decay (NMD), which controls mRNA quality and also degrades physiologic mRNAs to fine-tune gene expression in changing developmental or environmental milieus, requires that its targets be removed from the translating pool of mRNAs. Since how and where the decay steps of mammalian-cell NMD occur remains unknown, we developed the transcriptome-wide isolation and sequencing of direct NMD decay intermediates based on their co-immunoprecipitation with phosphorylated UPF1, which is the active form of this essential NMD factor. We show that NMD occurs cooperatively from both 5'- and 3'-ends. Data indicate that ribosome-bound decay intermediate 3'-ends are subject to the nontemplated addition and removal of mostly uridines but potentially all four nucleotides by, respectively, the TUT4/7 terminal uridylyl transferases and the Perlman syndrome-associated exonuclease DIS3L2, the latter of which functions to differing degrees with the exosome depending on the transcript. We further demonstrate that RNA structure can influence where nontemplated additions occur.

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128 A Hub for 3′-end Processing: Structural Insights into mRNA Polyadenylation

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Almost all eukaryotic pre-mRNAs must undergo 5′ capping, splicing and 3′-end processing before they can be transported to the cytoplasm for their translation into proteins. 3′-end processing involves over 20 different protein factors that also co-ordinate transcription termination. The cleavage and polyadenylation factor (CPF) is an essential component of the 3′-end machinery that cleaves pre-mRNA transcripts and adds the 3’ polyA tails. Despite its fundamental importance, we are still far from understanding the molecular mechanisms of CPF. Here, we identify a sub-complex of the yeast CPF, the polyadenylation module (pAm), which acts as a hub for protein-protein interactions. Using cryo-EM we determine a 3.5 Å structure of the Cft1-Pfs2-Yth1 subunits of pAm. This consists of 4 beta propellers in Cft1 and Pfs2 that are strikingly similar to other interaction hubs involved in DNA and RNA processing. The zinc finger Yth1 protein extends from the side, providing an RNA binding surface. Biochemical studies confirm the structural observations and indicate the important role of pAm as the scaffold element of CPF to assemble other CPF subunits, including the poly(A) polymerase, and accessory factors of the 3’end processing machinery on RNA. We now aim to understand how the enzymatic activities are regulated. Our most recent results will be presented.

129 Molecular basis for the recognition of the human AAUAAA polyadenylation signal

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Nearly all eukaryotic messenger RNA precursors must undergo cleavage and polyadenylation at their 3'-end for maturation. A crucial step in this process is the recognition of the AAUAAA polyadenylation signal (PAS), and the molecular mechanism of this recognition has been a long-standing problem. We have determined the cryo-electron microscopy structure of a quaternary complex of human CPSF-160, WDR33, CPSF-30 and an AAUAAA RNA at 3.4 Å resolution. Strikingly, the AAUAAA PAS assumes a conformation that allows this short motif to be bound directly by both CPSF-30 and WDR33. The A1 and A2 bases of the PAS are recognized specifically by zinc finger 2 (ZF2) of CPSF-30 and the A4 and A5 bases by ZF3. Interestingly, the U3 and A6 bases form an intramolecular Hoogsteen base pair and directly contact WDR33. CPSF-160 functions as an essential scaffold and pre-organizes CPSF-30 and WDR33 for high-affinity and synergistic binding to AAUAAA. Our findings provide an elegant molecular explanation for how PAS sequences are recognized for mRNA 3'-end formation.

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130 Structural basis of AAUAAA polyadenylation signal recognition by the human CPSF complex

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3' polyadenylation is a key step in eukaryotic mRNA biogenesis. The pre-mRNA 3' processing machinery is a macromolecular assembly consisting of more than twenty proteins that define the efficiency and the site of cleavage and polyadenylation. In mammalian cells, 3'-end processing is dependent on the recognition of the hexanucleotide AAUAAA motif in the pre-mRNA polyadenylation signal by the cleavage and polyadenylation specificity factor (CPSF) complex, which is also responsible for cleaving the pre-mRNA and recruiting the PolyA Polymerase for polyadenylation. Using cryo-electron microscopy we have elucidated the structural basis for AAUAAA motif recognition by the human core CPSF complex. The 3.1 Å resolution structure uncovers the molecular interactions responsible for base-specific recognition and reveals an unexpected intramolecular base-pair within the hexanucleotide motif, setting the stage for further structural and mechanistic studies of the mammalian polyadenylation machinery.

131 The structural basis of mRNA decapping: cap recognition and activation of the Edc1-Dcp1-Dcp2-Edc3 mRNA decapping complex with substrate analog poised for catalysis

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The 5' cap structure found on all eukaryotic mRNA transcripts is critical for their proper translation and stability. The conserved decapping enzyme Dcp2 recognizes and cleaves the 5' cap, committing the transcript to rapid degradation and removal from the translating pool. Dcp2 functions as the catalytic core of a dynamic, multi-protein mRNA decapping complex, which includes the essential activator Dcp1 and a dense network of protein coactivators that regulate decapping activity in the cell. Conformational flexibility of the multi-protein decapping complex, coupled with its weak affinity for the 5' cap substrate, has made it challenging to trap and structurally characterize the substrate-bound, catalytically-active conformation of Dcp2 to understand how decapping coactivators control Dcp2 conformational states and activity.

Here we will present a 2.8 Å resolution co-crystal structure of Dcp1-Dcp2 in complex with the conserved enhancer of decapping proteins Edc1 and Edc3, cap substrate analog, and essential metal ion. In combination with biochemical studies, the heterotetrameric Edc1-Dcp1-Dcp2-Edc3 structure shows how the 5' cap substrate is recognized and cleaved by the catalytically-active conformation of Dcp2 and how coactivators Edc1 and Edc3 can engage the decapping complex simultaneously to activate decapping. Our studies of the doubly activated decapping complex are consistent with the idea that decapping coactivators such as Edc1 and Edc3 function to alleviate an autoinhibited conformation of Dcp2 and to promote the catalytically-active form to achieve switch-like changes in decapping activity that regulate mRNA stability in the cell.

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132 Structure of the nuclear exosome captured on a maturing pre-ribosome

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To date, biochemical and structural studies on the eukaryotic complexes that synthesize and degrade RNAs and proteins have investigated these machines individually to gain insight on how they execute the many different steps in gene expression. The molecular basis of how these individual complexes participate in eukaryotic gene expression is physically linked and thereby influences each other. In this study we display for the first time a fascinating picture how a major RNA degradation machine, the eukaryotic exosome, is directly coupled to the protein-synthesis machine, the ribosome. The eukaryotic exosome is a 3'-5' ribonuclease of ancient evolutionary origin that is central to RNA processing and turnover. The largest and best-known exosome substrate is a precursor of the 5.8S rRNA (7S rRNA) that is part of a nuclear precursor of the large ribosomal subunit (pre-60S).

In particular, we studied how the nuclear exosome holo-complex recognizes and remodels a precursor of the large ribosomal subunit. We biochemically trapped a transient interaction between the nuclear exosome and its pre-60S substrate using a specific point-mutant version of the ribonuclease complex. This enabled us to solve the structure of this massive and flexible exosome processing intermediate by state-of-the-art cryo-EM at resolution ranging between 3.9 Å and 4.6 Å. The reconstruction allowed unambiguous fitting and refinement of all the known atomic models with good statistics. Our complete structural model not only explains the various effects of previously reported mutations and biochemical interactions at a mechanistic level, but also unravels completely unforeseen remodeling events for both complexes when engaged in catalysis. This exciting snapshot not only captures the transient interaction between the exosome and the pre-ribosome at pseudo-atomic resolution, but also reveals how the essential helicase Mtr4 interacts with the exosome core and assists in the degradation of structured RNAs. This study shows for the first time how this RNA degrading machine recognizes and processes a physiological ribonucleoprotein particle (RNP).

133 Helicase-Assisted Degradation of Structured RNA by the Mtr4-Exosome Complex

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The eukaryotic RNA exosome is an essential and conserved protein complex that can degrade or process RNA substrates in the 3' to 5' direction. The exosome includes a nine-subunit donut-shaped core (Exo9) that binds Dis3 (aka Rrp44), an endoribonuclease and processive exoribonuclease, and the Rrp6/Rrp47 heterodimer, a nuclear-localized distributive exoribonuclease. Cofactor proteins, including the small nuclear protein Mpp6, associate with the exosome and assist it in RNA decay in different subcellular compartments. Decades of study have revealed that the exosome acts on all classes of RNA in diverse model organisms and that the catalytic subunits primarily engage these substrates by first threading them through a prominent central channel in the non-catalytic Exo9 core. Structural and biochemical studies have demonstrated that this channel is wide enough to permit single-stranded but not double-stranded RNA to enter, presenting an obstacle for degradation of structured RNAs. One way this can be overcome is by extending the 3' end of structured RNA through unwinding by the essential RNA helicase Mtr4, or through a combination of polyadenylation and/or unwinding by the nuclear Trf/Air/Mtr4 (TRAMP) complex. The physical basis for recruitment of Mtr4 to the exosome and the interplay between the various biochemical activities contained within the Mtr4-exosome complex remain largely unexplored.

We used an engineered substrate to crystallize and solve the X-ray structure nuclear RNA exosome complex from S. cerevisiae bound to its cofactor Mpp6. Biochemical characterization of the Mpp6-exosome showed that Mpp6 and Rrp6/Rrp47 cooperate to physically tether Mtr4 to the complex. Furthermore, reconstitution of exosomes from human, S. cerevisiae, and S. pombe revealed that the Mpp6-exosomes stimulate Mtr4 helicase activity, enabling ATP-dependent degradation of structured substrates. These efforts motivated cryo-EM studies aimed at capturing this process, which resulted in structural models for Mtr4-nuclear exosomes engaged in processive RNA decay.

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134 Control of global mRNA turnover by protein acetylation

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Acetylation is a prevalent post-translational modification critical for epigenetic and transcriptional control of gene expression through modification of histones and transcription-related factors. We have recently shown that acetylation also regulates gene expression at the post-transcriptional level by controlling global poly(A) RNA stability (Sharma et al., Mol. Cell, 2016). Inhibition of histone deacetylases 1 and 2 (HDAC1 and 2) was found to induce widespread degradation of poly(A)-containing RNAs in mammalian cells. Acetylation-induced mRNA turnover critically depends on the integrity of the evolutionarily conserved CCR4-CAF1-NOT complex and involves p300/CBP-mediated acetylation of the exoribonuclease CAF1a. We propose that reversible acetylation acts as a major switch that promotes a dynamic mode of gene expression with high rates of transcription and mRNA turnover. In order to identify additional factors that mediate acetylation-induced mRNA turnover, we tagged endogenous NOT1 in HeLa cells and are currently analysing the CCR4-CAF1-NOT complex for acetylation-dependent changes by quantitative mass spectrometry. We further performed poly(A) RNA interactome capture and found increased binding of cytoplasmic polyadenylation element-binding protein 4 (CPEB4) to poly(A) RNA upon treatment with the class I-specific HDAC inhibitor Romidepsin. CPEB4 is a sequence-specific RNA-binding protein known to activate translation of mRNAs through poly(A) tail lengthening during meiotic and mitotic cell divisions and contribute to cancer progression. We identified p300/CBP as the acetyltransferases responsible for acetylation of CPEB4, and found that CPEB4 interacts with the CCR4-CAF1-NOT complex. Moreover, we can show that CPEB4 promotes mRNA degradation in a tethering assay. Using knock-down and crosslinking approaches, we are currently analyzing the spectrum of mRNAs targeted by CPEB4, and the impact of CPEB4 acetylation on its functions. The discovery of acetylation-induced mRNA turnover adds an unexpected post-transcriptional component to our fundamental concept of how acetyltransferases and deadenylases regulate gene expression.

Sharma S, Poetz F, Bruer M, Ly-Hartig TBN, Schott J, Séraphin B, Stoecklin G. Acetylation-Dependent Control of Global Poly-A RNA Degradation by CBP/p300 and HDAC1/2. Mol Cell 2016;63:927-38.

135 Cryo-EM structure of human telomerase holoenzyme and new insight into its assembly and function

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Due to the end-replication problem, telomeric caps at the ends of eukaryotic linear chromosomes are progressively shortened, which eventually leads to genome instability. To maintain genome integrity, the telomerase ribonucleoprotein (RNP) synthesizes telomeric repeats through its telomerase reverse transcriptase (TERT) and an integral RNA subunit (hTR) carrying the template for repeat synthesis[1]. In addition to TERT and hTR, the human telomerase holoenzyme consists of a number of other protein factors required for RNP assembly and localization. Telomerase has been shown to play important roles in cancer, aging and a number of human diseases. Due to its cellular scarcity and complex RNP assembly, the composition of human telomerase is still a matter of debate and available structural information on human telomerase is limited to a 25Å negative stain reconstruction[2]. Here we present the cryo-electron microscopy structure of substrate-bound human telomerase holoenzyme at subnanometer resolution, describing two flexibly RNA-tethered lobes: the catalytic core with telomerase reverse transcriptase (TERT) and conserved RNA motifs of telomerase RNA (hTR), and an H/ACA ribonucleoprotein (RNP). In the catalytic core, RNA encircles TERT, adopting a well-ordered tertiary structure. The H/ACA RNP lobe comprises two sets of heterotetrameric H/ACA proteins and one Cajal body protein, TCAB1, representing a pioneering structure of a large eukaryotic family of ribosome and spliceosome biogenesis factors. Our results reveal new insight into telomerase assembly and function and provide a structural framework for understanding human telomerase disease mutations.

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136 Direct observation of transcription and splicing dynamics in single human cells

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Transcription and splicing are dynamic processes which reflect the synergy involving chromatin architecture changes, epigenetic modifications and stochastic molecular activities. Single molecule kinetic measurement can aid our understanding on this complicate biological practice. We developed a quasi-genome-scale platform that enable the recording of transcription and splicing kinetics in single human cells. By labeling endogenous genes with MS2 stem loops in the introns, we recorded the nascent RNAs production process in >60 clonal populations for 15 unique genes at their endogenous loci. This approach relies on several methodological advances. First, we developed an approach for labeling genes at their endogenous loci with random insertion, followed by mapping of the labeling sites in a high-throughput manner. Second, we developed robust high-throughput single-molecule imaging and analysis. Finally, we implemented stochastic time-series analysis on a massive scale. This combined approach can in principle be scaled to thousands of genes. The view that emerges from these studies is that 1) transcription is mostly episodic and distinct among different genes, 2) transcription kinetics correlates with the distribution of RNA abundance in single cells, 3) Co-transcriptional splicing dynamics exhibits a stochastic distribution, ranging from minutes to more than an hour. Super-fast splicing events may reflect the occurrence of recursive splicing, which might be a prevalent mechanism exploited by long introns. These results provide a quantitative foundation for modeling RNA processing and gene regulation in living cells. We hope to establish a reference model and general framework for studying the property of RNA synthesis with single-molecule resolution.

137 Pervasive, coordinated protein level changes driven by transcript isoform switching during meiosis

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To better understand the gene regulatory mechanisms that program developmental processes, we carried out simultaneous, genome-wide measurements of mRNA, translation and protein through meiotic differentiation in budding yeast. Surprisingly, we observed that the levels of several hundred mRNAs are anti-correlated with their corresponding protein products. We show that rather than arising from canonical forms of gene regulatory control, the regulation of at least 380 such cases—or over 8% of all measured genes—involves temporally regulated switching between production of a canonical, translatable transcript and a 5' extended isoform that is not efficiently translated into protein. By this pervasive mechanism for the modulation of protein levels through a natural developmental program, a single transcription factor can coordinately activate *and* repress protein synthesis for distinct sets of genes. The distinction is not based on whether an mRNA is induced or not, but rather based on the type of transcript produced.

138 Global changes in mRNA abundance drive differential shuttling of RNA binding proteins, linking cytoplasmic RNA degradation to transcription

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mRNA turnover influences the overall abundance of mature mRNAs in a cell. Recent work has shown that the rate of mRNA decay impacts mRNA synthesis, expanding the scope of influence mRNA turnover has in defining the gene expression output of a cell. During gammaherpesvirus infection, widespread mRNA degradation initiated by the viral endonuclease SOX and completed by the cellular 5'-3' exonuclease Xrn1 in the cytoplasm elicits a correspondingly broad decrease in RNAPII-based transcription in the nucleus. Following this work, it remained unclear how these physically separated events were connected, although we previously demonstrated that it is generated through the degradation of cleaved mRNA fragments rather than depletion of specific transcripts. We therefore reasoned that the signal might be conveyed by one or more RNA binding proteins (RBPs) differentially trafficking from the cytoplasm to the nucleus upon release from mRNA fragments undergoing degradation. Here, we used an unbiased approach to reveal that accelerated cytoplasmic mRNA decay drives nuclear relocalization of many RBPs. These RBPs remained cytoplasmic when the mRNA fragments created by SOX were stabilized in cells lacking Xrn1, suggesting that they were in fact responsible for conveying mRNA abundance information between the two compartments. RBPs that bind mRNA 3'ends, including poly(A) and poly(U) binding proteins, were overrepresented within the group of RNA decay-induced shuttling proteins. We uncovered a new role for one of these RBPs, cytoplasmic poly(A) binding protein (PABPC), whose accumulation in the nucleus was necessary and sufficient to repress RNAPII promoter occupancy. We are currently exploring the mechanism of this PABPC-induced transcriptional repression, in addition to identifying other consequences of RBP nuclear relocalization during accelerated cytoplasmic mRNA decay.

139 Physiological relevance of the nucleo-cytoplasmic shuttling of the SR protein SRSF1

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The serine/arginine-rich (SR) family proteins constitute a diverse group of pre-mRNA splicing factors that are essential for viability. They have a modular structure consisting of one or two RNA recognition motifs (RRMs) and a C-terminal RS domain. A subset, of which SRSF1 is the prototype, is capable of nucleo-cytoplasmic shuttling. By contrast, SRSF2 is unable to shuttle due to the presence of a nuclear retention sequence (NRS) at its C-terminus. When the NRS normally present in SRSF2 is fused to SRSF1, it prevents shuttling of the SRSF1-NRS fusion protein (1).

We previously identified a role for SRSF1 in promoting translation, of specific mRNAs, particularly those encoding RNA processing factors and cell-cycle and centrosome-associated proteins (2,3). However, the physiological relevance of the nucleo-cytoplasmic shuttling of SR proteins is not clearly understood.

Here, we have developed a mouse model for a non-shuttling SRSF1 protein that is exclusively retained in the nucleus. For this, we have used CRISPR/Cas9 genome editing to knock-in the Nuclear Retention Signal (NRS), naturally present in SRSF2, at the C-terminus of the SRSF1 genomic locus. SRSF1-NRS homozygous mice are born at correct Mendelian ratios, but display numerous post-natal phenotypes, including severe hydrocephalus (an accumulation of cerebrospinal fluid (CSF) within the brain) and restricted growth, being on average 30% smaller than wild-type littermates. Hydrocephaly can be caused by many heterogeneous mechanisms, with defective cilia and related biogenesis pathways being often linked to this phenotype. We will discuss current experiments investigating different aspects of cilia biology in our knock-in mouse model (SRSF1-NRS), including gross function and molecular characterization of ciliated cells. We will also discuss ongoing experiments to investigate alterations in SRSF1-mediated mRNA translation in affected tissues in the SRSF1-NRS mouse model, using ribosome profiling techniques. In summary, we have created the first mouse model that allows us to comprehensively investigate the physiological relevance of the nucleo-cytoplasmic activity of the splicing factor SRSF1 during mouse development.

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140 Adenovirus co-opts cellular factors to mediate epitranscriptomic m6A modification of viral RNAs important for splicing and protein production

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The discovery of reversible N6-methyladenosine (m6A) modification of RNA has fundamentally altered our view of the central dogma of molecular biology. The m6A chemical modification is added post-transcriptionally to RNA, where it has been implicated in such diverse processes as RNA splicing, nuclear export, stability, and translation. Small DNA viruses that replicate in the nucleus have to employ cellular machinery to transcribe and translate their gene products. These viruses have developed ways to harness cellular RNA processing pathways. Shortly after the discovery of m6A on human RNA, it was demonstrated that Adenovirus RNAs are also marked by m6A. However, the effect of m6A modifications on Adenovirus has never been deciphered, and little progress has been made in understanding how these so-called "epitranscriptomic" modifications impact the life cycle of DNA viruses in the nucleus. Using methylated RNA Immunoprecipitation and Sequencing (meRIP-Seq) and meRIP-qPCR, we have identified the site-specific locations of m6A modifications within the Adenovirus transcriptome. Every major transcriptional unit contains at least one m6A peak, and these peaks are enriched near the 3' splice acceptors of the heavily spliced Adenovirus major late transcriptional unit (MLTU). Furthermore, we have shown that during infection Adenovirus recruits host factors involved in methylating RNA and binding methylated RNA to sites of active viral transcription. Knockdown or knockout of METTL3, METTL14, or WTAP, all critical components within the human m6A-specific methyltransferase complex, reduce viral late gene expression, protein production, and the production of infectious virus particles. In addition, loss of m6A function reduces the splicing efficiency of adenoviral late genes, while sparing early gene transcription and splicing. This result is phenocopied by knockdown of cellular YTHDC1, a known m6A-binding protein that associates with splicing factors. These data demonstrate that m6A modification of viral RNA is important during the late stage of Adenoviral infection. These experiments are among the first to show how a DNA virus co-opts cellular epitranscriptomic machinery in the host cell nucleus to mediate viral RNA biogenesis. Furthermore, these findings further validate the role of m6A in splicing of RNA transcripts.

141 Mechanisms of ALS/FTD-linked RNA binding protein aggregation

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Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are devastating neurodegenerative diseases characterized by RNA binding protein (RBP) aggregation within neural tissues, which contributes to ALS/FTD toxicity by largely unknown mechanisms. Among ALS/FTD-linked RBPs, Fused in Sarcoma (FUS), a nuclear RBP participating in splicing, forms cytoplasmic aggregates in a significant number of ALS/FTD patient brains. Moreover, FUS mutations have been linked to ~5% of familial, or inherited, ALS. Here we describe experiments aimed at understanding the mechanism of FUS aggregation and its underlying toxicity.

We first investigated the role of RNA in FUS aggregation. Using immunofluorescence (IF) and biochemical fractionation, we found that cellular RNAs are essential for FUS aggregation. Poly(A+) RNA showed marked co-localization by IF with FUS cytoplasmic aggregates, and we found substantially reduced insoluble FUS levels in RNase treated cell extracts. To determine if RNA serves a key role in FUS subcellular localization, we modified poly(A+) RNA distribution, and determined FUS localization by IF. Strikingly, we found that FUS nucleo vs cytoplasmic localization depends on poly(A+) RNA transport and turnover pathways, but not the XPOI-mediated pathway despite the presence of a nuclear export signal. Furthermore, we found increased FUS nuclear aggregate size and number after blocking nuclear RNA decay pathways. These data indicate that FUS localization and aggregation can be modified by RNA transport and turnover; deregulation of either leads to local RBP accumulation and elevate the risk of RBP aggregation. Intriguingly, these results were observed with both WT and ALS mut FUS, suggesting a convergent mechanism of WT and ALS mut FUS aggregation.

We next investigated what RNAs colocalize with ALS mut FUS in aggregates by isolating them followed by deep sequencing. While mut FUS associated with RNAs in an essentially stochastic manner, we nonetheless identified a subgroup of mRNAs that were more susceptible to mut FUS sequestration. Further analysis of the sequestered mRNAs showed enrichment for transcripts encoding mitochondrial proteins and splicing factors, consistent with previous studies showing mitochondrial impairment and splicing dysregulation in ALS. Our data together provide a novel link between mRNA homeostasis and RBP aggregation and its link to toxicity in ALS/FTD.

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142 The effect of CUG repeat binding molecule on the alternative splicing in myotonic dystrophy type 1

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Expanded r(CUG) repeats are the causative of the neurological disorder myotonic dystrophy type 1 (DM1). Pathologic features of DM1 include the formation of ribonuclear foci that contain expanded r(CUG) repeats, which sequesters MBNL1 protein and leads to misregulation of alternative pre-mRNA splicing. Small molecules able to bind to the r(CUG) repeats and improve the alternative splicing have therapeutic potential in the treatment of DM1. Herein, we report the synthesis of dimeric molecules DDAP having an amide linkage and CDAP with carbamate linkage, and their binding properties to r(CUG) repeat. The SPR assay, circular dichroism (CD) spectra, and ESI-TOF mass spectrometry showed the binding of DDAP to r(CUG)9 repeat. The amount of ribonuclear foci in DM1 cell model was reduced by DDAP. Studies using DM1 cell model and DM1 mouse model revealed that DDAP was effective for the amendment of the pre-mRNA splicing defects. The mechanism for the rescue was studied by filter binding assay in vitro and mini-gene based cell model, suggesting that DDAP bound to r(CUG) repeat at the low concentration and interfered MBNL1 sequestration, whereas at the higher concentration, DDAP was also suggested to affect splicing by other mechanisms.

143 Mechanstic consequences of mutations in *DDX3X* in patients with medulloblastoma or global developmental delay

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The *DDX3X* gene encodes DDX3, an RNA chaperone of the DEAD-box family. DDX3 facilitates proper translation initiation on a subset of mRNAs depending on 5′ UTR content, and is an essential gene. Our previous research found that DDX3 binds to transcript 5′ UTRs and to the small ribosomal subunit. We further found that mutations in *DDX3X* in medulloblastoma reduce its biochemical activity and shift start codon recognition on a group of mRNAs. Here, we present clinical and biochemical data on mutations in *DDX3X* found in a cohort of patients with global developmental delay. We find that biochemical activity is strongly affected by these mutations. We further find an association of specific mutation types with clinical features. Mechanisms by which these mutations could alter cellular state will be presented. Together, our work suggests how identical mutations to *DDX3X* could have differing outcomes, and emphasizes the importance of considering post-transcriptional effects on gene expression when analyzing clinical transcriptomics data.

144 Analysis of oxidatively modified RNA in human neurons reveals the correlation between NAT8L mRNA damage and multiple sclerosis pathophysiology

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The overproduction of the reactive oxygen species (ROS) is associated with neurological disorders, such as, Alzheimer's disease, Parkinson's disease and multiple sclerosis (MS). The ROS can damage every biomolecule including DNA, RNA and proteins. In the past the focus was mainly towards the studies of DNA and protein oxidative damage and their link to the disease pathogenesis, while the detrimental effects of RNA damage only began to be highlighted in recent years. We have recently demonstrated the presence of a high degree of RNA oxidative modification in the neurons of MS patients' postmortem brain. By identifying the oxidatively modified mRNA molecules during oxidative stress in the human neuronal cells, we aimed to delineate the relationships between specific mRNA oxidation, aberrant protein production, and neurodegeneration. We have identified the mRNA molecules that are selectively targeted during oxidative/inflammatory stress situation in human neurons by using immunoprecipitation followed by RNA sequencing (RNA-seq) analyses. Interestingly we've discovered that one of the selectively oxidized NAT8L (N-acetyltransferase 8 like protein) mRNA has been linked to a low protein production in human neuronal cells. NAT8L is responsible in the production and transfer of a major neuronal analyte N-Acetyl-L-aspartate (NAA). Studies have shown the reduced NAA level in MS brain is linked to a reduced myelin production and can contribute to the disease progression. We have also demonstrated the reduction in NAT8L protein level in an animal model of MS. We have discovered that mRNA oxidation induced reduced expression of NAT8L enzyme could impair NAA metabolism in neurons thereby debilitating the myelin sheath production. Combining our *in cellulo* data, animal model data and MS postmortem brain data, our study finds that damaged NAT8L mRNA is, at least, partially responsible in the progression of MS. To the best of our knowledge, our study is the first to directly study the connection of mRNA oxidative damage in the pathogenesis of MS. This study will shed light on the yet to be understood link between mRNA oxidation and neurodegeneration.

145 The oncofetal RNA binding protein IGF2BP1 enhances an aggressive tumor cell phenotype by impairing miRNA-directed downregulation of oncogenic factors

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The oncofetal IGF2 mRNA binding proteins (IGF2BPs) are upregulated in most cancers but their paralogue-specific roles in tumor cells remain poorly understood. In a panel of five cancer-derived cell lines, IGF2BP1 shows highly conserved oncogenic potential compared to the other paraloques. Consistently, the Crispr/Cas9 mediated deletion of IGF2BP1 impairs the growth and metastasis of ovarian cancer-derived cells in nude mice. Gene expression analyses in ovarian cancer-derived cells reveal that the knockdown of IGF2BPs is associated with the downregulation of mRNAs that are prone to miRNA regulation. All three IGF2BPs preferentially associate upstream of miRNA binding sites in the 3'UTR of mRNAs. The downregulation of miRNA-regulated target mRNAs of IGF2BP1 is abrogated at low miRNA abundance or when miRNAs are depleted. IGF2BP1 associates with these target mRNAs in RISC-free complexes and its deletion enhances their association with AGO2. The knockdown of most miRNA-controlled target mRNAs of IGF2BP1 impairs tumor cell properties. In four primary cancers, elevated synthesis of these target mRNAs is largely associated with upregulated IGF2BP1 mRNA levels. In ovarian cancer, the enhanced expression of IGF2BP1 and most of its miRNA-regulated target mRNAs is associated with poor prognosis. In conclusion, these findings indicate that IGF2BP1 enhances an aggressive tumor cell phenotype by antagonizing miRNA-impaired gene expression in a miRNome-dependent manner.

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146 Translation control of the immune checkpoint in cancer and its therapeutic targeting

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The immune system functions to recognise and provide protection from foreign invaders and internal threats including cancer. Nonetheless, cancer cells have developed mechanisms to escape immunosurveillance by changing the expression of immune suppressive mRNAs, although how this is molecularly achieved remains an outstanding question. Here we develop an in vivo mouse model to study oncogene cooperation in immunosurveillance. We show that MYC overexpression (MYC^{Tg}) synergizes with $KRAS^{G12D}$ to induce an aggressive liver tumour leading to metastasis formation and reduced mouse survival compared to $KRAS^{G12D}$ alone. Our findings unexpectedly show little change in the genome-wide transcriptional landscape of MYC^{Tg}; $KRAS^{G12D}$ tumours compared to $KRAS^{G12D}$, but rather a highly selective alteration in translational control of mRNAs, including programmed death–ligand 1 (PD-L1). PD-L1 translation is repressed in $KRAS^{G12D}$ tumours by functional, non-canonical upstream open reading frames (uORFs) in its 5' untranslated region (UTR), which is bypassed in MYC^{Tg}; $KRAS^{G12D}$ tumours to evade immune attack. Importantly, we show that this mechanism of PD-L1 translational upregulation is effectively targeted by a novel, clinical compound that inhibits eIF4E phosphorylation, eFT508, which reverses the aggressive and metastatic characteristics of MYC^{Tg}; $KRAS^{G12D}$ tumours. Together, these studies reveal how immune checkpoint proteins are exquisitely manipulated by distinct oncogenes at the level of mRNA translation and showcases how this knowledge can be exploited for new immunotherapies.

147 Oncogenic Variation of Ribosomal RNA in Human Cancer

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The ribosome is an RNA-protein complex essential for translation in all life. The structural and catalytic core of the ribosome is its four rRNAs: 18S, 28S, 5S, and 5.8S. While ribosomal protein gene mutations have been identified as oncogenic drivers of human cancers (*RPS5, RPS15, RPL10* and *RPL22*), the mutational and variation landscape of the rRNA has remained largely unexplored.

In an exploratory study of rRNA sequence variation in colorectal cancer (CRC), we discovered a single, cancer-specific point variation in the 18S rRNA *in 44% of patients* (N = 91 matched tumor-normal pairs, 4 cohorts, p = 3.81e-8). We identified the same variant in T-cell Acute Lymphoblastic Leukemia and Diffuse Large B-cell lymphoma patients, amongst others. Moreover, we validated the variant 18S in CRC cell lines and measured variant rRNA incorporation into mature and actively translating ribosomes.

To gain mechanistic insights into cancer-specific rRNA variations, we projected ribosomal protein mutations from taken from >28,000 cancer genomes onto the human ribosome structure. We found mutational hotspots in 3-dimensional clusters encompassing the known oncogenic driver mutations, as well as several novel inter-protein mutational clusters, such as at the uS9:RACK1 interface. The 18S rRNA cancer-variation co-clusters with a known RPS15 oncogenic hotspot at the P-site of the small subunit of the ribosome. Altogether, our findings support the hypothesis that cancer-variant rRNA is novel driver of oncogenesis.

Understanding cancer-variant ribosomes has paradigm-shifting potential in both its consequence to aberrant translation in cancer and as a novel chemotherapeutic target. Thus, this innovative stream of research has truly translational potential.

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148 Spatial and developmental regulation of A-to-I RNA editing in the brain

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Adenosine to inosine (A-to-I) RNA modification is particularly important in the regulation of genes involved in neurotransmission in the mammalian brain. Since inosine is read as guanosine during translation, A-to-I editing has the potential to change the read out of a gene. This modification, catalyzed by the ADAR enzymes, thereby provides flexibility in the proteome by expanding the variety of possible isoforms. Indeed A-to-I editing is a requisite for neuronal function, including key mediators of synaptic function. We have previously shown that editing is regulated during neuronal maturation. In mouse, only low levels of editing could be detected in the embryonic brain, while many sites are highly edited in the adult brain. Nevertheless, we know little about what regulates ADAR editing during brain maturation. The catalytically inactive ADAR3 family member has the potential to act as an editing inhibitor. We show that ADAR3 can inhibit editing at the I/M site of GABRA3, and several other efficiently edited transcripts expressed in the brain. The expression of ADAR3 protein during mouse brain development is initially high but declines with age post-birth, in negative correlation with the editing activity. These results indicate a potential role for ADAR3 as a negative regulator of editing during early brain development. In addition, we have used padlock probes specific for edited and unedited RNA, respectively, and in situ rolling circle amplification with fluorophore-conjugated probes to quantify mRNA expression and editing within single transcript. With this method, we can visualize the spatial distribution of edited transcripts in the entire brain and compare the efficiency of editing at different sites simultaneously. We show that editing is highly specific to certain brain regions with a unique pattern for each type of transcript, which does not necessarily follow the expression of the editing enzymes. For some transcripts, the spatial distribution of edited RNA also changes during neuronal development. We will discuss and unravel mechanisms used to regulate editing in the brain, both of individual transcripts and during development. This is important in order to understand the complexity of the brain as well as the development of neurological disorders.

149 Decreased A-to-I RNA editing as a source of keratinocytes dsRNA in psoriasis

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Recognition of dsRNA molecules activates the MDA5-MAVS pathway, and plays a critical role in stimulating type-I interferon responses in psoriasis. However, the source of the dsRNA accumulation in psoriatic keratinocytes remains largely unknown. A-to-I RNA editing is a common co- or post-transcriptional modification that diversifies adenosine in dsRNA, and leads to unwinding of dsRNA structures. Thus, impaired RNA editing activity can result in an increased load of endogenous dsRNAs. Here we provide a transcriptome wide analysis of RNA editing across dozens of psoriasis patients, and demonstrate a global editing reduction in psoriatic lesions. In addition to the global alteration, we also detect editing changes in functional recoding sites located in the IGFBP7, COPA, and FLNA genes. Accretion of dsRNA activates autoimmune responses, and therefore the results presented here are relevant to a wide range of autoimmune diseases.

150 Engineered RNase P ribozymes effectively inhibit the infection of murine cytomegalovirus in animals

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We had previously engineered new RNase P-based ribozyme variants with improved in vitro catalytic activity. In this report, we used R466-AS, a novel engineered variant with unique mutations at the catalytic domains, to target the mRNA of assemblin (AS) of murine cytomegalovirus (MCMV), which is essential for viral progeny production. Variant R466-AS cleaved AS mRNA sequence in vitro at least 150 times more efficiently than ribozyme M1-AS, which was derived from the wild type RNase P catalytic RNA sequence. In cultured MCMV-infected cells, R466-AS exhibited better antiviral activity than M1-AS and decreased viral AS expression by 98-99% and virus production by 7,000 folds. In MCMV-infected mice hydrodynamically transfected with M1GS-expressing constructs, variant R466-AS was more effective in inhibiting AS expression, blocking viral production, and improving animal survival than ribozyme M1-AS originating from the wild type RNase P catalytic RNA. These results provide direct evidence that engineered RNase P ribozyme variants with higher targeting activity in vitro are also more effective in inhibiting gene expression in animals. Furthermore, our findings imply the potential of engineering novel RNase P ribozyme variants with unique mutations to improve ribozyme activity for therapeutic application.

151 Therapeutic targeting of a thrifty microRNA in metabolic diseases

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The burgeoning epidemic of obesity and metabolic syndrome is accompanied by a rapid rise in type 2 diabetes and non-alcoholic fatty liver diseases (NAFLD/NASH) in the developed world, however the molecular underpinnings and genetic predispositions remain unclear. We recently used genome-wide association studies (GWAS) to identify the miR-128-1 microRNA as a key contributor to abnormal circulating cholesterol/lipids (Wagschal et al. *Nature Medicine* 2015). We have now found that miR-128-1 is genetically linked in Europeans to human evolutionary positive selection, as well as to obesity and type 2 diabetes. Our studies in diet-induced and genetic obesity mouse models show that antisense antagonism or genetic KO of miR-128-1 strongly decrease body fat, markedly improves glucose tolerance and insulin resistance, and lowers circulating cholesterol and triglycerides. We also find that miR-128-1 antagonism or genetic ablation potently decreases hepatic steatosis, inflammation and fibrosis in multiple NAFLD and NASH mouse models. Mechanistically, miR-128-1 acts as a thrifty microRNA by inhibiting the expression of a number of key metabolic regulators and enzymes involved in energy expenditure. Accordingly, we observe a marked increase in energy expenditure in response to antisense inhibition or genetic KO of miR-128-1 in obese mice using metabolic cage studies. Based on these findings, we suggest that miR-128-1 represents an attractive therapeutic target in metabolic diseases.

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152 High UGA nonsense mutation correction by a new readthrough molecule

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Nonsense mutations are responsible for about 10% of genetic disease cases. The consequence of a nonsense mutation is the silencing of the mutant gene. Several strategies have been developed to correct the presence of a nonsense mutation. In particular, the activation of premature termination codon (PTC) readthrough has been explored for more than 20 years. PTC readthrough is a natural mechanism that promotes the incorporation of an amino acid when the ribosome reaches the PTC. Recently, we built a screening system to identify compounds with a high capacity at correcting nonsense mutations in human cells. Using this screening system, we identified CNSM1 as an exclusive corrector of UGA nonsense mutations only. CNSM1 represents the first case of a corrector of nonsense mutations with an exclusive activity on one type of nonsense mutation. The efficacy of UGA nonsense mutation correction of CNSM1 is higher than the one promoted by G418. We validated these results on several cell lines and cystic fibrosis patient cells. The correction of UGA nonsense mutation was also effective in vivo on several mouse models harboring a nonsense mutation in TP53 or in mu opioid receptor. Finally, we investigated the mode of action of CNSM1 in order to understand how this molecule can correct UGA nonsense mutation only.

Today, no molecules are available for physicians and for patients affected by a genetic diseases caused by a nonsense mutation. With its high correction efficacy and low toxicity, CNSM1 could represent a strong drug candidate for the treatment of genetic diseases caused by a UGA nonsense mutations.

153 A translation inhibitor targets a bimolecular cavity between eIF4A and polypurine RNA

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A novel class of translation inhibitors isolated from *Aglaia* plants, exemplified by Rocaglamide A (RocA), exhibits antitumor activity by clamping eukaryotic translation initiation factor 4A (eIF4A) onto polypurine sequences in mRNA. This unusual inhibitory mechanism raises the question of how the drug imposes sequence selectivity onto a general translation factor. Here, we determined the crystal structure of the human eIF4A•ATP analog•RocA•polypurine RNA complex. RocA targets the "bimolecular cavity" formed characteristically by eIF4A and sharply-bent consecutive purines in bound RNA. The naturally selected mutations found in *Aglaia* eIF4A changed the cavity shape, leading to RocA resistance. This study provides the unique example of a drug targeting a bimolecular cavity, fitting into the space shaped cooperatively by protein and RNA with specific sequence.

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154 How ubiquitous and important are transient "invisible" RNA states for biological function?

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While widely accepted that RNAs are highly dynamic, it is becoming clear that non-protein coding RNAs such as riboswitches, viral RNAs, and triplex forming RNAs not only sample multiple conformations in solution but also such sampling might be necessary for function. These RNAs without their cognate ligands have traditionally been difficult to characterize structurally by conventional NMR and X-ray crystallography techniques. Both high-resolution x-ray crystallographic and SAXS analyses indicate that a number of such RNAs might adopt the same global conformation in the absence or presence of its cognate ligand. To better characterize the functional mechanism of how RNA in the unliganded state can adopt conformations close to the bound state, we probed various RNAs using a combination of ligation and new isotopic labeling strategies and newly developed NMR dynamics (Chemical Exchange Saturation Transfer, CEST) experiments that probe slow ms-ms motions to detect these otherwise invisible states. Our ligation approach combined with site-specific isotopic labeling significantly simplified the NMR spectra and allowed us to measure the conformational exchanges at atomic resolution. In this presentation we will showcase how ions can modulate the low populated states (<12%) with transient lifetimes (~ms) of the apo states that have NMR structural signatures close to but distinct from the holo-bound states. Finally we will discuss how such transient RNA apo states might play significant roles in ligand binding to ultimately sequester key signaling structural elements to modulate cellular function.

155 High temporal- and spatial resolution studies of a helix-to-coil transition that controls the switching mechanism of a riboswitch

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Riboswitches are structural elements that are found in the 5' untranslated regions of many messenger RNAs (mRNAs) and that undergo ligand-dependent structural rearrangements that regulate transcription, splicing, translation, and/or stability of the corresponding mRNA. Because they are typically found in bacteria, and because the majority of bacterial riboswitches lack counterparts in archaea and eukaryotes, riboswitches remain promising antibacterial drug targets. Architecturally, riboswitches are composed of a 'switching sequence' that overlaps with an upstream ligand-binding 'aptamer domain' and a downstream 'expression platform'. Ligand-dependent genetic control is achieved when binding of the ligand to the aptamer domain modulates the base-pairing interactions of the switching sequence such that the expression platform adopts a secondary structure that triggers expression or repression of the mRNA. In the prototypical adenine-responsive riboswitch that controls expression of the *pbuE* gene in *Bacillus subtilis*, adenine modulates the base-pairing interactions of the switching sequence so as to regulate transcription of the *pbuE* gene: In the absence of adenine, the switching sequence pairs with the expression platform to form a transcription terminator hairpin, whereas, in the presence of adenine, the switching sequence is prevented from pairing with the expression platform, allowing transcription to proceed. Using a new and powerful single-molecule biophysical approach, single-molecule field effect transistors (smFETs), we have investigated the dynamics of a helix-to-coil transition that controls the base-pairing interactions of the switching sequence of the adenine-responsive *pbuE* riboswitch at an unprecedented temporal resolution of 50 μsec per data point and a spatial resolution of a single base pair. Our studies have allowed us to investigate how adenine binding- and base-pairing interactions that are distal to the switching sequence collaborate to allosterically modulate the dynamics of the helix-to-coil transition and, consequently, control transcription of the *pbuE* gene.

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156 Co-transcriptional folding of a riboswitch controls the fate of the transcriptional machinery

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Folding of nascent transcripts can be modulated by properties of the RNA polymerase (RNAP) that carries out the transcription process. For example, the site-specific pausing of the RNAP and co-transcriptional RNA-protein interactions have been shown in several cases to be important for co-transcriptional RNA folding. In bacteria, riboswitches are genetic elements frequently found in the 5' untranslated regions of mRNAs, where they respond to cellular metabolites to regulate gene expression either at the level of transcription or translation. Since they change conformation "on-the-fly", riboswitches are tractable models for studying the role of the transcription process in guiding the folding of nascent transcripts.

The preQ1 riboswitch from *Bacillus subtilis* regulates gene expression by a transcriptional mechanism in which the binding of its ligand, the queuosine precursor $preQ_1$, causes a conformational change that leads to the formation of a terminator hairpin. Previous studies have deciphered the structure and dynamics of the isolated riboswitch aptamer, but less is known about the impact of the transcriptional machinery on riboswitch folding and vice versa. We have identified a strong pause by RNAP during riboswitch transcription that is stabilized by a previously characterized consensus pause sequence and the ligand-free conformation of the riboswitch. By using single molecule Förster resonance energy transfer (smFRET) analysis of the riboswitch in a paused elongation complex, we show that RNAP has a profound impact on RNA dynamics, stabilizing a tightly folded structure at this specific pause site. Molecular modeling and biochemical assays further support a specific functional interaction between the nascent transcript and the exit channel of RNAP through which ligand binding to the riboswitch unpauses the polymerase. We are currently using cryo-electron microscopy of the paused elongation complex to further pinpoint the specific structural rearrangements in both riboswitch and RNAP that are induced first by pausing, then by ligand binding. Finally, we are evaluating the impact of transcription factors on pausing and riboswitch folding. Our study provides significant insights into the mechanisms by which small RNA structures can regulate the function of the macromolecular transcription machinery, and vice versa.

157 Structural basis of tRNA-mediated transcription anti-termination by a T-box riboswitch

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Amino acids are among the most ubiquitous and essential nutrients. T-box riboswitches are bacterial cis-regulatory noncoding RNAs that regulate amino acid-metabolic genes through multipartite interactions with tRNAs [1]. T-boxes share a phylogenetically conserved architecture comprised of two domains — a 5' Stem I and 3' antiterminator domain and a linker. Stem I selectively docks a cognate tRNA via sequence- and structure-specific interactions [2-4]. The antiterminator probes the molecular volume of the docked tRNA 3' end to sense aminoacylation [5]. This readout dictates the formation of either an intrinsic transcription terminator or antiterminator. The architecture of a full-length T-box complex and detailed interactions between the tRNA 3' region and the antiterminator remain poorly understood.

We define a minimal region of the T-box both necessary and sufficient to selectively bind an uncharged tRNA, and report a 2.7 Å co-crystal structure of the complex. The structure reveals how tRNA 3' end is buried inside the antiterminator such that a conserved G•U wobble pair at the base of helix A2 abuts the ribose 3'-OH of the tRNA terminal adenosine. This juxtaposition creates steric clash between the universal amino group of the esterified amino acid and the uridine nucleobase, thus providing a general mechanism to reject any aminoacyl-tRNA. Interestingly, the clash is exasperated by the outward shift of the wobble uridine into the major groove. We compare this RNA-based steric device to ribosome-bound RelA, which positions a beta strand to reject aminoacyl-tRNA in stringent response [6-7].

Further, we report a cryo-EM structure of a full-length T-box riboswitch-tRNA complex, which reveals a surprisingly ordered inter-domain linker. Together, the structures show that extensive intermolecular stacking allows Stem I and antiterminator domains of the T-box to sandwich the uncharged tRNA to form a 30-bp continuous stack, to stabilize the antiterminator to transcribe downstream genes.

1. Grundy & Henkin, Cell 1993; 2. Grigg & Ke, Structure 2013; 3. Zhang & Ferré-D'Amaré, Nature 2013; 4. Zhang & Ferré-D'Amaré, Structure 2014; 5. Zhang & Ferré-D'Amaré, Mol Cell 2014; 6. Brown et al., Nature 2016; 7. Loveland et al., eLife 2016. *Supported in part by the intramural research program of NIDDK, NIH.*

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158 New tRNA interaction sites in the US T-box riboswitch

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T-box riboswitches regulate the expression of essential amino acid-related genes in Firmicutes and Actinobacteria by monitoring the aminoacylation status of a cognate tRNA. In T-box RNAs with a canonical Stem I, the Specifier Loop interacts with the anticodon of the cognate tRNA and the terminal loop and AG bulge form a loop-loop structure that interacts with the tRNA elbow. The Ultrashort (US) Stem I class of *ileS* T-box riboswitches, which regulate isoleucyl-tRNA synthetase genes, have an alternate Specifier Loop structure and lack the elements necessary for interaction with the tRNA elbow, but contain the highly conserved Stem II and Stem IIA/B pseudoknot, whose role in tRNA recognition remained unknown. We have now demonstrated that both Stem II and Stem IIA/B of the US *ileS* RNA contribute to $tRNA^{Ile}$ affinity. Additionally, using selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE), crosslinking, and mutational studies, we identified two new interaction sites, one between the S-turn element in Stem II and the tRNA T arm and the other between the pseudoknot and the tRNA D loop. This is the first example of tRNA recognition by an S-turn motif or a pseudoknot element, revealing new types of RNA-RNA interactions. These data provide the first biochemical evidence for the functional role of Stem II and the pseudoknot, which are present in the majority of T-box RNAs but absent in the *glyQS* RNAs that have been used for most of the current biochemical and structural analyses. We hypothesize that these interactions are important for the recognition and binding of the cognate tRNA and discrimination against non-cognate tRNA in those T-box RNAs in which they are present, and propose that structural variability in T-box riboswitches indicates alternate solutions to the tRNA recognition problem.

159 A structural basis of gene regulation by guanidine riboswitches

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Bacterial genes encoding proteins that are involved in guanidine detoxification in bacteria are subject to regulation by riboswitches. Three guanidine riboswitches have been identified. We have solved high resolution crystal structures for the guanidine-II and guanidine-III riboswitches. The former comprises two stem-loops that interaction via loop-loop interaction and this creates specific binding pockets for two guanidine molecules. The guanidine- III riboswitch adopts a pseudoknot structure that includes a triple-helix, and a left-handed helical ramp. The riboswitches use the Hoogsteen edge of guanine to hydrogen bond the ligand, together with π-cation interactions. Both have side openings that allow small side chains access.

L. Huang, J. Wang and D. M. J. Lilley: The structure of the guanidine-II riboswitch *Cell chem. biol*. **24**, 695–702 (2017).

L. Huang, J. Wang, T.J. Wilson and D. M. J. Lilley: The structure of the guanidine-III riboswitch *Cell chem. biol*. **24**, 1407-1415 (2017).

160 Crystal structure of an essential RNA domain from IRES of hepatitis A virus in complex with a synthetic antibody fragment

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RNA domains of picornaviral internal ribosome entry sites (IRESs) play a vital role in initiating and regulating the cap-independent translation of the viral genome, however, our understanding of the relationship between the three-dimensional structure of such RNA domains and their biological function remains elusive. We have crystallized the domain V from the IRES of hepatitis A virus - a picornavirus that contains a type III IRES, using a synthetic antibody fragment as an RNA crystallization chaperone and solved the structure of the antibody-RNA complex at 2.54-Å resolution. The RNA adopts a T-shaped structure, topologically organized by a lone-pair tri-loop motif. Despite a lack of primary sequence homology, we observed a striking similarity between the overall architecture of domain V and a circularly permuted form of the J-K domain from the IRES of encephalomyocarditis virus that contains a type II IRES, suggesting a conserved biological role of these domains. Our work represents the first high-resolution crystal structure determination of a picornaviral RNA domain and underscores the value antibody assisted RNA crystallography.

161 The Structure of an Extended RNA Kink-Turn Reveals a Potential Tool for RNA Design

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Kink-turns are an RNA structural motif important for stabilizing the global architectures of folded RNAs, providing a sharp bend between two RNA helices. Diverse versions of the kink-turn motif have been characterized in terms of their dynamic and structural properties, revealing differences in their ability to transition from an extended (unkinked) conformation to the sharply bent kinked conformation mainly through single-molecule experiments. The underlying structural differences between these two states remains unknown, as the structure of a kink-turn in the unkinked or extended state have never been observed. Here we present the structure of a kink-turn in an extended state solved by x-ray crystallography, revealing a conformation in which the emerging helices are nearly coaxial. The extended kink turn structure is well defined and appears to be an authentic alternate conformation. Within this conformation, an alternate base-pairing scheme is adopted and two defining noncanonical G-A base pairs are not formed. We hypothesize that the formation of the G-A pairs is a critical event that underlies the switch from an extended to a highly kinked state. The ability of kink-turns to adopt two different but defined conformations may be a useful tool in the future for 3D RNA design and nanotechnology applications that require an RNA switch.

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162 A structurally complex fluorescent aptamer is improved with rational design and structure guided engineering

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Fluorescent proteins revolutionized cell biology by making it possible to visualize large complexes and even single proteins within living cells. A toolkit of fluorescent RNA tags would help answer some of the more pressing questions in RNA cell biology, facilitating *in vivo* analyses of RNA interactions, localization and traffic. Since there are no known naturally fluorescent RNAs, several groups have applied *in vitro* selection to produce aptamers that selectively bind and activate the fluorescence of small molecules. Although not optimized for fluorescence activation, fluorescent aptamers have been used to visualize RNA within eukaryotic cells and have also been employed as reporters for biosensors. Recently, the final pool of the *in vitro* selection of RNA Mango (Mango-I) was subjected to a fluorescence based reselection resulting in three fluorescent aptamers (Mango-II, Mango-III and Mango-IV). All three aptamers have improved fluorescence properties relative to Mango-I while maintaining a small size (< 30 nt) and high binding affinity (< 10 nM). Mango-III was especially intriguing since it was the brightest of the Mango aptamers (43,000 M^{-1} cm^{-1}) and has the lowest sequence similarity to Mango-I. To gain understanding as to why this aptamer is so much brighter than Mango-I we determined its co-crystal structure with TO1-Biotin at 2.35 Å-resolution. The structure reveals a complex RNA fold with numerous tertiary interactions spanning a 24-nt conserved core. The binding pocket imposes a planar conformation to the heterocycles of the TO1-Biotin fluorophore by forming a *trans*-Watson-Crick A·U cap over the fluorophore heterocycles. We mutated the cap nucleotides to all sixteen possible base combinations and tested for binding and fluorescence enhancement. One cap mutant is 22% brighter than Mango-III-wt and maintains a high binding affinity to TO1-Biotin. Using *in vitro* compartmentalization coupled to fluorescence sorting, structure guided engineering was performed. Four highly similar sequences were obtained, confirming the utility of the cap mutant while improving the helical linker contacts. Our results show that a complex, information rich fluorescent aptamer can be further improved through both rational design and structure guided engineering.

This work was supported by the National Heart, Lung, and Blood Institute, intramural research program.

163 Size, Shape and Sequence-dependent Immunogenicity of RNA Nanoparticles

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RNA molecules have emerged as promising therapeutics. Like all other drugs, the safety profile and immune response are important criteria for drug evaluation. However, literatures on RNA immunogenicity have been controversial. Here, we used the approach of RNA nanotechnology to demonstrate that the immune response of RNA nanoparticles is size, shape, and sequence-dependent. RNA triangle, square, pentagon and tetrahedron with same shape but different sizes, or same size but different shapes were used as models to investigate the immune response. The levels of pro-inflammatory cytokines induced by these RNA nanoarchitectures were assessed in macrophage-like cells and animals. It was found that RNA polygons without extension at the vertexes were immune-inert. However, when single-stranded RNA with a specific sequence was extended from the vertexes of RNA polygons, strong immune responses were detected. These immunostimulations are sequence-specific, since some other extended sequences induced little or no immune response. Additionally, larger size RNA square induced stronger cytokine secretion. 3D RNA tetrahedron showed stronger immunostimulation than planar triangular RNA. These results suggest that the immunogenicity of RNA nanoparticles is tunable to produce either a minimal immune response that can serve as safe therapeutic vectors, or a strong immune response for cancer immunotherapy or vaccine adjuvants.

164 Evolution of Structural Complexity as a Response to Increasing Length

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RNA is widely considered to be an early, if not the earliest molecule to transmit heritable information and perform catalysis. The length of the earliest evolving RNA molecules is thought to have been limited, with increases in length and activity over time. Combined phylogenetic and structural evidence suggests that complex modern RNAs contain structural elements that were present in their shorter ancestral RNA. Understanding both the origin and evolution of life, therefore, requires an understanding of how polymer length impacts the evolution of RNA structure and function. The extent to which increases in length favor either elaboration upon structural solutions available to short RNA, or entirely new structures inaccessible to short RNA is not well understood. To test the consequences of increasing length in RNA evolution, we evolved populations of ligase ribozymes in vitro. We initially evolved populations of two lengths independently, a population of short (20N) and long (80N) ligases, with fully randomized sequences of respective length N. We analyzed the populations using high throughput comparative sequence analysis. We observe evolution of recurring structures and detect both short and long ligase ribozymes that favor the same ligation junction and form identical secondary structures surrounding the junction. Structures of low activity ribozymes evolved in the 20N population are present as structural components of larger, more active, ribozymes in the 80N population, directly demonstrating the potential for evolution through elaboration upon existing structures. In this instance, elaboration occurs through the addition of a stem that converts a conserved terminal loop into an internal loop. We are performing additional evolution experiments to probe the limits of structural elaboration upon this motif. We also tested the activity of other previously evolved ligase motifs and observe a consistent increase in activity as a function of length. By combining our results with previous characterizations of ligase activity, we observe a length-activity correlation across nine orders of magnitude in activity. These insights into how polymer length impacts evolution improve our understanding of the mechanisms available during early evolution and provide guidance in interpretation of the molecular record present in modern RNA structures.

165 Evolutionary plasticity of the NHL domain underlies distinct solutions to RNA recognition

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RNA-binding proteins regulate all aspects of RNA metabolism. Their association with RNA is mediated by RNA-binding domains, of which many remain uncharacterized. A recently reported example is the NHL domain, found in prominent regulators of cellular plasticity such as the C. elegans LIN-41. We employ an integrative approach to dissect the RNA specificity of LIN-41. Using computational analysis, structural biology, and *in vivo* studies in worms and human cells, we find that a positively charged pocket, specific to the NHL domain of LIN-41 and its homologs, recognizes a stem-loop RNA element, whose shape determines the binding specificity. Surprisingly, the mechanism of RNA recognition by LIN-41 is drastically different from that of its more distant relative, the fly Brat. Our phylogenetic analysis suggests that this reflects a rapid evolution of the domain, presenting an interesting example of a conserved protein fold that acquired completely different solutions to RNA recognition.

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166 The Atlas of DROSHA Cleavage Sites on Pri-miRNAs

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The Microprocessor complex consists of RNase III DROSHA and dsRNA binding protein DGCR8. The first step of miRNA maturation is the cleavage of a primary microRNA (pri-miRNA) by DROSHA in the Microprocessor complex. The cleavage site choice is critical as it determines the seed sequence which defines target specificity of miRNA. Although cleavage sites can be inferred from small RNA sequencing data, the inference is often inaccurate, since terminal modification can occur after processing. In this study, we investigated pri-miRNA processing for all of 1,881 human pri-miRNA entries both *in vitro* and *in vivo*, and determined the cleavage sites directly by sequencing the processed RNA fragments. First, we prepared expression constructs for all human pri-miRNA entries (from miRBase 21). Second, the processing efficiency and cleavage sites were determined by *in vitro* processing and sequencing. Third, the pri-miRNA constructs were ectopically expressed to examine pri-miRNA processing in cells. From this comprehensive approach, we identified the DROSHA cleavage sites for the entire set of human miRNAs for the first time. The results revealed that a substantial number of entries do not qualify as genuine miRNAs, which helps to refine the current miRNA database. Furthermore, by comparing the datasets, we identified the processing events that are alternatively regulated in cells. This study provides comprehensive information about the DROSHA cleavage sites and offers insights into the regulation of miRNA biogenesis.

167 Tertiary Structure Differences in Pri-miRNAs Promote Alternative Drosha Cleavage and Expand Target Repertoires

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Background and Hypothesis: MicroRNAs (miRNA) biogenesis starts with Drosha cleavage. The fidelity of this initial cleavage is critical for the posterior cleavage by Dicer as well as for establishing the seed sequence that determines the miRNA target specificity. To understand how pri-miRNA sequence and structure impact on the choice of Drosha cleavage sites, we study the processing of the three mir-9 paralogs, which harbor the same sequence of miR-9 but differ in loop and surrounding sequences.

Study Design and Methods: Here, we systematically explore how different pri-miRNA paralogs of miR-9 are processed. To this end, we have analyzed the relative abundances of canonical and alternative cleaves in pri-mir-9-1, pri-mir-9-2 and pri-mir-9-3 by in vitro cleavage assay. In parallel, the in vivo cleavage was measured by deep sequencing the products. We also generated chimeric and hypothesis-driven mutations of pri-miRNA structures to study the contribution of each structural element to Drosha cleavage fidelity. Finally, we have evaluated the differential targeting between the miR-9-can and miR-9-alt, which are products of canonical and alternative Drosha cleavages.

Results and Conclusions: Interestingly, we reveal that pri-miR-9-1 has a unique Drosha cleavage profile due to its kinked tertiary structure which is confirmed by small-angle X-ray scattering (SAXS). Pri-miR-9-1, but not pri-miR-9-2 or pri-miR-9-3, generates abundant miR-9-alt isoform with a shifted seed sequence that expands the scope of its target genes. Further analysis in gliomas cells and patient samples indicates a distinct role of miR-9-alt in tumorigenesis. Finally, we generalize our conclusion by demonstrating that the tertiary structure is a major determinant of Drosha cleavage fidelity on all pri-miRNAs.

Relevance and Importance: 1) Our results demonstrate for the first time that pri-miRNA tertiary structure impact Drosha processing. 2) We provide compelling evidence that isomiRs play important roles in a physiological condition. 3) More than 30% of miRNA genes are members of families. Our results indicate that individual members can exert a different function via distinct Drosha processing. 4) We provide new guidelines in shRNA design to reduce heterogeneous processing and its associated off-target effects.

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168 Neuronal miRNAs collaborate to strongly repress many shared targets

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miRNAs are key post-transcriptional regulators in many biological processes, including differentiation of muscle and nerve cells, that function by repressing a broad range of mRNAs. Distinct miRNAs have hundreds to thousands of conserved binding sites in the transcriptome, but binding of a miRNA to a single mRNA site generally has only a modest effect on target expression. We hypothesized that miRNAs might exert greater effects through co-targeting of individual mRNAs by two or more different miRNAs. We compared target sets of conserved miRNAs and identified hundreds of significant co-targeting relationships between specific pairs and groups of miRNAs. Interestingly, these co-targeting miRNA pairs and groups, including one comprised predominantly of neuronal miRNAs, tend to have similar expression profiles across tissues, further supporting the notion that they act in concert. We explored a possible functional relationship in neuronal differentiation between one pair of miRNA genes with unrelated seed sequences but a highly significant co-targeting relationship: *mir-138* and *mir-137*. We generated CRISPR-mediated miRNA knockout cell lines in the murine CAD cell culture model of neuronal differentiation. We observed that *mir-138* KO cells were unable to differentiate and project neurites following serum withdrawal, whereas *mir-137* KO cells had no observable defect in differentiation. Further characterization of the *mir-138* KO cells by RNA-seq showed that loss of *mir-138* resulted in a de-differentiated state compared to WT cells. Intriguingly, addition of a miR-138 or miR-137 mimic, but not mimics of other neuronal miRNAs, could rescue the neurite growth phenotype and allow *mir-138* KO cells to differentiate. To assess the level of repression that is possible by a group of co-targeting miRNAs, we designed cell-based reporters containing the 3' UTRs of highly targeted genes with well-known roles in neuronal differentiation. We show that miRNAs acting in concert achieve much greater levels of repression than is typically observed for individual miRNAs. Our results contribute to an understanding of how miRNAs may act in co-targeting groups and collaborate to more strongly drive neuronal differentiation and perhaps other processes.

169 Divergent Roles of the miRNA Argonaute Proteins in *C. elegans* Aging

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Although highly related proteins often perform redundant functions, there are rare cases of homologous proteins taking on opposing roles in certain contexts. We discovered one such example where the activities of Argonaute-like-gene 1 *(alg-1*) and *alg-2* diverge in adult *C. elegans*. These Argonaute (AGO) proteins are specific to the miRNA pathway and seem to perform overlapping and complementary roles in regulating gene expression during embryogenesis and larval development. Surprisingly, we found that loss of *alg-1* leads to a shorter lifespan and loss of *alg-2* results in an extended lifespan. Gene expression analyses revealed that distinct sets of genes are mis-regulated in each of the AGO mutant backgrounds. Consistent with the longevity phenotypes of *alg-1* and *alg-2* mutant animals, many of the differentially expressed genes are regulated by the insulin/ IGF-1 signaling (IIS) pathway. Furthermore, genetic experiments demonstrate that the long lifespan of animals deficient in insulin receptor activity (*daf-2* mutants) is partially dependent on *alg-1*, while the extended lifespan of *alg-2* mutants requires the FOXO DAF-16 transcription factor. These findings prompt the question of how two proteins that are over 80% identical in amino acid sequence and exhibit similar expression patterns and functions during development take on opposing roles in adulthood. To address this problem, we have used CRISPR to fuse fluorescent tags to the endogenous *alg-1* and *alg-2* genes, which will enable detailed analyses of the expression and activity of these AGOs in aging animals. These strains will allow us to test the hypothesis that in adults ALG-1 and ALG-2 bind distinct miRNAs and targets, which contributes to their opposing longevity roles. To understand the molecular basis for the different activities of these two AGOs, regulatory and coding sequences will be swapped between *alg-1* and *alg-2* to identify the elements responsible for their divergent roles in adult animals. Overall, I aim to elucidate how two miRNA AGOs promote opposite longevity fates in *C. elegans.*

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170 MiR-320c targets RAP1 in platelets and reduces Platelet Activation in Storage

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***Background*:** A small GTPase protein, the Ras-related protein 1 **(**RAP1), abundant in platelets is known to be activated following agonist induced platelet activation, suggesting that RAP1 downregulation could in turn reduce platelet activation in storage. Our objective of this study is to identify RAP1 regulating miRNAs and their role in platelet activation during storage.

***Methods*:** We applied MS2-TRAP (tagged RNA affinity purification) methodology to enrich miRNAs that target the 3' untranslated region (3'UTR) of RAP1 mRNA in two mammalian cell lines followed by miRNA identification by microarray of total RNA extracted from the miRNA-enriched samples. Data analyses were done using different bioinformatics approaches. The direct miR-320c:RAP1 3'UTR interaction was confirmed by using a luciferase reporter gene expression system in a mammalian cell line. Subsequently, platelets were transfected with miR-320c to evaluate RAP1 downregulation by this miRNA and its effect on platelet activation.

***Results*:** Six miRNAs (miR-320c, miR-181a, miR-3621, miR-489, miR-4791 and miR-4744) were identified to be enriched in the two cell lines tested. We selected miR-320c for further evaluation. Direct interaction of miR-320c with RAP1 3¢UTR was confirmed by using dual luciferase reporter assay system. Further, in platelets treated with miR-320c, RAP1 protein expression was decreased and concomitantly, platelet activation was also decreased.

***Conclusion*:** Overall, the results demonstrate that miRNA-based RAP1 downregulation in stored platelets reduces platelet activation.

171 TRBP and PACT pose stoichiometric questions for Dicer complex assembly

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The RNase III enzyme Dicer catalyses a key step in microRNA (miRNA) biogenesis. In humans, Dicer associates with two homologous proteins, TRBP and PACT, both of which comprise three dsRNA-binding domains (dsRBDs) that are connected by flexible linkers. The two N-terminal dsRBDs of TRBP and PACT are canonical domains, which bind dsRNA, while the C-terminal dsRBD is non-canonical and interacts with proteins, including Dicer. Currently, much remains unknown about how the protein-RNA and protein-protein interaction properties of TRBP and PACT affect the assembly and function of the Dicer complex.

We have elucidated the 3D structures of the homodimerisation domains of TRBP and PACT and determined that these domains self-associate via an unusual asymmetric binding mode that is conserved from flies to humans. We have identified conserved and divergent residues on the homodimerisation interface and demonstrated that mutation of these sites modulates the self-association properties of TRBP and PACT. We have also investigated the RNA-binding properties of TRBP and PACT. Individually, each of the canonical dsRBDs interacts with precursor miRNAs (pre-miRNAs) with micromolar affinity while multidomain constructs bind the same pre-miRNA more tightly. Analysis of the stoichiometry of dsRBDs-pre-miRNA interactions show that multiple dsRBDs can associate with a single pre-miRNA.

Together, our data reveal that the protein-RNA and protein-protein binding properties of TRBP and PACT pose stoichiometric questions for Dicer. The dsRBD-dsRBD interaction surface involved in homodimerisation of TRBP and PACT overlaps with the Dicer-binding surface. Moreover, the binding of multiple dsRBDs to a pre-miRNA would compete with the interaction with Dicer. These results suggest that the dissociation of interactions involving canonical and non-canonical dsRBDs may be a key step in the assembly of a functional Dicer complex.

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172 Influence of Anticancer Therapeutics on Differential Expression of SnoRNAs, Ribosome Methylation, and Nucleolar Stress

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While the influence of DNA damage has been extensively studied as one cellular response to anticancer therapeutics such as cisplatin, recent data indicate important roles of non-DNA targets, including RNA and proteins. To gain insight into the RNA responses induced by this drug, full RNA-seq analysis of triple negative breast cancer MDA-MB-468 cells treated at therapeutic concentrations of cisplatin between 30 minutes and 24 hours was performed. The resulting data provide insight into the complex nature of the cellular response to cisplatin, including observation of novel responses. In one unexpected response, the expression of numerous snoRNAs decreases as early as 30 minutes post-treatment. Located in the nucleolus, snoRNAs are necessary components in ribosome processing. A subgroup of the downregulated snoRNAs direct modification of helix 69 on the 28s ribosome, and correct modification of helix 69 is necessary for proper ribosomal formation and translation termination. Further quantification of methylation at helix 69 and other locations suggests that cisplatin induces changes in snoRNA expression and leads to dysregulation of rRNA modification, likely altering ribosome activity upon drug treatment through a nucleolar response. Additional signatures of nucleolar response mechanisms support this new pathway for platinum-based therapeutics to influence RNA processes.

173 Comparative study of the adenine riboswitch in Apo and bound state using relaxation dispersion

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Riboswitches are structured *cis*-regulators mainly found in the untranslated regions of messenger RNA. They consist of an aptamer domain that can sense and bind specific ligand by adopting conformational changes, thus regulate the behavior of expression platform. As the model systems for studying riboswitch structures and functions[1], we studied conformational dynamics of the *add* adenine riboswitch from *V. vulnificus*. We investigate the conformational motions of both apo and bound *add* adenine riboswitch aptamer domain to capture the microsecond-to-millisecond dynamics using Car-Purcell-Meiboon-Gill (CPMG) relaxation dispersion. Our preliminary results indicate that the millisecond motions in P1 and P3 will slow down upon ligand binding while the motions in loops and the binding pocket is similar to those in the absence of ligand. Combined with structural information in all four states[2], our data suggest that altered dynamic landscape in P1, not the binding pocket, is a direct consequence of the formation of the three-base triple interactions and the P1-P3 stacking, which constitute the "switching" action triggered by ligand binding.

1. Porter, E.B., Marcano-Velazquez, J.G. & Batey, R.T. The purine riboswitch as a model system for exploring RNA biology and chemistry. *Biochim Biophys Acta* **1839**, 919-930 (2014).

2. Stagno, J.R. et al. Structures of riboswitch RNA reaction states by mix-and-inject XFEL serial crystallography. *Nature* **541**, 242-246 (2017).

174 Unravelling the direct role of miRNAs during neurogenesis by identifying functional miRNA/mRNA complexes in the mouse brain

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Posttranscriptional regulation via microRNAs (miRNA) is a well-accepted concept. While the molecular mechanisms of miRNA functions have been mainly studied *in vitro*, *in vivo* studies are underrepresented. miRNA regulation has been implicated in neurogenesis, the process of neuronal stem cells giving rise to the diversity of neuronal and glial subtypes. Indeed, most of the known mammalian miRNAs are expressed in the brain; several of them being brain-specific. Moreover, miRNAs have been demonstrated to regulate neurogenesis, e.g. controlling neural stem cell determination or proliferation. However, a clear picture of the dynamic expression, activity and function of miRNAs during neurogenesis is still pending.

A major restriction for studying miRNA function in complex *in vivo* contexts has been the lack of appropriate tools to analyse miRNA expression patterns and study their respective function in defined cell populations at defined developmental stages. To overcome these limitations, we take advantage of Ago-APP, a novel tool that allows direct isolation of miRNAs from cells and tissue. Ago-APP is based on a peptide (T6B, derived from a GW182 protein) that binds all Argonaute proteins with high affinity. Hence, expression of this peptide in the cell population of interest allows efficient isolation of miRNAs by immunoprecipitating Ago-miRNA complexes.

We introduced the T6B peptide into neuronal stem cells of the postnatal murine forebrain to investigate miRNA expression during the determination and differentiation of olfactory bulb interneurons *in vivo*. Subsequently, we isolated Ago-bound miRNAs from neuronal stem cell progeny at several stages of the differentiation process from stem cells over migratory precursors to mature OB neurons. Small RNA sequencing will provide us a temporal view of the miRNA expression-pattern.

In a next step, we will investigate as well the temporal changes of the mRNA expression pattern at different stages of neuronal differentiation using RNA-Sequencing. This will allow us to correlate changes in miRNA/mRNA expression and predict inhibitory miRNA/mRNA interactions. The function of the most potential predicted miRNAs will then be further investigated by gain- and loss-of-function studies.

In summary, this approach will provide deeper insight into the function of miRNAs in a physiological context such as neurogenesis.

175 3′ Uridylation Expands miRNA Target Repertoire

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Advancements in next generation sequencing lead to identification of a vast amount of miRNA isoforms (isomiRs), most of which arise from post-maturation sequence modifications. One prevalent modification is tailing, in which nontemplated Uracil (U) and/or Adenine (A) are detected at the 3' end of miRNAs. Recent studies demonstrate that the change of the isomiR profile is a hallmark for many cancers, suggesting isomiRs play a unique role during tumorigenesis. Despite their importance, little is known as to how the function of tailed isomiRs differs from that of the canonical miRNAs.

We applied human miR-27a as a model to investigate the function of isomiR, as overexpressing miR-27a in 293T cells results in abundant isomiR expression. To our surprise, a target lacking seed pairing but having base-pairing at the 3' end of miRNA was subject to miR-27a repression to a degree comparable to that of a wild-type target. Interestingly, an "AA" motif located immediately upstream of the target site is critical for the repression, suggesting that the U-tail at the 3' of miRNA base-pairs to the "AA" motif and stabilizes the association between the miRNA and the target. In support of this idea, the U-tailed miRNAs, but not the canonical miRNA, were enriched when we pull down target-associated miRNAs by biotinylated probes. In addition, this repression is abolished in cells lacking the uridylation enzyme TUT4 and TUT7. Together, these findings suggest a model in which uridylation of miRNA enables its association and repression of novel targets in addition to canonical seed-matched targets. Further analyses of patient samples in TCGA indicate this type of regulation happens in vivo and potentially plays an important role during tumorigenesis.

Our study revealed a novel mechanism for miRNA to regulate non-canonical targets and furthered our understanding on the function of tailed isomiRs.

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176 Exploring the new role of oncogene UNR/CSDE1 at multiple layers of mammalian small RNA pathways

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Small RNAs are defined by their length (19–34 nucleotides) and association with Argonaute (AGO) family proteins, classifying them into miRNAs, siRNAs and piRNAs. In animals, especially in *Drosophila*, pathways associated with these small RNAs have been extensively studied and relevance of core components such as AGO proteins are well established, at least, in part, due to their non-redundant functions among pathways. But in mammals, the AGO proteins, in particular AGO2, have common roles in both siRNA and miRNA pathways, which makes it difficult to dissect their functional relevance and individual physiological significance in disease and development. Therefore, we decided to work on deciphering the complexities of both these pathways in mammals. Since, the mode of target repression differs between the siRNA and miRNA pathway, though commonly mediated by AGO2, we hypothesized that the phenomenon might depend on interaction of AGO proteins with relevant accessory factors, which are largely unknown until date. In order to identify such factors, we employed mouse Embryonic Stem Cells (mESCs) and performed RNA pull downs to purify protein complexes associated with both siRNA and miRNAs, commonly referred as siRISC and miRISC, respectively. Following LC/MS analysis and confirmation by western blotting, we identified multiple protein factors and one among them being CSDE1, an RNA binding protein (RBP) associated with both complexes, while others associated with only miRISC. The interaction of CSDE1 with si/miRISC is RNA-independent and cells depleted with CSDE1 showed impaired small RNA mediated gene silencing, suggesting the involvement of CSDE1 at the target repression level. We observed CSDE1 interaction with multiple components of both small RNA pathways in different cell compartments, implicating its functional relevance at various stages of small RNA-mediated gene regulation. Further, we have found that CSDE1 maintains the steady state levels of AGO proteins by inhibiting both proteasome and lysosome mediated degradation. Altogether, our results provide insights into the contribution of accessory factors in defining functional outcome between siRNA and miRNA mediated gene silencing in mammals.

177 Chemically Synthesized Long dsRNAs are a Potent RNAi Therapeutic Platform Circumventing Innate Immune Response

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RNA interference (RNAi) is a sequence-specific gene silencing phenomenon in cells, initiated by double-stranded RNA (dsRNA). However, the major problem associated with dsRNA triggers for therapeutics is that those are recognized by specific pattern recognition receptor (PRR) as a pathogen-associated molecular patterns (PAMP), activating the innate immune system. Especially, long linear dsRNA structure (above 30bp) has not been considered as a potential candidate for RNAi therapeutics due to activation of sequence-nonspecific innate immune response. Nonetheless, short dsRNAs have been used widely without triggering any innate immune responses. The long dsRNA used earlier in the immune stimulation studies, however, mainly comprised of in vitro transcribed products, that possess 5' triphosphate, a crucial component of PAMP. With the progress in the RNA synthesis facilities, we tried to re-elucidate the innate immune stimulation using chemically synthesized dsRNA without the key 5' triphosphate in various structures. Herein, we show that against conventional concept, innate immune stimulation is reduced as the length of the chemically synthesized dsRNA increases, maintaining a potent target gene silencing effect. Using Next-Generation Sequencing we demonstrate the longer dsRNA is subject to cleavage, which might explain the previous result. We find out one of the dsRNA sensors plays a crucial rule for recognizing chemically synthesized long dsRNA structure. Furthermore, the dsRNAs with 3' 2nt overhangs on both sense and antisense strands can also abolish the innate immune stimulation. In conclusion, the current introduction of long dsRNA structure with little innate immune stimulation may highlight the development of the next generation nucleic acid therapeutics platform with various functionalities.

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178 Investigating the tissue specific roles of miRNAs in *C. elegans*

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MicroRNAs (miRNAs) are small non-coding RNAs that carry out post-transcriptional gene regulation by guiding Argonaute proteins to the 3′untranslated regions (3′UTRs) of mature mRNAs. In the nematode *C. elegans* the Argonaute proteins ALG-1 and ALG-2 act redundantly to execute the miRNA pathway. The loss of both proteins leads to embryonic lethality during morphogenesis, with defects in muscle and epidermal tissue formation, suggesting an essential role for miRNA-based gene regulation in tissue development and cell identity.

We previously profiled the tissue specific transcriptomes of several somatic tissues in *C. elegans* and found that alternative polyadenylation (APA), a mechanism by which the same gene is expressed with different 3'UTR isoforms, is pervasive, and used to bypass regulation by miRNAs in tissue specific manner. Although very important, only a handful of interplays between miRNAs and APA have been highlighted so far, and thus the extent of these interactions remain largely unknown.

We adapted a technique previously developed in our lab to isolate and sequence high-quality tissue specific miRNA targets from the muscle and intestinal tissues of *C. elegans*. We prepared transgenic strains expressing tissue specific GFP tagged ALG-1 and used them to perform tissue specific RNA-immunoprecipitations, followed by high-throughput sequencing. Our study validated previously predicted and wet-bench identified miRNA targets, and found that the number of genes regulated by miRNAs in each tissue correlates with their tissue specific transcriptome size.

Importantly, we detected an unusual abundance of RNA binding proteins (RBPs) within our top hits in both datasets, including several splicing factors, suggesting that perhaps miRNAs could also contribute at some level to tissue specific isoform diversity. Our approach highlights intricate muscle and intestine miRNAs/APA interaction networks, which allow us to better understand the roles of miRNAs in producing and maintaining tissue identity.

179 QsRNA-seq: a method for high-throughput profiling and quantifying small RNAs

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The finding that small non-coding RNAs (sRNAs) can affect cellular processes by regulating gene expression had a significant impact on biological research and clinical diagnosis. Yet, the ability to quantify and profile sRNAs, specifically miRNAs, using high-throughput sequencing is especially challenging because of their small size and repetitive nature. We developed QsRNA-seq, a method for preparation of sRNA libraries for high-throughput sequencing that overcomes this difficulty by enabling separation of fragments shorter than 100nt long that differ only by 20nt in length. The method supports using unique molecular identifiers for quantification. We show that QsRNA-seq gives very accurate, comprehensive and reproducible results. Using QsRNA-seq to study the miRNA repertoire in C. elegans embryo and L4 larval developmental stages, enabled extending the list of miRNAs that are expressed in a developmental-specific manner. Interestingly, we found that miRNAs 23nt long are predominantly expressed in developmental stage L4, suggesting a possible connection between the length of miRNA and its developmental role.

180 Knockdown of Piwi-like proteins in the mouse hippocampus enhances the memory of conditioned fear

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Piwi-interacting RNAs (piRNAs) are a unique class of small regulatory RNAs which interact specifically with the Piwi-like proteins. Through this interaction, piRNAs can modulate gene expression via RNA interference and epigenetic mechanisms. The mammalian Piwi pathway has been defined by its role in transposon control during spermatogenesis, and despite an increasing number of studies demonstrating its expression outside the testes, relatively little is known about its function in mammalian somatic tissues. We have discovered that the Piwi-like genes *Piwil1* and *Piwil2* are expressed in mouse neurons, and are upregulated by neuronal activation. Simultaneous knockdown of *Piwil1* and *Piwil2* in the mouse hippocampus enhances fear learning and the memory of conditioned fear without affecting generalised anxiety. Additionally, preliminary results from small RNA sequencing indicate that the expression of some hippocampal piRNAs is responsive to the fear conditioning paradigm. Ongoing work in our laboratory aims to further investigate the dynamics of neuronal Piwi proteins and piRNAs during neural plasticity and learning, and to investigate the regulatory targets of the Piwi pathway in the mammalian brain.

181 Small nucleolar RNAs are processed to small RNAs and associate with *Saccharomyces cerevisiae* ribosomes in a stress-dependent manner

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Small nucleolar RNAs (snoRNAs) belong to the group of evolutionary conserved non-coding RNAs. They are found in Archea and Eukarya and they role is guiding chemical modification of other classes of RNAs. snoRNAs operate as ribonucleoprotein complexes (snoRNP), in which, RNA molecules act as guides, targeting modification site and protein performs actual modification. snoRNAs were initially discovered in nucleolus and thought to exclusively target RNAs inside this sub-nuclear compartment. However, now it is clear that numerous snoRNAs do not possess target RNAs and might possess alternative functions outside nucleolus. Moreover, under certain stress condition snoRNAs are processed to smaller fragments called snoRNA-derived RNAs (sdRNA). sdRNAs function as miRNA in *Giardia lamblia* or human. However, the function of sdRNA in organisms lacking miRNA machinery, like yeast *Saccharomyces cerevisiae* is still vague.

Our previous work focused on identification of ribosome-associated noncoding RNAs (rancRNAs) revealed that both, full length snoRNAs as well as their derivatives, sdRNAs, interact with yeast ribosomes. Our current preliminary studies indicate that yeast sdRNAs function as inhibitors of global translation by interactions with the ribosomes. To verify the hypothesis if snoRNAs and sdRNAs interact with the ribosomes in a stress-dependent manner, we have investigated changes in the snoRNA and sdRNA levels in the cytosol, ribosomal and post-ribosomal fractions isolated from yeast under variety of environmental growth conditions. The absolute levels of snoRNAs and sdRNAs were quantified with the means of stem-loop reverse transcription (SL-RT) followed by droplet digital PCR technology (ddPCR). Our results show that snoRNAs are indeed present outside the nucleus and their level in the cytosol differs when yeast is subjected to environmental changes. Moreover, we have observed that snoRNA are subjected to the stress-dependent processing in the cytosol, most prominent during pH and salinity stress. Most of the snoRNA processing products (sdRNAs) are associated with the ribosomes and this association as well as the concentration of individual sdRNAs in the cytosol is stress-dependent.

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182 Functional Characterization of Bacterial Regulatory RNAs Informed by *in vivo* Antisense Hybridization Assays

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Increased understanding of the roles bacterial small RNAs (sRNAs) play in pathogenesis and stress response pathways has prompted an interest in high throughput methods to understand their networks. Transcriptomics studies relying on RNA ligation have made considerable progress in identifying sRNA partners; however, such interactome studies are often limited to highly expressed sRNAs or to those that are bound by user-defined proteins. Inspired by base-pairing mechanisms of sRNA-mRNA regulation, we have developed a high throughput *in vivo* synthetic biology tool to simultaneously measure the likelihood of any number of user-defined RNA regions to establish base pairing with a cognate RNA oligonucleotide. We will discuss our application of this system to evaluate the hybridization landscape of approximately 900 RNA regions in 71 confirmed, but mostly mechanistically under-characterized, *Escherichia coli* sRNAs. Included in this library of sRNA regions are approximately 40 previously-mapped binding sites in 16 sRNAs, deliberately interrogated for characterization of hybridization patterns of functional RNA regions. Importantly, we determine *in vivo* characteristics indicative of known binding sites and use these hybridization patterns to identify likely functional regions and corresponding computationally-predicted mRNA targets of previously uncharacterized sRNAs. Upon experimentally validating proposed mRNA targets via in vitro binding shift assays, we find that the use of *in vivo* hybridization insights captures unique sRNA:mRNA pairs that would likely not be considered as true interactions for validation based on traditional free energy rankings of computational sRNA target prediction algorithms. Recognizing the sensitivity to regulatory interactions *in vivo*, we further exploit the high throughput method to quantify the functional influence of the global chaperone protein Hfq on these sRNAs by comparing aforementioned hybridization landscapes to landscapes derived from experimentation in an isogenic Hfq-deficient strain. Interestingly, we find a large number of sRNAs exhibit no Hfq dependency in their ability to engage in antisense hybridization, suggesting that Hfq selectively alters RNA hybridization to increase favorability of some sRNA:mRNA interactions, likely via a previously proposed unfolding chaperoning mechanism.

183 Determination of the human Dicer interactome during viral infection reveals potential RNA interference restriction factors

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RNA silencing or RNAi is a conserved mechanism mediating suppression of gene expression and relying on the generation of small RNAs (sRNAs) of 21-30 nucleotides in length. These sRNAs are processed from long double-stranded RNA precursors through the action of type III ribonucleases called Dicer. Well-established in plants and invertebrates, the importance of RNAi as an antiviral defense system remains hotly debated in mammals. Indeed, in the later viral infection triggers an innate immune response based on the production of type I interferon (IFN) that triggers the expression of hundreds of IFN-stimulated genes (ISGs) to block viral replication and/or lead to programmed cell death. Although all components of the RNAi pathway are conserved in mammals, infection of somatic cells with various viruses results in low accumulation of virus-derived siRNAs (vsiRNAs). Moreover, only a modest effect of Dicer deficiency on viral replication has been reported suggesting that mammals might have evolved the IFN-based response to replace RNAi as the major defence system against viruses. Nonetheless, recent studies have shown that antiviral RNAi may be important in undifferentiated cells such as in oocytes, or mouse embryonic stem cells. Those observations led to the hypothesis that the IFN system, which is attenuated in undifferentiated and germ cells, may inhibit or mask antiviral RNAi in mammalian somatic cells.

To test this hypothesis, we performed Dicer immunoprecipitation followed by mass spectrometry analysis in mock- and virus-infected HEK293 cells with the aim to identify factors that could restrict Dicer antiviral activity. These analyses allowed us to retrieve a number of Dicer-interacting proteins that were significantly enriched or sometime exclusively found in the context of virus infection. Among these proteins were known partners of Dicer such as PACT, or the well-known ISG PKR, as well as new ones such as the RNA helicase DHX9 or ADAR1. We validated these interactions by western blot analysis and showed that they were maintained upon infection with different positive strand RNA viruses. The biological function of those interactions as well as their potential role to restrict antiviral RNAi in somatic mammalian cells will be discussed.

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184 Investigating small RNA involvement in the nonstop decay pathway in Caenorhabditis *elegans*

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In *C. elegans,* endogenous siRNAs known as 22G RNAs regulate expression of thousands of protein coding transcripts in developing sperm and egg cells, and their loss leads to infertility. While the biological roles of these small RNAs are becoming more well understood, we are working to understand the factors that control which genes are targeted for regulation by 22G RNAs. Previous research in the Youngman lab identified a gene of unknown function (F43E2.6) that is targeted by 22G RNAs only in polymorphic wild strains of worms that lack a stop codon. Conversely, when the stop codon is present, there is low 22G RNA production. We are investigating whether the loss of a stop codon alone is sufficient to target an mRNA for silencing by 22G RNAs. In addition to 22G RNAs, F43 is targeted by a Dicer product, and RDE-1-bound Dicer products can trigger 22G RNA synthesis in the classical exogenous RNAi pathway. This leads to two alternative hypotheses to explain the biogenesis of 22G RNAs at F43: the loss of the stop codon, or the targeting by the Dicer product in complex with RDE-1. To determine whether production of 22G RNAs at the F43 locus is independent of the Dicer product, we first generated *rde*-1;F43(nonstop): a strain with the nonstop codon in absence of the Dicer pathway. We are currently deep sequencing small RNAs from this strain to ask whether production of 22G RNAs is independent of Dicer. In parallel, we are using CRISPR-engineered strains to ask whether loss of the stop codon alone is sufficient to trigger 22G RNA production at this locus. Taken together, these results will allow us to assess whether transcripts that lack a stop codon are targeted by 22G RNAs as part of the nonstop decay pathway in worms.

185 Hierarchical roles of mitochondrial Papi and Zucchini in *Bombyx* germline piRNA biogenesis

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PIWI-interacting RNAs (piRNAs) are small regulatory RNAs that bind to PIWI proteins to control transposons and maintain genome integrity in animal germ lines. piRNA 3' end formation in the silkworm *Bombyx mori* was shown to be mediated by the 3'-to-5' exonuclease Trimmer (Trim; known as PNLDC1 in mammals), and piRNA intermediates are bound with PIWI anchored onto mitochondrial Tudor domain protein Papi. However, whether the Zucchini (Zuc) endonuclease and Nibbler (Nbr) 3'-to-5' exonuclease, which both have pivotal roles in piRNA biogenesis in *Drosophila*, are required for piRNA processing in other species remains unknown. Here we show that the loss of Zuc in *Bombyx* had no effect on the levels of Trim and Nbr, but resulted in the aberrant accumulation of piRNA intermediates within the Papi complex, and these were processed to form mature piRNAs by recombinant Zuc. Papi exerted its RNA-binding activity only when bound with PIWI and phosphorylated, suggesting a hierarchical process of the complex assembly. Both the 5' and 3' ends of piRNA intermediates within the Papi complex showed hallmarks of PIWI 'slicer' activity, yet no phasing pattern was observed in mature piRNAs. The loss of Zuc did not affect the 5'- and 3'-end formation of the intermediates, strongly supporting the idea that the 5' end of *Bombyx* piRNA is formed by PIWI slicer, but independently of Zuc, whereas the 3' end is formed by Zuc endonuclease. The *Bombyx* piRNA biogenesis machinery is simpler than that of *Drosophila*, because *Bombyx* has no transcriptional silencing machinery that relies on phased piRNAs.

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186 GRSF1 mediates miRNA-dependent gene regulation in mitochondria

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MicroRNAs (miRNAs) are small noncoding RNAs that influence cellular events by binding target mRNAs and generally triggering their degradation or suppressing their translation. Recent studies have revealed that other types of RNAs, such as long noncoding RNAs and circular RNAs, are also targeted by functional Argonaute (AGO)-associated miRNAs. Regarding their cellular location, mature miRNAs are found in diverse subcellular compartments in the nucleus and cytoplasm. Several studies have identified hundreds of nuclear DNA-encoded miRNAs in mitochondria in different mammalian cells and tissues. However, it is still unclear how those miRNAs are mobilized into mitochondria and whether they contribute to maintaining mitochondrial function and gene expression patterns. Our preliminary data found dozens of mitochondria-targeting miRNAs (mitomiRs) highly abundant in the mitochondrial matrix, the innermost space of mitochondria. Among those miRNAs, we identified several that interacted strongly with a mitochondrial RNA-binding protein, G-rich RNA sequence-binding factor 1 (GRSF1). We studied these interactions by employing methodologies such as RIP-qPCR, miRNA pull-down, and anisotropy. Interestingly, the most promising GRSF1-interacting miRNAs (miR-23a-3p, miR23b-3p, miR-221-3p, and let-7b-5p) displayed a shared sequence motif in the middle of the miRNA sequence. As previously reported, only AGO2 (but not other AGO proteins or miRNA machinery components) was found in isolated mitochondria, and bound miRNAs miR-181c and miR-1; however, it is not clear how AGO2 might influence the function of interacting miRNAs or how mitomiRs might modulate mitochondrial gene expression patterns. Ongoing experiments are aimed at testing the hypothesis that GRSF1 associates with mitomiRs and to mediate their loading onto AGO2 and influence mitochondrial gene expression programs.

187 RNA-seq of Cellular and Extracellular Vesicle RNAs using Thermostable Group II Intron Reverse Transcriptase

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Thermostable group II intron reverse transcriptases (TGIRTs) are ancient enzymes that are thought to be evolutionary ancestors of modern reverse transcriptases (RTs) and have novel biochemical properties that are advantageous for RNA-seq. These include higher fidelity and processivity than retroviral reverse transcriptases, as well as a novel template-switching activity for attachment of RNA-seq adapters to nucleic acid templates without RNA tailing or ligation, enabling RNA-seq library construction from small amounts of starting material in <2 h. Utilizing the beneficial properties of TGIRTs, we developed a new RNA-seq method (TGIRT-seq), which enables: (i) quantitative profiling of protein-coding and long ncRNAs in the same RNA-seq as tRNAs and small ncRNAs; (ii) gives full-length, end-to-end reads of tRNAs and other structured small ncRNAs; and (iii) enables high-throughput mapping of post-transcriptional modifications by distinctive patterns of misincorporation, none of which is possible with retroviral RTs. Validation of the TGIRT-seq method using fragmented human reference RNAs with ERCC spike-ins demonstrated advantages compared to the widely used TruSeq v3 method, including higher strand-specificity, more uniform 5'- to 3'-gene coverage, detection of more splice junctions, particularly near the 5' ends of genes, and elimination of biases inherent in TruSeq. A recent improvement minimizes formation of primer dimers to improve recovery of miRNAs. The ability of TGIRT-seq to construct comprehensive RNA-seq libraries from small amounts of starting material makes it particularly useful for the analysis of extracellular RNAs in human plasma and exosomes (Qin et al. RNA *22*, 111, 2016; Shurtleff et al. PNAS 114, E8987, 2017). We found that exosomes and other extracellular vesicles (EVs) contain predominantly small non-coding RNAs, particularly full-length tRNAs, along with smaller amounts of mRNAs or fragments therof. In recent work, we used TGIRT-seq to analyze the RNA content of different classes of EVs and to follow correlated changes in cellular, exosomal, and other EV RNAs during *in vitro* differentiation of K562 cells into megakaryocytic- and erythroid-like lineages. Our results suggest regulated changes in the RNA content of exosomes and other EVs correlated with changes in gene expression during differentiation.

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188 Disentangling MicroRNA Regulation: Differentiating Direct and Indirect Targets

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MicroRNAs (miRNAs), a class of small non-coding RNAs, are critical regulators of post-transcriptional gene expression. Each miRNA negatively regulates hundreds of mRNAs (direct targets), primarily by accelerated mRNA decay. The repression of direct targets induces downstream regulatory cascades, consequently altering the expression of downstream genes (indirect targets), the majority of which is likely mediated by transcriptional regulation. Together, these direct and indirect targets form a complex regulatory network that defines miRNA's roles in cells. Despite extensive literature on miRNA targeting, efforts to differentiate direct and indirect targets are lacking, limiting our ability to study miRNA function at the systems level. We developed an experimental strategy to reliably identify and differentiate direct and indirect targets of miRNAs by decoupling post-transcriptional and transcriptional regulation. We measured changes in mRNA abundance using RNAseq, and changes in transcription using PROseq, a method that maps engaged RNA polymerase genome-wide. Post-transcriptional changes were computed by subtraction of transcriptional changes, determined with PROseq, from changes in mRNA abundance. To establish the efficacy of this approach, we compared transcriptional and post-transcriptional alterations in stable cell-lines expressing different miRNAs, including miR-1 and miR-122. We identified widespread transcriptional and post-transcriptional changes upon expression of either miRNA. Notably, most post-transcriptionally repressed mRNAs contained predicted miRNA target sites, as expected for direct targets. Furthermore, using CLIPseq, we found that direct targets exhibited enrichment of Argonaute (AGO) binding, an essential component of the silencing machinery, while indirect targets showed negligible enrichment of AGO binding. These results demonstrate the efficacy of our approach in identifying and distinguishing direct and indirect targets. Expression of miR-122 resulted, predominantly, in repression of a set of direct targets, with minimal indirect targeting. In contrast, miR-1 demonstrated extensive direct and indirect targeting, including substantial indirect post-transcriptional regulation. Together, these results suggest that different miRNAs manifest different degrees of direct and indirect targeting; we suspect such differences are likely dependent on the combination of cell-type and miRNA examined. Our approach provides a powerful method to reveal miRNA regulatory networks with precision. We are currently applying these methods to study functions of miRNAs *in vivo* using miRNA knockout mouse models.

189 Role of specific non-coding RNAs and associated proteins in DNA double-strand break repair

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The WD40 domain-containing protein WRAP53β controls trafficking of splicing factors, the telomerase enzyme and small Cajal body-associated (sca) RNAs to Cajal bodies, and its functional loss has been linked to carcinogenesis, premature aging, and neurodegeneration. WRAP53β acts as an essential regulator of DNA double-strand break repair, targeting the E3 ligase RNF8 to DNA lesions by facilitating the interaction between RNF8 and its upstream partner MDC1. By focusing on the RNA-binding properties of WRAP53β, we found that after the depletion of members of the non-coding scaRNA family there is a consistent impairment of accumulation of several DNA repair factors at damage sites and an extensive heterochromatinization of the nucleus. Interestingly, we also found that other proteins associated with scaRNAs and WRAP53β, rapidly and transiently localize to DNA breaks in a PAR-dependent manner. Like depletion of scaRNAs, depletion of these proteins impairsproper accumulation of repair factors to double strand breaks, suggesting a role for these factors in DNA repair, possibly in association with non-coding RNAs. Taken together, our findings indicate that scaRNAs and associated proteins may play a role in DNA double-strand break repair by regulating chromatin state at the sites of DNA damage.

190 Identification of proteins involved in the regulation of viral microRNA biogenesis

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Among the different classes of small non-coding RNAs participating in post-transcriptional gene silencing, microRNAs (miRNAs) represent one of the most studied and well-characterized. These small regulatory RNAs allow cells to fine tune the expression of target mRNAs by translation inhibition and destabilization. Recently some DNA viruses have been described to use the cellular machinery to express their own miRNAs thereby highlighting a new mode of host/virus interaction. The oncogenic Kaposi's sarcoma associated herpesvirus (KSHV) expresses twelve miRNAs, which are all clustered within 3kb at the same genomic locus. They have been shown to be involved in the regulation of multiple cellular targets that are implicated in immune response, cell proliferation or cell death and participate in the transformation of the cell by the virus. Interestingly, although they all derive from the same primary transcript, the level of accumulation of each individual KSHV miRNAs varies widely indicating that there is some level of post-transcriptional regulation at play. We recently showed that the secondary structure of the pri-miRNA can explain some of the differential accumulation of these viral miRNAs, but it seems clear that there are also other factors involved. We have therefore used an RNA pull-down approach followed by mass spectrometry analysis in order to identify for each individual KSHV pre-miRNA the putative co-factors that could modulate their processing by Drosha. We also used two cellular pre-miRNAs, Let-7 and miR-155, as controls for our experiment. This analysis allowed us to generate lists of proteins binding either to individual or several pre-miRNAs and that could favor or hinder their biogenesis. We will present the detailed results and functional validation of this analysis, with a special focus on miR-155 and its viral orthologue miR-K11 that are especially important for KSHV biology.

191 The Sm-core mediates the retention of partially-assembled spliceosomal snRNPs in Cajal bodies until their full maturation

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Cajal bodies (CBs) are nuclear non-membrane bound organelles where small nuclear ribonucleoprotein particles (snRNPs) undergo their final maturation and quality control before they are released to the nucleoplasm. However, molecular mechanism how immature snRNPs are targeted and retained in CBs has yet to be described. Here, we microinjected and expressed various snRNA deletion mutants as well as chimeric 7SK, Alu or bacterial SRP non-coding RNAs and provide evidence that Sm and SMN binding sites are necessary and sufficient for CB localization of snRNAs. We further show that Sm proteins, and specifically their GR-rich domains, are important for accumulating snRNPs in CBs. Consistently, core snRNPs containing the Sm proteins, but not naked snRNAs, restore the formation of CBs after their depletion. Finally, we show that immature but not fully assembled snRNPs are able to induce CB formation and that microinjection of an excess of U2 snRNP-specific proteins, which promotes U2 snRNP maturation, chases U2 snRNA from CBs. We propose that the accessibility of the Sm ring represents the molecular basis for the quality control of the final maturation of snRNPs and the sequestration of immature particles in CBs.

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192 Exploring microRNA cooperation: mapping the Pax6 3′ untranslated region regulatory landscape

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P*AX6* is a highly dosage-sensitive homeodomain transcription factor essential for development of the eye, nose, central nervous system, gut and endocrine pancreas, and is mutated in the haploinsufficiency disease aniridia. Though the physiological mechanisms regulating precise PAX6 expression levels have not been elucidated, post-transcriptional regulation of *Pax6* by miRNAs (miRNA) may represent an important mechanism for maintaining the correct dosage of Pax6. Several microRNAs have been implicated in regulating PAX6 in different cellular contexts. Notably, miRNA-7 has been implicated in transcriptional regulation of *Pax6* in regions of the brain and in the endocrine pancreas. Despite this, the physiological relevance of miRNAs in the fine-tuning and homeostasis of PAX6 *in vivo* remains poorly understood.

We characterized an 876bp mouse *Pax6* 3' untranslated region (3'UTR), and have identified 47 miRNAs predicted to target putative miRNA recognition elements (MREs) in the *Pax6* 3'UTR. Many of these miRNAs displayed distinct expression patterns in *Pax6*-expressing tissues and cells, which may enable cell-type specific optimization of PAX6 through cooperative miRNA regulation. MicroRNA trapping by *in vitro* RNA affinity purification (miTRAP) was used to identify miRNAs interacting with an exogenous *Pax6* 3'UTR-containing transcript in α-TC1-6 cells. Of the miRNAs having predicted MREs in the *Pax6* 3'UTR, 25 were found to interact and cluster into three regions where miRNAs are predicted to cooperatively regulate *Pax6.* Both miR-7a and b were found to interact with the *Pax6* 3'UTR and we have identified two MREs for miR-7 that are highly conserved between orthologous *Pax6* 3'UTR sequences. *Pax6* 3'UTR luciferase reporters suggest that both miR-7 recognition elements function additively to regulate PAX6 levels. To explore the biological relevance of the miR-7 MREs, we generated mice harboring mutations that disrupt one or both sites within the *Pax6* 3'UTR using CRISPR/Cas9 gene editing.

Our findings suggest that, *in vivo*, microRNA regulation can be mediated through redundant interactions. Since aniridia is the result of insufficient PAX6 protein, understanding how miRNAs negatively regulate *PAX6* may enable the development of therapies to block these interactions and de-repress *PAX6*.

193 Characterization of Short Interspersed Nuclear Elements During Herpesviral Infection

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Approximately half of mammalian genomes are comprised of retrotransposons. Among these, a third are Short Interspersed Nuclear Elements (SINEs), which are non-coding RNA elements transcribed by RNA polymerase III (Pol III) that serve as non-autonomous retrotransposons. While their transcription is generally repressed, SINE transcription can be de-repressed under select circumstances, such as embryonic development, viral infection, and other cell stresses. Emerging evidence suggests these elements play dynamic roles as repressors of RNA polymerase II transcription during the heat shock response and can shape species-specific stem cell gene expression.

We previously reported a marked up-regulation in the transcription of murine B2 SINE elements during infection with Murine Gammaherpesvirus 68 (MHV68), a close relative of the oncogenic human herpesviruses. In fibroblasts, we reported that B2 RNAs contributed to the activation of the NF-kB pathway in the cytoplasm of infected cells and were co-opted by MHV68 to enhance viral gene expression. We have now extended these studies to primary bone marrow-derived macrophages and immortalized mouse macrophages in order to address two main questions. To explore the mechanism by which B2 SINEs are transcriptionally upregulated during viral infection, we performed inhibitor screens of a wide variety of kinase pathways and have examined the involvement of the Pol III repressor Maf1. Thus far, we have been unable to identify a particular kinase pathway involved. Unexpectedly, we see that Maf1 appears to play a negligible role in B2 SINE induction. To look for evidence of interplay between B2 SINEs and the host immune response, we monitored differences in transcription of interferons (IFNs) and interferon stimulated genes (ISGs) during infection in the presence or absence of B2 SINEs. Interestingly, we observe that transcription of IFN decreases while levels of some ISGs go up. Our ongoing work will attempt to further describe the viral and host requirements for retrotransposon induction, as well as how SINE RNAs modulate the viral fitness and host response during herpesvirus infection.

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194 Is there an association of *Caenorhabditis elegans* small noncoding RNAs with mRNA quality control pathways?

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Small noncoding RNAs play diverse roles in the regulation of gene expression. In *C. elegans*, endogenous short-interfering RNAs (endo-siRNAs) known as 22G RNAs target thousands of protein-coding mRNAs in pathways known to defend against double-stranded RNA and other "non-self" RNA. 22G RNAs are synthesized by RNA-dependent RNA Polymerase (RdRP) enzymes from mRNA templates; however, the full complement of upstream events that can trigger the biosynthesis of these 22G RNAs is not well-characterized. Using a comparative genomics approach in wild isolates of *C. elegans*, the Youngman Lab has identified a locus (F43E2.6) at which the loss of the stop codon is correlated with a resulting increased production of 22G RNAs. The F43 3'UTR has no in frame stop codons, which introduces the possibility of a novel link between 22G RNAs and a quality control decay pathway characteristically triggered by a lack of a stop codon. In order to ask whether nonstop alleles are generally associated with increased targeting by 22G RNAs, we are using next-generation sequencing to characterize the small RNA transcriptomes of *C. elegans* strains that carry a variety of nonstop alleles. In this initial scan, we have chosen to focus on genes expressed in the germline, where 22G RNAs are most concentrated. To further ask whether 22G RNA synthesis can be triggered by other classes of aberrant transcripts, we are also characterizing the small RNA transcriptomes of worms carrying a well-studied series of nonsense alleles in the germline-expressed gene *sel-1*. Together, these experiments will provide insight into whether small noncoding RNAs might play a role in multiple mRNA quality control pathways in the nematode.

195 The interaction of FUS, U7 snRNP and hnRNP UL1 during the cell cycle of human cells

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FUS plays a role of a positive regulator that activates replication-dependent histone gene expression, in complex with U7 snRNP in the S phase of the cell cycle (1). In contrast, in G2 and G1 phase FUS interacts with heterogeneous ribonucleoprotein (hnRNP) UL1 (1). hnRNP UL1 is known to repress histone gene expression by interaction with U7 snRNP in cell cycle-arrested condition (2). Therefore, we suggested that FUS can mediate U7 snRNP/hnRNP UL1 complex assembly outside of S phase in order to inhibit histone synthesis that could be harmful to the cell.

To confirm the diversified interaction in vivo, we performed proximity in situ ligation assay (PLA) in cells synchronized to different phases of the cell cycle. To further examine the mutual interactions between FUS, U7 snRNP and hnRNP UL1, vectors encoding different fragments of FUS and hnRNP UL1 were prepared. The protein fragments were then transiently expressed in HeLa cells followed by immunoprecipitation and RNA immunoprecipitation. Next, we determined which fragment of FUS and hnRNP UL1 interacts with each other as well as with U7 snRNA, using Western blot and RT-qPCR.

To analyze how interactions of FUS, U7 snRNP and hnRNP UL1 are switched during the cell cycle, we checked posttranscriptional modifications of proteins in different phases of the cell cycle. First, the phosphorylation status of both proteins in cells synchronized to G1, S and G2 phase were analyzed by Phos-tag gel electrophoresis. Second, the interactions between FUS, U7 snRNP and hnRNP UL1 were tested in cells with transient overexpression of FUS and hnRNP UL1 mutants of RGG motifs responsible for arginine methylation.

1. Raczynska et al., 2015, Nucleic Acids Res. 43(20):9711-28.
2. Ideue et al., 2012, PNAS 109(15):5693-8.
3. Zhou et al., 2013, PLoS Genet. 9(10):e10038952013.

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196 Structural basis of G-quadruplex unfolding by the DEAH/RHA helicase DHX36

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Guanine-rich nucleic acid sequences challenge the replication, transcription, and translation machinery by spontaneously folding into four-stranded structures known as G-quadruplexes, the unfolding of which requires forces greater than what most polymerases can exert. Eukaryotic cells host numerous helicases capable of unfolding G-quadruplexes. One such helicase is DHX36, a member of the DEAH/RHA family of helicases, which binds both DNA and RNA G- quadruplexes with uniquely high affinity is consistently found bound to G- quadruplexes in cells and is a major source of G-quadruplex unfolding activity in HeLa cell lysates. DHX36 is a multi-functional helicase that is implicated in G-quadruplex-mediated transcriptional and post-transcriptional regulation, and is essential for heart development, hematopoiesis and embryogenesis in mice. Here, we report the co-crystal structure of bovine DHX36 bound to a DNA comprised of a G-quadruplex and a 3' single-stranded segment. We show that the N-terminal DHX36 specific motif folds into a DNA-binding-induced α-helix that together with the oligonucleotide-binding subdomain selectively binds parallel G-quadruplexes. These structural elements provide the mechanical resistance against which the helicase core, bound to the 3' single-stranded DNA segment, can exert unfolding force. Comparison with our unliganded and ATP-analog-bound DHX36 structures, together with single-molecule FRET analysis of DHX36 mutants suggests that G-quadruplex binding alone induces helicase core rearrangements that drive G-quadruplex unfolding by one residue at a time.

197 RNA-Seq of *in vitro* transcription reactions yields new mechanistic and practical insights.

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T7 RNA polymerase is an ideal model system for transcription and, at the same time, is widely used in research and biotech to produce synthetic RNA for a wide variety of systems and applications. RNA synthesis is a complex biochemical and multi-step process, and is well known to produce both shorter and longer than expected RNA products. In the current work, RNA-Seq of *in vitro* transcription reactions is compared with classical gel electrophoresis under a variety of conditions. This approach yields not just lengths (as with gels), but also detailed sequence information, providing information on the nature and formation of undesired RNA products, and yielding new mechanistic understandings in transcription. The results lead us to new models for the formation of undesired transcription products, with implications for fundamental mechanisms in transcription. The results may also provide practical guidance on how best to avoid undesired products, potentially leading to increased yield and purity of the desired run-off RNA. This approach is readily extendable to a wide variety of systems, and we expect generalized conclusions from these initial studies.

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198 RNA-binding protein Sfpq regulates skeletal muscle metabolism through promoting long-gene expression

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The RNA-binding protein Splicing factor proline/glutamine rich (Sfpq) has been reported as multifunctional in mRNA processing. Recently, we found that Sfpq facilitates the expression of extra-long genes (>100kbp) through promoting transcriptional elongation and comprehensively regulates subsets of genes essential for neuronal development and maturation. Besides neuron, muscle tissue is known to express several extra-long genes-including *dystrophin* (*Dmd*), *titin* (*Ttn*), *laminin α2* (*lama2*), and *sarcoglycan delta* (*Sgcd*)-that have a genomic size from 200 kbp to 2.2 Mbp and can cause muscular dystrophies when they are mutated. To decipher the physiological roles of Sfpq in muscle, we generated skeletal-muscle-specific *Sfpq*-knockout mice ($Sfpq^{SM\text{-}KO}$). *Sfpq* disruption impaired long-gene expression, including *Dmd*, in muscle cells; however, $Sfpq^{SM\text{-}KO}$ mice did not exhibit the typical dystrophic phenotypes. Instead, $Sfpq^{SM\text{-}KO}$ mice caused metabolic myopathy: glycogen accumulation and decreased abundance of oxidative phosphorylation complexes in skeletal muscle. In Gene Set Enrichment Analysis (GSEA) of RNA-seq data from *Sfpq-/-* primary myotubes, downregulated gene subsets were highly enriched in metabolism related pathways that is consistent with metabolic myopathy phenotype observed in $Sfpq^{SM\text{-}KO}$ mice. Down-regulated genes in GSEA highly contains long genes > 100 kbp. These findings uncovered the unknown regulatory mechanism of energy metabolism in skeletal muscle by RBP Sfpq through promoting mRNA expression.

199 EhLINE1 and EhSINE1 expression profiling using Next Generation Sequencing reveals uneven transcript distribution and antisense expression of translationally-silent regions

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Entamoeba histolytica is a primitive parasitic protist. 11% of its genome is comprised of retrotransposons (EhLINEs and EhSINEs). LINE and SINE copies are generally maintained in a transcriptionally silent state with few copies being active. To determine the transcriptional status of *E. histolytica* retrotransposons, RNA-Seq was carried out in triplicate and reads corresponding to EhLINE1 and EhSINE1 were elucidated. Of total 947 copies of EhLINE1, and 493 copies of EhSINE1, 41 LINE1 and 129 SINE1 copies were shown to be transcribed. Of 41 expressed LINE1, 20 were full length while the rest had deletions/truncations. Uneven read distribution of ORF1 and ORF2 was observed with ratio of 1:40 respectively. Reads were coming from three regions of EhLINE1: 1) 5' to 1478 bp; 2) 2400 to 3761 bp; 3) 3990 to 3', corresponding to ORF1, RT and EN domains respectively. Northern analysis with double-stranded probes from different regions (1, 1-2, 2 and 3) showed 1.5-kb bands hybridizing with probes1 and 2, while no signal was obtained with other probes. This corroborated with RNA-seq data, except region3 where no band was seen in northern. It is possible that transcripts of region3 were short and heterogeneous. Read-through transcription was observed for just 2 copies of LINE1 in direct and antisense direction which showed very limited role of neighboring genes in driving the expression of EhLINE1. Further, we looked for antisense expression of EhLINE1 and EhSINE1. Interestingly, 70% of EhLINE1 and 30% of EhSINE1 expressed copies showed antisense expression, with LINE1 at a significantly high level compared with sense strand. Only the reverse transcriptase (RT) and Endonuclease (EN) region of LINE1 showed antisense expression whereas in SINE1 it was seen throughout the element. Antisense transcription possibly explains our western blot data that show constitutive expression of ORF1p, while ORF2p is undetectable. According to the current paradigm of LINE transcription, these elements are transcribed into a single polycistronic transcript from a 5'end internal promoter. Our data suggest a complex transcriptional profile whereby transcripts corresponding to different regions of EhLINE1 accumulate to different levels, and antisense transcripts presumably attenuate translation of some of the transcripts.

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200 Retrocopies nested in other genes and their functions

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Reverse transcription of mature mRNA and subsequent reintegration of cDNA into new genomic localization result in generation of protein coding genes' duplicates, called retrocopies. Part of them, previously considered as nonfunctional pseudogenes, is transcriptionally active and therefore they have potential to play various molecular roles.

In our research, we are focused on retrocopies localized in introns and/or exons of other genes and their possible functions. Based on human retrocopy repertoire from RetrogeneDB2 and Ensembl genes annotations, we found that nearly half of the human retrocopies (47%) overlaps protein coding or non-coding genes and the majority of them is localized in introns. Considering the hypothesis that intronic elements may be involved in transcriptional interference, we decided to study possible interactions between expressed retrocopies and host genes. We searched for retrocopies localized not only in introns, but also downstream of at least one shorter gene isoform. We found 51 retrocopies meeting these requirements. To further investigate their putative regulatory role, we deleted the chosen retrocopy from its host gene in CRISPR-Cas9 experiment. In result, an expression pattern of host gene was changed, as one of the shorter transcripts was barely detectable. The observation suggests that retrocopy transcription may play a role in the regulation of host gene's isoforms expression.

We also analyzed retrocopies integrated, entirely or partially, into exons of protein coding or non-coding genes. Bioinformatic analyses of protein coding transcripts allowed us to identify 18 cases in which retrocopies delivered a start/stop codon, exon or part of functional domain to known proteins. Another interesting group consists of 169 retrocopies, which are incorporated into exons in antisense orientation in comparison to their parental genes. Utilizing the transcript support level and length of retrocopy-derived sequence, we selected 15 strong candidates which could potentially act *in trans* as natural antisense transcripts (trans-NATs) regulating expression of their parental genes.

201 NHP25, a critical factor, regulates a Novel mRNA Polyadenylation Machinery in *Arabidopsis thaliana*

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Polyadenylation is a critical step for nascent messenger RNA (mRNA) maturation. In yeast and metazoans, polyadenylation is mediated by CPSF (cleavage and polyadenylation specificity factor), CstF (cleavage stimulation factor), and other assistant factors. CPSF and CstF complex recognize the poly(A) signal *AAUAAA* and GU-rich motif locating in the upstream and downstream of cleavage site, respectively. After the recognition of these complexes, pre-mRNA is cleaved, and poly(A) tail is added. Although most orthologs involved in polyadenylation exist in plant, the polyadenylation mechanism is still unclear. Here, *nhp25* was identified *in silico* and showed embryonic lethal. The truncation mutant *nhp25-1* showed many biological defects such as abnormal leaf development, late flowering, and abnormal floral buds initiation. RNA profiling of *nhp25-1* revealed that many transcripts showed variable 3' end lengths compared to that in wild type. This result indicated a defective polyadenylation machinery in *nhp25-1*. Furthermore, the *cis*-element *AA(G/N)AAA* was identified as the prime motif which bound by NHP25, and this motif was randomly dispersed within the 200 bp regions surrounding of cleavage site in genes showed viable transcripts length in *nhp25-1*. Interestingly, for the transcripts with unchanged 3' end lengths in *nhp25-1,* this *AA(G/N)AAA* motif majorly enriched at 20bp upstream of cleavage site. This interesting result suggested a novel polyadenylation machinery in plant. The distribution of *AA(G/N)AAA* might be an important issue for determining cleavage site, and the binding of NHP25 on this motif is critical for polyadenylation machinery. Moreover, many floral initiation genes showed cleavage site shifts in *nhp25-1* mutant. These poly(A) site shifts generated different developmental phenotype when we transformed the 3'end isoforms into Arabidopsis. Thus, we speculate that NHP25 is an important regulator that controls a novel poly(A) site selection machinery and mediates floral bud formation through regulates floral genes post-transcriptionally.

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202 Withdrawn

203 HSV1 recruitment of RNA Polymerase II mimics liquid-liquid phase separation

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Over the past decades, the paradigm for understanding the forces driving the formation of sub-nuclear compartments has undergone a major shift. Recent work has convincingly shown that some sub-nuclear compartments have the characteristics of a separate liquid phase, and it has been argued that such liquid-liquid demixing is a mechanism to accomplish either the sequestration of specific macromolecules to a specific area, or to the increase the local concentration of biomolecules to facilitate chemical interactions. The formation of these nucleoplasmic structures are thought to be driven by weak, multivalent interactions that generally map to intrinsically disordered regions of proteins.

During lytic infection, Herpes Simplex Virus type 1 generates replication compartments in the host that effectively recruit many nuclear factors, such as RNA Polymerase II (Pol II). These compartments appear to have many for the descriptive properties associated with phase separated domains: They are highly spherical, they can undergo fusion events, and they have a refractive index that is very different from the surrounding nucleoplasm. Here we show that, despite this appearance, from the point of view of individual Pol II molecules, replication compartments do not behave as a separate phase. Rather, the accumulation of Pol II can be explained by direct non-specific interactions with the viral DNA, resulting in orders of magnitude higher local enrichment without invoking liquid demixing. We propose that such a strategy allows the virus to effectively compete with the host for cellular important resources.

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204 RNAPII ubiquitination promotes co-transcriptional RNA processing

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Numerous links exist between co-transcriptional RNA processing and the transcribing RNAPII. Reversible phosphorylation of the RNAPII C-terminal domain links transcription with RNA processing and surveillance activities. In addition, the catalytic NTD subunit is ubiquitinated on K1246, which is located close to the DNA entry path. Ubiquitinated RNAPII was UV crosslinked to the nascent transcript and found to be enriched over exon 2 regions of intron-containing pre-mRNAs and at poly(A) proximal sites. A mutation $K_{1246}R$ blocked ubitiquination and the distribution of the ubiquitin-resistant RNAPII was reciprocal to Ub-RNAPII around 3' splice-sites. In cells expressing only RNAPII $K_{1246}R$, co-transcriptional splicing was globally impaired. Our results indicated that splicing-associated transcriptional pausing is enforced by RNAPII ubiquitination, promoting co-transcriptional splicing. Accumulation of ubiquitinated RNAPII was also observed ~150 nt upstream of the poly(A) site. In contrast, RNAPII $K_{1246}R$ was depleted at this position relative to total RNAPII, but showed increased occupancy beyond the poly(A) site. These data indicate that RNAPII ubiquitination is associated with slowed elongation or transient pausing upstream of the poly(A) site. We note that an RNAPII elongation checkpoint was previously identified at a similar location upstream of the poly(A) site in human cells (Laitem et al. NSMB, 2015). Notably, polymerases that have passed this region are not only deubiquitinated, but are also largely dephosphorylated on the CTD. Moreover, total RNAPII occupancy is reduced, indicating greater processivity. RNA association of the RNAPII CTD was assessed by *in vivo* crosslinking of a version in which a protease cleavage site was introduced into the linker between the NTD and CTD. This showed elevated association of the CTD with the nascent transcript immediately upstream of the poly(A) cleavage site. We speculate that following satisfaction of a checkpoint, RNAPII elongation is released by deubiquitination, while CTD dephosphorylation facilitates transient interactions with the nascent transcript, promoting polyadenylation signal recognition, and/or off-loading of the polyadenylation factors. We are currently investigating the links between RNAPII ubiquitination and recruitment of the cleavage and polyadenylation machinery.

205 Transcriptional control and biogenesis of intronic RNase P RNA in Drosophila

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RNase P, an essential ribonuclease, is present in all domains of life and is required for the removal of 5' leader sequences from pre-tRNAs. The ribonucleoprotein form of the enzyme is comprised of a catalytic RNA (RNase P RNA, RPR) and a variable number of protein subunits (RNase P Proteins, RPPs). Although RPR is typically a Pol III transcript in most organisms, we found that the Drosophila RPR is inserted into a recipient gene intron and is transcribed by Pol II. By analyzing genomes of other insects and crustaceans, we discovered that this genetic change in the regulation of RPR originated in an ancestor of this major group of animals some 500 million years ago. Transcription and processing from an intron necessitates a distinct mode of biogenesis for the inserted RPR. I am using a genetic approach to uncover the factors involved in RPR biogenesis and to test the functional consequence, if any, of the change in transcriptional control from Pol III to Pol II. Using reporter genes assayed in Drosophila S2 tissue-culture cells, I found the 5' end of the pre-RPR is processed by XRN2/Rat1, and I am currently investigating the exosome and Rexo5 as 3' end-processing candidates. To investigate the involvement of the nine predicted RPPs in RPR maturation, I depleted each individually and assessed RPR maturation by northern blot analysis. Results from this study indicated that selected RPPs are required for RPR maturation and/or stability. Interestingly, however, when I switch RPR transcription back to the ancestral Pol III-mode, RPR is stable and unaffected when RPPs are depleted. This observation inspires the question of whether the Pol III-RPR is active. According to preliminary data, when the Pol III-RPR is expressed as a transgene in S2 cells, it co-purifies with the holoenzyme complex. I am using CRISPR/Cas9 to engineer flies with an ancestral, Pol III-regulated gene, to examine functional consequences from the transcriptional rewiring. Overall my work is expected to provide insights into small RNA biogenesis and how the switch in gene transcription contributed to the evolutionary history of RPR.

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206 Dissecting the mechanisms of RNA polymerase II (Pol II) pausing using RNA aptamers

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Transcriptional regulation plays a fundamental role in controlling cellular fate and function. Such regulation is achieved by a precise coordination between the initiation, elongation and termination phases of transcription. However, pausing of RNA polymerase II (Pol II) near the Transcription start site (TSS) of majority of promoters in metazoans has been shown to be a critical rate limiting step during transcription elongation. Pol II pauses 20-50 bp downstream of the TSS upon interaction with the DRB sensitivity inducing factor (DSIF) and Negative elongation factor (NELF), and requires the kinase activity of Positive transcription elongation factor b (P-TEFb) for pause release. To understand the roles of these factors in pause establishment and release, it is necessary to perturb their interactions and/ or activities *in situ* and observe the changes by high resolution assays. To achieve such perturbation, we have selected RNA aptamers against *Drosophila* transcription factors that bind to their targets with high affinity (K_d ~10-20 nM). In this study, we have tested the functional activities of the DSIF and P-TEFb aptamers using *in vitro* pausing and kinase assays respectively. Our results indicate that the DSIF aptamer interferes with Pol II pausing and competes with DSIF's binding to the transcription elongation complex as well as destabilizes the bound DSIF. In addition to this we have found that the P-TEFb aptamer can interfere with the kinase activity of P-TEFb with an IC_{50} of ~14 nM. These aptamers have been expressed in sufficient levels in the S2 cell nucleus and their effects are studied using genome-wide assays like precision nuclear run-on sequencing (PRO-seq) that provides a genome wide distribution of elongating and paused RNA polymerase. A preliminary survey indicates some changes in pausing upon the expression of the DSIF aptamer. This kind of perturbation strategy employing RNA aptamers would allow us to interfere with specific macromolecular interactions in the genomic context circumventing the possible secondary effects that could occur upon complete protein removal.

207 Live cell analysis of Imd2 expression suggests post-transcriptional regulation in response to intracellular purine levels

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IMP dehydrogenase (IMPDH) catalyzes the first committed step of GTP biosynthesis from the purine nucleotide precursor inosine monophosphate, and thus helps regulate the balance of ATP and GTP. Mutations in the human IMPDH1 gene cause autosomal dominant retinitis pigmentosa type 10 (adRP10) and Leber congenital amaurosis type 11 (LCA11), progressive and congenital blindness disorders, respectively. Evidence suggests that IMPDH is an RNA-binding protein and the disease mutations are located in its putative nucleic acid-binding domain, not the catalytic domain. In *S. cerevisiae*, IMPDH is produced from the *IMD2*, *IMD3*, and *IMD4* genes. Imd2 is uniquely resistant to the IMPDH inhibitor mycophenolic acid (MPA) and transcription of its gene is induced by MPA. Imd2 transcription is regulated by intracellular GTP levels through the use of alternative transcription start sites (TSS) that elicit or bypass transcription termination in the 5'-UTR and early ORF. When GTP is replete, TATA-proximal TSS are used and the transcript contains a Sen1-dependent terminator resulting in attenuated transcripts. When GTP is depleted by MPA treatment, TATA-distal TSS are used, bypassing the terminator and producing full length mRNA. We showed previously that simultaneous treatment with MPA and guanine results in the predominant use of upstream, non-productive TSS, as expected since guanine can be directly incorporated into GMP by HGPRT. Yet live cell microfluidic analysis of Imd2-GFP protein expression reveals that MPA+guanine treatment increases Imd2 protein level beyond that seen by MPA alone. These paradoxical results suggest post-transcriptional regulation of Imd2 expression occurs, possibly in response to ATP level. Intriguingly, Imd2-GFP levels cycle when cells become confluent, and the onset of cycling is delayed by exogenous adenine. Preliminary data also suggest that an adRP10 mutation in *IMD2* disrupts the regulation of Imd2 expression in response to both GTP and ATP levels. We are further exploring the complex mechanism of regulation of this key enzyme in nucleotide metabolism.

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208 The involvement of polyadenylation factors PCFS4 and CstF64 in proper flower development in Arabidopsis thaliana

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Eukaryotic messenger RNAs are produced from primary transcripts as a result of extensive processing events, including addition of the cap structure at the 5' end, splicing, and cleavage and polyadenylation at the 3' end of pre-mRNAs. The poly(A) tail protects mRNA from degradation, and it is required for translation initiation. About 70% of Arabidopsis genes have more than one polyadenylation site and alternative selection of polyadenylation sites can change the mature transcript (e.g. inclusion or exclusion of regulatory elements, shortening of 3'UTRs). This process is called alternative polyadenylation (APA). One of the best studied example of APA in plants is connected with the control of flowering time. PCFS4 is an important factor involved in this process. PCFS4 is an Arabidopsis homologue of the yeast polyadenylation factor Protein 1 (Pcfs11) that is a subunit of the Cleavage Factor 1 complex (CFI). However, unlike the yeast Pcfs11, its plant homolog is not essential for viability, but instead plays a regulatory role in pre-mRNA processing of the specific subset of plant genes. In addition to PCFS4 in Arabidopsis there are three other Pcfs11-like proteins: PCFS1, PCFS2 and PCFS5. We show that PCFS4 directly interacts with another polyadenylation factor, CstF64, and this interaction is crucial for the regulation of flowering time in Arabidopsis plants. Both polyadenylation factors, PCFS4 and CstF64, are also required for proper development of flowers.

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209 Structure of a paused RNA polymerase stabilized by an RNA hairpin and transcription factor NusA

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Transcriptional pausing by RNA polymerases (RNAPs) is a key mechanism to regulate gene expression in all kingdoms of life, and is a prerequisite for transcription termination. The essential bacterial transcription factor NusA stimulates both pausing and termination of transcription, thus playing a central role. Here we report single particle electron cryo-microscopy (cryo-EM) reconstructions of NusA bound to paused *E. coli* RNAP elongation complexes with and without a pause-enhancing RNA hairpin in the RNA exit channel. The structures reveal four interactions between NusA and RNAP that suggest how NusA stimulates RNA folding, pausing and termination. An asymmetric translocation intermediate of RNA and DNA converts the active site of the enzyme into an inactive state, providing a structural explanation for the inhibition of catalysis. Comparing RNAP at different stages of pausing provides insights on the dynamic nature of the process and the role of NusA as a regulatory factor.

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210 Exploring the Capping Code: CleanCap™ Co-transcriptional Capping Allows the Syntheses of Cap 0, Cap 1, Cap 2 and $^{m6}A_m$ Capped Messenger RNAs

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Messenger RNA (mRNA) therapy is a popular platform technology for expressing proteins in cells or *in vivo* because there is minimal risk of insertional mutagenesis. mRNA transfection is used to express proteins for genome editing, protein replacement, vaccines and antibody expression. To avoid an innate immune response, transfected mRNAs should mimic the 5' cap structure of non-immunogenic endogenous mRNAs.

During eukaryotic RNA capping, Cap 0 (^{m7}GpppN) is formed as an intermediate. Methylation of the 2' ribose position of the first cap-proximal nucleotide forms Cap 1 (^{m7}GpppN$_m$N). In ~50% of transcripts, the 2' ribose position of the second cap-proximal nucleotide is also methylated to form Cap 2 (^{m7}GpppN$_m$N$_m$). N6-methylation of adenosine at the first cap-proximal nucleotide (^{m6}A$_m$N) is the second most frequently found modification in mRNA and occurs in conjunction with Cap 1 (and potentially Cap 2).

The immunogenic role of mRNA caps requires elucidation. Viral attenuation occurs after deleting methyltransferases that RNA viruses encode to convert Cap 0 to Cap 1. IFITs bind Cap 0 and activate antiviral translational repression. Thus, Cap 1 (and possibly Cap 2) marks endogenous mRNAs as "self" RNAs. The role of Cap 2 and ^{m6}A$_m$ is poorly understood because such capped mRNAs have not been produced synthetically at scale. In a recent study, ^{m6}A$_m$ caps increased stability and translation and decreased mRNA de-capping (Mauer et al., Nature 2016).

Traditional co-transcriptional capping utilizes ARCA (Anti-Reverse Cap Analog) to produce immunogenic Cap 0 with poor capping (~70%) and low yield. Post-transcriptional enzymatic capping to produce Cap 0 or Cap 1 is hindered by highly structured 5' ends, requires further purification and is expensive. Methods to produce Cap 2 mRNAs have not been commercially available. We developed CleanCap™, a novel co-transcriptional capping method to yield Cap 0, Cap 1, Cap 2, ^{m6}A$_m$ or unnatural caps. Capping with CleanCap™ is reproducibly efficient (90-99%), less expensive than enzymatic capping and is done in a "one pot" reaction without additional purification. Our studies in a THP-1 Dual monocyte cell line indicate that these various CleanCap™ mRNAs exhibit altered expression and immunogenicity. Further *in vivo* studies to characterize these mRNAs are ongoing.

211 Enzymatic Site-Specific Labeling of RNA for Affinity Isolation of RNA-Protein Complexes

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Intricate networks of RNA-protein complexes underlie cellular homeostasis through their roles in gene regulation and downstream cellular processes. Therefore, identification of these RNAprotein interactions is crucial to our understanding of cell biology and disease. Building on our recently developed enzymatic technique, RNA-TAG (Transglycosylation At Guanosine), we demonstrate the site-specific labeling and affinity purification of cellular RNA transcripts. This technology takes advantage of a bacterial tRNA guanine transglycosylase (TGT) for the incorporation of nucleobase derivatives bearing functional probes, such as biotin, into an encodable RNA stem loop recognition motif. Using this methodology, we have demonstrated that an RNA of interest can be selectively biotinylated in live mammalian cells or cell lysate. Affinity purification enables high levels of enrichment of the target RNA complexes. Through the development of RNA-TAG, we aim to create a robust, convenient methodology for the elucidation of RNA function via affinity purification of cellular RNA-protein complexes.

212 Aminoglycoside receptors reveal patterns in RNA recognition and conformational change

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Elucidating the critical elements in folding and recognition of dynamic RNA structures remains a challenging problem. These challenges hinder efforts to understand RNA biology at the molecular level and to fully pursue RNA as a potential drug target. We have developed a method to use pattern-recognition of RNA with small molecules (PRRSM) to decipher patterns in RNA recognition as well as structural classification. In this application, we use aminoglycoside derivatives as receptors and fluorescently-labeled RNA constructs as analytes. This method allows for rapid assessment of patterns in small molecule:RNA recognition via plate reader measurements and principal component analysis, revealing similarities and differences in the recognition of distinct RNA structures. We have discovered that: 1) RNA secondary structure classes dictate the first level of classification, even with variation in sizes and sequences; 2) slightly denaturing conditions (PEG, 37C) allow for increased differentiation of *individual* RNA constructs, presumably due to increased RNA dynamics; and 3) these classifications can be used to predict secondary structure at the fluorescently labeled site, leading to rapid observations of riboswitch conformational changes and revealing nucleotides critical for functional folding. We are currently expanding this procedure to include diverse small molecule scaffolds and more complex RNA structures.

213 Isolation, Enrichment, and Identification of Acetyl-CoA Modified RNAs

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Acetyl-CoA is an evolutionarily conserved biomolecule that occupies a central position in the cell; connecting the metabolism of glucose, fatty acids, and amino acids. Moreover, acetyl-CoA also plays an important epigenetic role in gene expression and cellular differentiation processes. Recently, several groups have found that a 3′-dephosphoacetyl-CoA (dCoA) modification is incorporated on the 5′ terminus of RNAs both *in vivo* and *in vitro.* However, the conditions under which the RNA is modified, and the biological role this modification plays in gene expression remain unknown. Additionally, the abundance of dCoA is estimated to be only 80-120 modifications per cell, requiring novel sensitive approaches to capture the RNAs of interest. This study utilizes a bio-orthogonal approach to identify RNAs of interest in *E. coli*. Cells were cultured in the presence of panthetheine analogues (containing either an azide or alkynl moiety), which are processed *in vivo* by cellular biosynthetic pathways into mature dCoA. Future work will focus on enrichment of modified RNAs for RNA-Seq, as well as determining the conditions under which this modification is altered. The completion of this work will provide chemical tools for identifying RNAs with acetyl-CoA modifications and will help elucidate the importance of metabolic changes on gene expression and cellular identity.

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214 Cell – SELEX with Modified Nucleotide

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Our main research interest is the development of a biosensor that detects foodborne pathogens by using DNA aptamers for food safety. DNA aptamer that has high affinity and specificity to the target pathogens is identified by Cell – SELEX, Systematic Evolution of Ligands by EXponential enrichment. Our target cell is *Escherichia Coli* K12. The DNA aptamers selection for deoxyuridine aptamers (dU-Ap) uses deoxyuridine instead of thymidine as well as the other three common dNTPs. The 2'-H in deoxyribose gives DNA more chemical stability than the 2'-OH present in the RNA structure. However, RNA is able to form more conformations due to this structural difference with DNA. Therefore, the dU-Ap that are identified will have the stability of DNA and the structural variety of RNA providing for a broader selection of aptamers and a higher specificity when binding to *E. coli*. This project compares the sequences, stability, and structure of the two DNA aptamers: deoxythymidine aptamers (dT-Ap) and deoxyuridine aptamers (dU-Ap).

215 SPR-SELEX: Determination of DNA Aptamers that Bind to *E. coli* for Biosensor

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Pathogenic *Escherichia coli* (*E. coli*) strains can cause food poisoning. One possible way to prevent food poisoning is by detecting the harmful *E. coli* via biosensor with DNA aptamer. The DNA aptamer sequence that has high affinity and specificity to the target is determined by the systematic evolution of ligands by exponential enrichment (SELEX) technique. However, the current SELEX procedure is time-consuming because the selection progress cannot be monitored in real time. Surface plasmon resonance (SPR) measures the molecular binding kinetics in real-time without any labeling. In this project, we show the new SELEX methods combined with SPR technique (SPR-SELEX) to identify the DNA aptamers. The SPR-SELEX is a faster aptamer selection method compared to current SELEX.

216 Detection of the m6A-regulatory-enzymes activities using an endoribonuclease, MazF

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N^6-methyladenosine (m6A) is the most abundant internal RNA modification, which is mainly found at the RRACH sequence (R: G/A, H: U/A/C). The modification regulates various physiological processes. The levels of m6A methylation are controlled by m6A regulatory enzymes, such as FTO and ALKBH5 demethylases or METTL3/METTL14 methyltransferases. To characterize these m6A regulatory enzymes and to find their inhibitors, it is required to develop new methods to easily detect their enzymatic activities. Here, we constructed a convenient strategy to detect enzymatic activities of these demethylases and methyltransferases in a high-throughput manner without using any specific apparatus or radioisotopes.

MazF is a bacterial toxin involved in growth regulation responded to stress. It has been shown that MazF cleaves single-strand RNA at ACA triplet in a sequence-specific manner. The cleavage of RNA fragment by MazF can be easily detected by FRET-based plate assay as well as by polyacrylamide gel electrophoresis (PAGE). Using these methods, we herein clarified that MazF is an m6A-sensitive endoribonuclease specific for ACA sequences. The sensitivity of MazF to m1A but not to 5mC was also demonstrated. In addition, we established a new method for evaluation of m6A demethylases and methyltransferases in a high-throughput manner, which could be applicable to inhibitor screening.

217 An RNA Chemical Proteomics Approach Reveals the N^6-methyladenosine (m^{6}A) interactome

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Chemical modifications on biological macromolecules play a critical role in cellular physiology. In particular, RNA is extensively modified with a diverse array of post-transcriptional modifications, including "epitranscriptomic" modifications that occur on mRNA. The functions of epitranscriptomic modifications, including their effects on protein translation and mRNA stability and localization, remain largely unknown. This gap in our knowledge stems in large part from the major challenge in understanding the effect of these modifications on protein-RNA interaction networks. Here, we develop a chemical proteomics approach relying upon diazirine-containing RNA probes and quantitative proteomics to profile 'readers' of N^6-methyladenosine (m^{6}A), the most abundant internal mRNA modification in eukaryotes. In addition to identifying known m^{6}A binders, including YTH-domain proteins and ALKBH5, we identify novel disease-associated proteins that 'read' this modification. Interestingly, we also find that m^{6}A disrupts RNA binding by proteins commonly found in stress granules. Our results reveal the m^{6}A-regulated protein-RNA interactome in human cells, and provide a general framework for investigating the function of diverse RNA modifications.

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218 The understudied splicing kinase PRPF4 as a medicinal chemistry target

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The Pre-mRNA Processing Factor 4 kinase (PRPF4) is an enzyme that has been implicated in chromatin remodeling and downstream splicing regulation. The protein has been linked to cancer development and drug resistance in ovarian and breast cancers, however, PRPF4 is still considered an understudied kinase. We are studying structural and molecular aspects of PRPF4 function using a medicinal chemistry approach. For this, we are combining the development of a chemical probe to PRPF4 with phenotypic characterization in triple negative breast cancer cells lines. Previously published data pointed to a potent PRPF4 kinase inhibitor compound (IC_{50} = 16 nM in enzymatic assay) but failed chemical probe criteria, requiring improvements in selectivity and cellular permeability. Using this compound as a starting point, we designed a series of 42 new compounds with improved cellular penetrance and selectivity but with lower potency (K_D = 84 nM by ITC). We are currently improving selectivity over two detected off-targets (data from a 320 kinases panel) using structure-based design aided by protein crystallography and molecular docking. On the cellular aspect we have generated stable cancer knock down MDA-MB231 (triple negative) cell lines where we have observed cell cycle arrest. Next, we will continue the improvement of our chemical series and we will subject the knockdown cells to RNA-seq in order to evaluate overall splicing chances. Ultimately, we will compare de splicing map of the genetic knockdown of PRPF4 to the inhibition by a chemical probe.

219 Discovery of selective RNA-binding small molecules by affinity-selection mass spectrometry

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Recent advances in understanding the relevance of non-coding RNA (ncRNA) to disease have increased interest in drugging ncRNA with small molecules. The recent discovery of ribocil, a structurally distinct synthetic mimic of the natural ligand of the flavin mononucleotide (FMN) riboswitch, has revealed the potential chemical diversity of small molecules that target ncRNA. Affinity-selection mass spectrometry (AS-MS), a biophysical high-throughput screening (HTS) technique, has historically been applied to protein drug targets, but is also theoretically capable of finding small molecules binding to ncRNA.

Here we report the first application of the Automated Ligand Detection System (ALIS), an indirect AS-MS technique, for the selective detection of small molecule-ncRNA interactions. The wild-type FMN Riboswitch and a variety of mutant and FMN riboswitches were each screened against an array of 53,000 antibacterial-active drug lead compounds, and screening results for each riboswitch variant were compared to determine target-ligand selectivity profiles. Relative binding affinities and binding competition against the natural ligand were also investigated with ALIS. Detailed structural analysis of the ligand-RNA binding modes was conducted using other biophysical techniques, including NMR and X-ray crystallography. Crystal structures reveal that different types of ligands can induce distinct conformations of the FMN riboswitch, and are consistent with different activity profiles exhibited by these ligands. Our findings validate the ALIS platform for high throughput screening for RNA-binding small molecules, and represent a novel strategy that can be used to screen ncRNAs against large arrays of small molecules to find new therapeutics.

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220 Targeting therapeutically relevant non-coding RNA with small molecules: Identification of selective RNA-binding compounds using ALIS

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Approximately 60% of DNA is transcribed as RNA, but only 1-2% codes for proteins. Genetic linkage studies of single nucleotide polymorphisms show that the numbers of non-coding RNAs (ncRNA) involved in cellular processes are similar to the numbers of protein coding genes. The function of ncRNA and its interactions with small molecules is relatively unexplored, and understanding the druggability of ncRNA with small molecules will likely open up new therapeutic approaches for various diseases.

In order to understand ncRNA-small molecule druggability, we have investigated whether the Automated Ligand Identification System (ALIS) can be validated for detection of RNA-small molecule interactions. ALIS is an affinity-selection mass spectrometry platform capable of high-throughput screening for small molecules that bind to proteins, and has been routinely used to detect hundreds of thousands of protein drug target-small molecule interactions per instrument per day. We have validated the ALIS system with naturally occurring ncRNA bacterial regulatory elements (i.e. riboswitches) and known small molecule drug leads.

In order to extend this work and explore the small molecule binding profiles of other ncRNA targets, we next identified over 40 ncRNA sequences from a range of ncRNA classes and disease correlations. Using ALIS, these 40 ncRNA targets have each been screened against chemically diverse small molecule collections, functionally annotated collections from previous phenotypic screens, and collections enriched in RNA-binding properties (60,000+ compounds total). To date, we have generated millions of screening data points from which we are revealing new targets and mechanisms involving small molecule-ncRNA interactions. Here, we outline our approach, results so far, and discuss their implications for wider small molecule drug discovery efforts.

221 Theophylline-dependent riboswitches as tools to control gene expression in *Arabidopsis thaliana*

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Regulation of gene expression upon binding of a ligand to mRNA transcripts is an interesting and promising approach for applied and basic biology. Ligand-dependent regulation of gene expression is inherent to natural riboswitches, which are widely found in different organisms from bacteria to eukaryotes. Riboswitches typically comprise a ligand-binding aptamer domain linked via a communication module to an output domain, also called expression platform. Binding of a metabolite to the aptamer domain results in a conformational change within the expression platform thereby affecting the expression of the corresponding gene. Based on the principles of natural riboswitch regulation, a versatile set of engineered riboswitches (aptazymes) has been developed by the combination of RNA-based ligand sensing and regulating domains.

Aptazymes composed of a self-cleaving ribozyme and a theophylline aptamer have been shown to mediate the regulation of gene expression in a number of model organisms, including *E. coli*, yeast, *C. elegans* and human cells. Switching direction and efficiency was found to depend on the position of the aptazyme in either the 3'-UTR or the 5'-UTR of the transcripts. We aimed to establish aptazymes as a tool for the external regulation of transgene expression in plants. Switching efficiency is optimized by altering the position and testing different combinations of aptamer, communication module and ribozyme.

Here we present the detailed characterization of a theophylline-induced aptazyme inserted in the 3' UTR of GFP reporter gene in *Arabidopsis thaliana*. The riboswitch allowed theophylline-induced downregulation of GFP transcript and protein levels in a dose- and time-dependent manner with a maximum 10.4-fold change in transcript level that translated to maximally 33.3-fold changes of the protein levels. Besides the regulation of the reporter gene GFP we also demonstrate the functionality of the riboswitch for conditional complementation of seedling-lethal *One Helix Protein1* mutants.

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222 Riboglow: A multi-color riboswitch-based platform for live cell imaging of mRNA and small non-coding RNA in mammalian cells

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Spatiotemporal dynamics of coding and non-coding RNAs play central roles for many cellular processes in mammalian and bacterial cells. For example, mRNAs are transiently sequestered in granules upon different stressors in mammalian cells. Currently, visualization of RNA in cells is largely limited to methods that require fixing and staining of cells, with the notable exception of the recently developed "Spinach/Broccoli" and "Mango" aptamers, and the MS2-GFP system.

We developed a complementary platform to visualize RNA live in mammalian cells that combines small size, robust applicability and multi-color fluorescent tags. A small RNA aptamer is attached as a fusion to an RNA of interest and addition of an organic probe induces fluorescence. This platform is based on the bacterial cobalamin riboswitch where the riboswitch RNA functions as the RNA aptamer tag. We synthesized variants of cobalamin that are covalently attached to a series of organic fluorophores. Cobalamin itself functions as a fluorescence quencher and we show that the fluorescence signal increases upon binding of the cobalamin-fluorophore probes to the riboswitch RNA. Importantly, the riboswitch binds cobalamin, but not the fluorophore, allowing us to alter fluorophores easily without compromising RNA-probe affinity.

We have demonstrated that mRNA recruitment to stress granules can be visualized by the riboswitch-based RNA tag in live cells using two different probes with different colors. Furthermore, we have tagged U1 snRNA using our riboswitch tag and demonstrated recruitment to cytosolic granules (U-bodies) in live cells. Together, we have developed a small and versatile RNA-tag that can be attached to diverse RNAs of interest while only minimally disrupting function.

223 A novel tool for riboswitch discovery: the Shifted-Reverse PolyAcrylamide Gel Electrophoresis.

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During the previous decades, new classes of noncoding RNAs having important regulatory functions have been discovered including riboswitches. Riboswitches are RNA molecules mostly found within the 5′ untranslated region (UTR) of mRNAs. They are composed of an aptamer domain that can bind specifically to a ligand and of an expression platform that allows to turn on or off gene expression according to a conformational change of the motif. Numerous riboswitches have already been discovered in almost all studied bacteria, but limits associated with a bioinformatic approach make it increasingly difficult to discover new riboswitches.

The goal of my project is to discover new riboswitches by a method developed in our laboratory called the Shifted-Reverse Polyacrylamide Gel Electrophoresis (SR-PAGE). This technique is based on the ability of riboswitches to change their conformation upon binding of the ligand. This structural change induces a modification of migration in a native polyacrylamide gel. We apply this method on RNA libraries made with several genomic DNA from different organisms including pathogenic bacteria like Mycobacterium tuberculosis, as well as archaea and eukaryotes. Next-generation sequencing is used to verify libraries and riboswitch enrichment. Several ligands can be tested simultaneously for the new riboswitch selection. This is done together with glycine, a ligand for a known riboswitch that is used as a positive control. Furthermore, a thiamine pyrophosphate (TPP) riboswitch-based degenerated library was converted for preferential thiamine binding, instead of TPP with SR-PAGE.

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224 Optimization of CRISPR/Cas9-mediated base editing for targeted genetic screens

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Cas9, an RNA-guided DNA endonuclease, has proven to be a powerful tool for inducing locus specific effects in mammalian cells, both *in vivo* and *in vitro*. It is used routinely for knockout and knock in generation, as well as transcriptional activation and silencing, endogenous labeling and most recently, DNA base editing. Fusion of nuclease-dead or nickase Cas9 with cytidine or adenine deaminases facilitates the conversion of bases within a targeted locus from C to T or A to G, respectively. Recent advances have seen the engineering of a variety of base editors capable of inducing a range of mutations, from single base pair changes to widespread mutagenesis of target loci. Further, these mutations can be induced at high efficiency and without the introduction of potentially toxic double strand breaks. Thus, base editing provides an attractive platform for genetic screening approaches that were previously untenable with standard Cas9. Here, we present our optimization of base editing in human and mouse cancer cell lines, and demonstrate its potential for functional genomic screening.

225 New biophysical approaches to study IRES/ribosome interactions

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Translation initiation, in both eukaryotes and bacteria, requires essential elements such as mRNA, ribosome, initiator tRNA and finally initiation factors. For each domain of life, canonical mechanisms and signals are observed to initiate protein synthesis. However, in some cases other ways of initiation can be used, as for viral mRNAs.

Viruses hijack cellular machinery to translate some of their mRNAs through a non-canonical initiation pathway using Internal Ribosome Entry Site (IRES), a highly structured RNAs which can directly recruit the ribosome.

Here we took advantage of innovative biophysical approaches to study interactions between the intergenic IRES from the cricket paralysis virus (CrPV) and the eukaryotic yeast ribosome. Isothermal Titration Calorimetry (ITC) and kinetic ITC (kinITC) provided thermodynamic and kinetic data. A comparison is made with data collected on a biosensor using the new switchSENSE technology, based on electroswitchable DNA chips.

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226 *In vitro* characterization of Cas12a (Cpf1) endonucleases as tools for molecular biology

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Cas12a (Cpf1) proteins are RNA-guided endodeoxyribonucleases from Class II, TypeV CRISPR systems. Cas12a orthologs require short ~40 nt single guide RNAs for dsDNA cleavage activity, can process polycistronic guide RNA transcripts into individual mature guide RNAs, and generate a staggered cut distal to protospacer adjacent motifs (PAMs). This contrasts with Cas9 RNA-guided nucleases from Type II CRISPR systems that require 2 guide RNAs supplied individually or fused into a single ~100 nt guide RNA, require additional factors for guide RNA maturation, and cleave dsDNA proximal to a PAM sequence.

Because of their inherent versatility and potential usefulness for manipulating DNA *in vitro* and *in vivo*, we aimed to characterize *in vitro* properties of Cas12a in detail. Here we present data consistent with other recent reports showing that Cas12a has collateral nuclease activity which degrades non-target DNA in *trans* and that is activated only upon binding and cleaving a target DNA. In addition we show data suggesting that some C-rich PAM sequences can be tolerated by Cas12a orthologs in addition to the canonical T-rich PAMs.

227 Nanoimaging of structural domains in long single-stranded RNA molecules

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Determination of the secondary structure of long single-stranded RNA molecules (>1 kb) is generally a tedious process. We are endeavoring to use Atomic Force Microscopy (AFM) imaging combined with automated data analysis algorithms as a high-throughput approach to characterize and model RNA structure. To obtain images of RNA secondary structure, the RNA molecules are diluted in a low salt solution which leads to a loss of tertiary structure, but preservation of much of the native secondary structures due to the hierarchical nature of RNA folding. To analyze these molecules, volume profiles are generated using a MATLAB-based algorithm to calculate the relative volume of secondary structural domains, which is then used to estimate the range of nucleotides in each one. Proof-of-concept for this approach was evaluated by comparing AFM data to the largest RNA molecule with an existing 3D structure, the human 28S ribosomal RNA (rRNA), in which we were able to identify 5 out of the 6 canonical domains. In addition, we further apply these procedures to identify and model structural domains in viral RNA molecules. We have identified large structural domains encompassing the 5' and 3' splice sites of the influenza NS mRNA. These domains are much larger (>200 nt) than previously reported structures in these RNA molecules and both have branched and unbranched conformations. Structural models are proposed for the varying conformations and validated with the use of minimal domain constructs. These findings demonstrate the utility of using AFM for identifying and modeling RNA secondary structure, as well as identifying co-existing domain conformations in a population of RNA molecules.

228 Identification of Rbfox associated proteins in striated muscles by BioID

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Rbfox family proteins contain a single highly conserved RNA recognition motif domain that binds preferentially to the UGCAUG, and affect the target exon inclusion or skipping in a position-dependent manner. Our lab has previously reported that both Rbfox1 and the Rbfox2 genes undergo tissue-specific alternative splicing and produce multiple isoforms specific to brain, heart and skeletal muscle (Nakahata and Kawamoto, 2005). However, the function of Rbfox isoforms and their protein interactions remains to be elucidated. Here, we used a proximity-labeling proteomics approach called BioID (Proximity-dependent Biotin identification, Roux et al. 2012) to identify the protein interaction network of individual Rbfox2 isoforms. This technique is particularly useful in identifying weak or transient interactions in living cells that are not detected by co-immunoprecipitation or yeast two-hybrid systems.

We used a C2C12 myoblast cell line to perform all our Rbfox2 BioID experiments since C2C12 cells can be differentiated into myotubes in culture, similar to primary myoblasts. In these cells, individual Myc-BirA*Rbfox2 isoforms are localized identical to Rbfox2 isoforms harboring the N-terminal Myc tag, and the BirA* fusion does not appear to alter the splicing activity of Rbfox2 isoforms. Next, we tested the biotinylation of endogenous proteins in cells expressing Myc-BirA*Rbfox2 isoforms, either in the presence or absence of exogenous biotin, using western blots probed with streptavidin. The results indicate that in the presence of exogenously added biotin, Myc-BirA*Rbfox2 isoforms induce biotinylation of a wide range of endogenous proteins. These biotinylated proteins reside in the same cellular compartment and colocalize with Myc-BirA*Rbfox2 isoforms by immunofluorescence microscopy. These results suggest that Myc-BirA*Rbfox2 can be targeted to a same cellular location as Myc-Rbfox2 fusion proteins and biotinylate endogenous proteins. We then used affinity capture to isolate the biotinylated proteins in undifferentiated and differentiated C2C12 cells expressing individual Myc-BirA*Rbfox2 isoforms and identified them by mass spectrometry. The identified proteins are highly enriched in gene ontology terms related to mRNA metabolic processes, post-transcriptional regulation of gene expression and spliceosome assembly.

229 Using CRISPR-Cas9 reagents for transcriptional activation to improve understanding of gene function

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The CRISPR-Cas9 system has been adapted to generate CRISPR activation (CRISPRa) technology used for transcriptional activation and upregulation of gene expression. CRISPRa is one of the most promising applications, because genes are upregulated from their endogenous promoter and genomic context which is a significant improvement over traditional vector-based gene overexpression technologies. In the past, gene overexpression was performed with open reading frame expression vectors. However, some genes are difficult to clone, others are difficult to express exogenously and expression from non-native promoters can result in aberrant phenotypic effects. The development of synthetic guide RNAs in CRISPRa for robust gene transcriptional activation will be described.

230 Thermal profiling for tracking of interactions between RNA-binding proteins and their RNA targets.

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RNA-binding proteins (RBPs) are key players in cell biology and gene regulation. The study of RNA-protein interactions in vivo is complex, and current techniques have been challenging for non-abundant targets and often relied on the insertion of tags. To address these issues, we are exploring thermal proteome profiling (TPP), which is based on the temperature-sensitive stabilisation of proteins by binding of their endogenous ligand(s), e.g. RNA, and assessment by mass spectrometry (Savitski M.M., et al., 2014). We want to know whether TPP can be applied specifically in the investigation of RBP-RNA interactions on a proteome-wide scale in an unbiased manner ('meltRBP').

We piloted the methodology with the help of a well-characterised interaction: iron regulatory proteins (IRPs) and their RNA targets containing the iron-responsive element (IRE). IREs are stem loops of 25 to 30 nucleotides that are commonly found in the untranslated regions (UTRs) of mRNAs. Most IRE-containing mRNAs encode proteins that are important in iron metabolism. Iron regulatory proteins, specifically IRP1 and IRP2, bind IREs in iron-deficient cells and induce differential gene expression depending on the location of the IREs in the UTRs (Hentze, M. W., et al., 1987). When iron levels are high in cells, IRP1 assembles an iron-sulphur cluster (4Fe-4S) and this alters its conformational state rendering it inactive for IRE binding, while IRP2 RNA-binding activity is diminished by proteasomal degradation (Wallander, M.L., et al., 2006).

Specifically, we utilised alterations in cellular iron availability and mutagenesis of the IRE of HIF2a mRNA. Inclusion of these controls suggests that meltRBP is able to specifically identify the IRP1-IRE interaction in Hela cells by determining a shift in IRP1's melting behaviour. With the help of mass spectrometry, we also expect to be able to determine whether other proteins are potential IRE-binding proteins.

In summary, this project aims to adapt an innovative proteomic technique to the study of endogenous RBP-RNA interactions.

Hentze, M. W., et al., Science, 1987. 238: 1570-1573.

Savitski, M.M., et al., Science, 2014. 346(6205):1255784.

Wallander, M. L., et al., Biochimica et Biophysica Acta, 2006.1763: 668-689.

231 VIR-CLASP reveals unexpected interactions between host-encoded pioneer RNA binding proteins and the pre-replicated RNA genome of Chikungunya virus.

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The success of an RNA viral pathogen to infect a cell can be defined by its ability to co-opt host factors, compete for endogenous gene expression machinery, and ultimately copy and re-package its genome at the expense of cellular resources and processes. Indeed, a virus that is able to replicate its genome marks a milestone in its lifecycle, having bypassed innate immune pattern recognition pathways and is on its way to gathering sufficient viral and host proteins for packaging and assembly. Thus, a particularly vulnerable phase of infection comprises of the steps prior to viral replication. Yet our molecular understanding of host-pathogen interactions, particularly between the viral RNA genome and cellular proteins that would interact with it, are lacking in large part due to an inability to distinguish interactions with first-generation viral genomes versus newly synthesized transcripts or subgenomic fragments. To specifically characterize interactions that occur between pre-replicated viral RNA genomes and cellular proteins, we developed a novel approach termed VIR-CLASP (Virus Induced Ribonucleoside-analog enhanced Cross-Linking And Solid-phase Purification). VIR-CLASP combines photochemical biology and RNA isolation methods with mass spectrometry to broadly identify proteins that directly interact with viral RNA only during the earliest events of infection, between nucleic acid entry and replication. Using this approach, we investigated early host-pathogen interactions during infection of human cells with Chikungunya virus (CHIKV), a zoonotic positive-sense ssRNA pathogen that is re-emerging as a global threat. We report the identification of hundreds of direct interactions between host cellular proteins and pre-replicated CHIKV genome, consisting of canonical RNA-binding proteins (RBPs) as well as non-canonical pioneer RBPs. Of these, we characterize and functionally validate the biological impact of three classes of RBPs, and find that they play critical antagonistic or facultative roles during replication - particularly in the first replication step, the synthesis of the minus-sense template strand of CHIKV. As VIR-CLASP does not rely on sequence-specific isolation of viral nucleic acids, our approach is utilizable to potentially all RNA viruses of interest.

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232 MicroRNA-focused CRISPR-Cas9 Library Screen Reveals Fitness-Associated miRNAs

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MicroRNAs (miRNAs) are posttranscriptional gene regulators that play important roles in the control of cell fitness, differentiation, and development. The CRISPR-Cas9 gene-editing system is composed of the Cas9 nuclease in complex with a single guide RNA (sgRNA) and directs DNA cleavage at a predetermined site. Several CRISPR-Cas9 libraries have been constructed for genome-scale knockout screens of protein function; however few libraries have included miRNA genes. Here we constructed a miRNA-focused CRISPR-Cas9 library that targets 1,594 (85%) annotated human miRNA stem-loops. The sgRNAs in our LX-miR library are designed to have high on-target and low off-target activity, and each miRNA is targeted by 4-5 sgRNAs. We used this sgRNA library to screen for miRNAs that affect cell fitness of HeLa or NCI-N87 cells by monitoring the change in frequency of each sgRNA over time. By considering the expression in the tested cells and the dysregulation of the miRNAs in cancer specimens, we identified five HeLa pro-fitness and cervical cancer up-regulated miRNAs (miR-31-5p, miR-92b-3p, miR-146b-5p, miR-151a-3p, and miR-194-5p). Similarly, we identified six NCI-N87 pro-fitness and gastric cancer up-regulated miRNAs (miR-95-3p, miR-181a-5p, miR-188-5p, miR-196b-5p, miR-584-5p, and miR-1304-3p), as well as three anti-fitness and down-regulated miRNAs (let-7a-3p, miR-100-5p, and miR-149-5p). Some of those miRNAs are known to be oncogenic or tumor-suppressive, but others are novel. Taken together, the LX-miR library is useful for genome-wide unbiased screening to identify miRNAs important for cellular fitness and likely to be useful for other functional screens.

233 Mapping Neurotransmitter Identity in Whole-Mount Drosophila Brain Using Fluorescence in situ Hybridization

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Understanding how neurons communicate with each other is an important goal in neuroscience. A critical step is to identify and survey the spatial distribution of neurons that express specific chemical molecules in the brain. Fluorescence in situ hybridization (FISH) is a powerful technique used for neuron cell type identification by localizing specific mRNA in neurons. Recent advancements in FISH in the drosophila whole-mount brain have made identification of specific mRNAs possible at the cellular level [1]. Here, we present distributions of eight different neurotransmitter phenotypes by probing mRNAs from genes involved in specific pathways. Using an optimized fluorophore combination scheme to detect neuronally expressed transmitters simultaneously, we identify neurons that have coexpression of more than one transmitter-specific marker. To further accommodate the need to identify the transmitter phenotype in thousands of transgenic lines, we also improve the throughput of FISH experiments. We expect that our approach will be of broad utility in drosophila brain neuroscience studies for understanding brain-wide neuronal connections.

1. Long X, Colonell J, Wong AM, Singer RH, Lionnet T (2017) Quantitative mRNA imaging throughout the entire Drosophila brain. Nat Methods 14: 703-706.

234 Identifying Novel Genes Involved in the miRNA Pathway

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MicroRNAs (miRNA) are small (~22-nt), endogenous, noncoding RNAs which bind to complementary sites found within the 3'UTR of target mRNAs. This interaction promotes accelerated mRNA decay and/or translational repression of the transcript. To exert their regulatory function, miRNAs require the activity of additional factors. Although the core components required for miRNA biogenesis and gene silencing have been established, many secondary regulatory factors have also been identified, and more are expected to exist. The identification of novel factors involved in miRNA mediated repression will help elucidate the multiple levels of regulation that exist in the miRNA pathway and potentially answer other longstanding questions.

To identify novel miRNA pathway genes, we performed a high throughput RNAi screen of a pooled lentiviral short hairpin RNA (shRNA) library containing ~55,000 shRNAs targeting ~11,000 genes. We developed a fluorescent reporter cell line sensitive to RNAi events that alter miRNA-mediated repression to perform the screen. Fluorescence activated cell sorting (FACS) was used to sort cells with increased or decreased miRNA mediated repression and high throughput sequencing was used to identify what gene targets were enriched compared to an unsorted background. We were successfully able to identify enrichment of known miRNA pathway genes as well as potential novel candidates.

235 Harnessing RNA-targeting CRISPR-Cas enzymes to study RNA biology

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There is a continuing need to understand how RNA-mediated gene regulation helps to control fundamental biological processes such as cell-fate decision and maintenance, and is dysregulated in diseases such as cancer. Understanding these processes would greatly benefit by access to robust programmable sequence-specific RNA binding molecules. Prokaryotic CRISPR-Cas adaptive immune systems have recently revealed new opportunities to create a versatile range of tools to specifically target RNA to understand these biological processes. My lab has been studying the molecular mechanisms by which CRISPR-Cas proteins such as Cas9 and Cas13 are able to target RNA, and we are using these proteins to develop a number of applications including RNA detection, RNA imaging and manipulation of RNA metabolism. I will present some of our work aiming to understand how Cas9 and Cas13 are able to specifically interact with RNA, and how we can exploit these properties to develop tools to detect/image RNA and manipulate RNA function in living cells.

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236 Simultaneous transcriptome profiling and antibody repertoire sequencing in single cells.

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High throughput single-cell RNA sequencing (scRNA-seq) technology has provided important insights into cellular complexity and transcriptome dynamics. However, current implementations of this technology are only designed to capture information from the 3' or 5' ends of A-tailed messenger RNA transcripts. We have developed a versatile, high throughput scRNA-seq technology that surmounts this limitation and allows investigation of all regions of the mRNA, as well as measurement of all types of RNA transcripts present in the cell. It is a high throughput microfluidics based approach that is flexible, and inexpensive, and can be performed on mixed populations of cells without the need to experimentally purify or separate the cells. In addition to providing complete single cell transcriptome data our method allows targeting of specific transcripts of interest in the cell. The efficiency transcriptome measurement versus specific targeting can be tuned as per the requirement of the study.

Using this technology we were able to sequence full segments of the dsRNA viral genome of the reovirus strain type 3 Dearing (T3D) as well as the transcriptome of the infected host cells. By allowing simultaneous measurement of virus and host transcriptome at single cell level, our technology opens new avenues for studies in host-virus interactions, a field that has been often limited by the observer needing to choose either to analyze the diversity of the host or virus transcriptome.

We also applied our technology to successfully identify and measure natively paired, variable region heavy and light (VH:VL) amplicons within the transcriptome of human B lymphocyte cells in a mixed human peripheral blood mononuclear cell (PBMC) population. We were able to simultaneously measure full transcriptome data of all cell types in the PBMC population as well as sequence the full human immune repertoire.

In addition to immunological studies, our single cell sequencing technology can provide in-depth insight into regulation of non-coding RNAs in individual cells, an aspect of single cell genomics that currently remains unexplored.

237 A sensitive tool to measure phototoxicity during live-cell super-resolution imaging.

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Super-resolution imaging in living cells opens up new opportunities to explore molecular dynamics of previously inaccessible cellular structures and mechanisms. However, these methods use substantial amounts of light, such as 405nm wavelength, to photo-activate the fluorophores in order to reach nanometer resolution. Unfortunately, 405nm light induces DNA damage mostly through the generation of reactive oxygen species. Existing phototoxicity assays lack accuracy and underestimate the stress induced by light exposure during imaging. **Therefore we developed imaging tools to quantify phototoxic mediated stress-response associated with live-cell Photo-Activated Localization Microscopy (PALM).** We created a live-cell stress-response reporter by using CRISPR/Cas9 technology to incorporate 24 MS2 repeats into the 3'UTR of the endogenous p21 cell-cycle arrest gene in human U2OS cells. We validated the system by measuring increased frequency and duration of transcriptional bursting from the p21 reporter upon induction of p53. Furthermore, we found that even low doses of 405nm light were sufficient to induce a significant increase in p21 transcription. We further developed a rapid and ultrasensitive phototoxicity sensor based on changes in genomic binding dynamics of BAF180, which remodels chromatin at DNA damage sites. Live-cell single molecule tracking of BAF180 revealed an immediate increase in its residence time on chromatin after 405nm exposure. These alterations in BAF180's genome binding dynamics likely reflect global changes in genome architecture due to phototoxicity. Taken collectively, our results indicate that minimal light exposure at 405nm induces a stress-response which could bias live-cell PALM imaging.

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238 Nanopore sequencing reveals isoform-specific alterations in human bronchial epithelial cells with U2AF1 S34F mutations

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Next-generation genomic studies have revealed a widespread pattern of recurrent mutations in pre-mRNA splicing factors across various cancer types. The impact of these cancer-specific mutations on the transcriptome has been studied to some extent, yet the function of most mutant associated transcripts has not been interrogated. Understanding the role of these cancer-specific transcripts in oncogenesis could shed light on mechanisms of tumor development and new therapeutic targets. *U2AF1* mutations in the context of lung adenocarcinoma (ADC) is one example in which the functions of aberrant transcript isoforms have not been completely elucidated. *U2AF1* is an essential splicing factor required for recognizing the AG dinucleotide at the 3' terminus of introns. The most common *U2AF1* mutation in lung ADC is S34F, which is associated with transcriptome-wide splicing and polyadenylation alterations of certain genes. Although S34F-associated altered RNA processing events have been characterized, the full-length transcript isoform context of these events has not been identified. Moreover, it is unclear to what extent S34F-associated splicing and polyadenylation co-occur. To close this gap in knowledge, we are sequencing full-length mRNA isoforms with long-read sequencing technologies. With this data, we can use bioinformatic tools to determine the full connectivity of splicing and polyadenylation. We will present a preliminary analysis of ~4 million and 1.5 million Oxford MinION reads from HBEC cell lines with wild-type and mutant *U2AF1*, respectively, to identify U2AF1 S34F-associated differential alternative splicing, polyadenylation, and isoform usage. Our preliminary analysis has revealed splicing patterns in genes such as UPP1 and BUB3 that are consistent with *U2AF1* S34F primary tumor samples from lung ADC patients from The Cancer Genome Atlas. Moreover, our long-read sequencing approach provides deeper insight on alternative-splicing events previously classified as nonsense-mediated decay targets. Last, we have resolved a number of alternative polyadenylation events associated with S34F that were previously misclassified due to the limitations of short-read illumina sequencing. Altogether, these results are the first steps in describing isoform-specific changes and functional outcome of S34F-associated splicing.

239 Systematic evaluation of CRISPR-Cas systems for genome editing

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While CRISPR-Cas holds tremendous potential for genome engineering, it is unclear how well each system performs against one another in both NHEJ-mediated and HDR-mediated genome editing. Here, we performed a systematic comparison of five different CRISPR-Cas systems by targeting 90 distinct sites in genes with varying expression levels. For a fair comparison, we selected sites that are either perfectly matched or have overlapping seed regions for Cas9 and Cpf1 (Cas12a). We observed that the editing activities of the smaller Cas9 enzymes from Staphylococcus aureus (SaCas9) and Neisseria meningitides (NmCas9) were less affected by gene expression than the other larger nucleases. We also found a trade-off between cleavage efficiency and target specificity. Notably, Cpf1 was able to introduce precise genome modifications at an overall higher rate than the Cas9 from Streptococcus pyogenes (SpCas9) when ssODNs were used as templates. Collectively, our work has delineated design parameters for each CRISPR-Cas system and will help guide the design of future genome engineering studies.

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240 A streamlined cost-effective method for multiplexed transcriptomics

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RNA-seq has become the gold standard for assaying global gene expression. As the cost of sequencing decreases with improved technology, library preparation accounts for a growing fraction of experimental costs. However, the standard RNA-seq library preparation protocols in use are often constrained to a smaller set of research applications due to larger amounts of RNA input and prohibitive cost per sample library preparation. To address these issues, previous research groups have developed a method called RNAtag-Seq for simultaneously generating many RNA-seq libraries in a single preparation. Here our goal is to improve this method by streamlining the protocol, optimizing the efficiency of ligation reactions, which together would lower RNA input thresholds. Additionally, our cloning strategy would be broadly applicable to many assays in to investigate RNA regulation even for precious samples.

241 Multipotency of NG2 cells after cerebral ischemia: new elements of reactive gliosis

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NG2 cells, a fourth glial cell type in the adult mammalian central nervous system, produce oligodendrocytes in the healthy nervous tissue, and display large differentiation potential under pathological conditions. However, their differentiation capacity is still poorly described in the context of focal cerebral ischemia (FCI). To study the effect of FCI we used transgenic mice, which enables genetic fate ‑ mapping of Cspg4 ‑ positive cells and their progeny, based on the expression of red fluorescent protein tdTomato. Differentiation potential of tdTomato-positive cells from control and post-ischemic brains was determined using the single cell RT-qPCR and immunohistochemistry. To analyze the changes of expression patterns caused by FCI we utilized self-organizing Kohonen maps, enabling us to divide NG2 cells and oligodendrocytes into subpopulations based on similarities of expression profiles of individual cells. We identified three subpopulations of NG2 cells emerging after FCI: proliferative; astrocyte-like and oligodendrocyte-like NG2 cells, phenotypes which were further confirmed by immunohistochemistry. We also utilized EdU labeling to disclose, that NG2 cells can differentiate directly into reactive astrocytes without proliferation. The difference between astrocyte-like NG2 cells and classical reactive astrocytes was determined using single cell RNA-Seq. Oligodendrocytes themselves formed four subpopulations, which reflected the process of oligodendrocytes maturation. Taken together we identified several yet unknown differences between the expression profiles of NG2 cells and oligodendrocytes, and characterized specific genes contributing to oligodendrocyte maturation and phenotypical changes of NG2 cells after FCI. Moreover, our results point to the ability of NG2 cells to acquire multipotent phenotype after FCI, which is documented by generation of reactive astrocytes.

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242 Integrated analysis of eCLIP profiling for 150 RNA binding protein targets reveals RNA processing regulatory networks and novel protein functions

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RNA binding proteins (RBPs) bind to RNA transcripts to regulate each step of RNA processing, including pre-mRNA alternative splicing, RNA stability and localization, and control of translation. Thus, robust, unbiased genome-wide methods to characterize RNA processing regulation is essential to a molecular understanding of RBP regulatory networks and the development of novel therapeutic targets. We recently described enhanced crosslinking and immunoprecipitation (eCLIP), which enabled robust profiling of RBP targets at scale. We have now used eCLIP to profile 150 RBPs in K562 and HepG2 cells, the largest resource of RNA binding protein targets using a single methodology in the same cell types. Integration of these datasets has provided new insights into the functional role of individual RBPs in regulating splicing; for example, we observe that AQR eCLIP signal indicates association with introns post-lariat formation and can be utilized to identify branch points with single-nucleotide resolution. Further, the ability to directly compare across datasets suggests new general principles of RNA processing not seen from single-RBP approaches, including the widespread presence of single RNA targets that dominate signal for many RBPs. Incorporation of new approaches to quantify binding to retrotransposable and other multi-copy RNAs identified widespread association with these elements and enabled the identification of novel regulators of ribosomal RNA processing and L1 retrotransposition. Finally , we performed integrated analyses of RBP targets with orthogonal datasets profiling RNA transcripts upon RBP knockdown, in vitro RBP binding motifs, and RBP subcellular localization to build a global integrated picture of RNA processing, which revealed a novel RNA element in mitochondria that correlates with the role of DHX30 in mitochondrial RNA processing. In summary, we anticipate that the ENCODE eCLIP dataset will provide a significant resource to the RNA community, enabling both broad computational analyses as well as specific targeted studies focusing further on individual RBPs of interest.

243 High-throughput multiplexed imaging of hundreds of high-abundance RNA species with MERFISH and Expansion Microscopy

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The intracellular organization of the transcriptome – where individual mRNAs are found within a cell - has been discovered to be a powerful regulator of post-transcriptional dynamics which in turn plays an important role in a variety of biological processes from cell motility to neuronal polarization. Image-based approaches to single-cell transcriptomics, in which RNA species are identified and counted in situ via imaging, emerge as a powerful complement to single-cell RNA sequencing by providing the spatial landscape of the transcriptome in their native contexts. Recently our lab has introduced Multiplexed Error-Robust Fluorescence In Situ Hybridization (MERFISH) and demonstrated the ability to count and map hundreds to a thousand of RNA species in tens of thousands of single cells per single-day measurement with single-molecule sensitivity and resolution. In MERFISH, RNAs are identified via a combinatorial labeling approach that encodes RNA species with error-robust barcodes followed by sequential rounds of single-molecule FISH (smFISH) to read out these barcodes. The accuracy of RNA identification relies on spatially separated signals from individual RNA molecules, which limits the density of RNAs that can be measured and makes imaging of a large number of high-abundance RNA species challenging. Here we combined MERFISH with Expansion Microscopy to substantially increase the total density of RNAs that can be measured. Using this approach, we demonstrate accurate identification and counting of ~130 high-abundance RNA species, with a near 100% detection efficiency. The total density of RNAs measured here is more than 10-fold higher than previously reported. Further, we also incorporated immunofluoresence into MERFISH measurements via oligo-conjugated antibodies, whose signals were also read out by smFISH following MERFISH measurements. With these advances, we measured and quantified spatial patterns of hundreds of RNA species inside of cells such as cultured human osteosarcoma (U-2 OS) cells and cultured hippocampal neurons. These advances increase the versatility of MERFISH and will facilitate its application to a wide range of biological problems and in many different biological systems.

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244 Withdrawn

245 High Throughput Analysis of Various RNA Species

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Advanced Analytical Technologies, Inc, Ankeny, IA, United States Minor Outlying Islands

Quantitative and qualitative assessment of nucleic acids is an essential step in researching a variety of biological and biomedical samples. Obtaining reliable measurements of sample integrity is a challenge in and of itself, as there is some variation in standards between different types of nucleic acids. For example, the quality and concentration of total RNA, mRNA, and small RNA is crucial when preparing next-generation sequencing (NGS) libraries. Similarly, the amount of microRNA present in a sample is crucial when preparing small RNA libraries. In short, profiling input RNA in a cost and time efficient manner is essential when evaluating if samples are worth using in future experiments.

The Fragment Analyzer™ (Advanced Analytical Technologies, Inc) has proven to be an indispensable instrument for the reliable qualification and quantification of nucleic acids. When employed in most RNA-related pipelines, such as NGS library construction and RNAi, the Fragment Analyzer can assess all sample types throughout construction, from the initial assessment of sample integrity through final qualification. It is a flexible platform that automates the analysis of a variety of nucleic acids over a wide range of concentrations and sizes. Data will be presented showing sizing and quantification reproducibility of both small and large in vitro transcribed/synthetic RNA fragments. Data will also be presented to show general purity and how lack of template DNA can be evaluated as both peak and contaminate within the same run. The versatility of the Fragment Analyzer allows for the efficient qualification and quantification of traditionally challenging nucleic acids for RNAi and other downstream applications.

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246 RNP-MaP identifies networks of combinatorial control by RNA-binding proteins and highlights functional domains in non-coding RNAs

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RNA-protein complexes (RNPs), which are tied together by interactions between RNA and RNA-binding proteins (RBPs), are critical in biology. Unsurprisingly, mutations that alter RBP genes or disrupt the sequences recognized by RBPs contribute to hundreds of human diseases. RBPs rarely act alone when regulating RNA functions, but existing methods do not report on how multiple RBPs coordinate on an RNA without extensive prior knowledge of the RNP complex. We have developed a novel approach, called RNP mapping by mutational profiling (RNP-MaP), to comprehensively identify protein-binding sites and to characterize RNP networks on an RNA in a single, straight-forward experiment.

RNP-MaP combines live-cell chemical probing with a simple sequencing readout to locate protein interaction sites within any RNA and with single nucleotide resolution. Moreover, RNP-MaP enables simultaneous detection of multiple protein-binding events within individual RNA molecules and reveals where proteins cooperate to form functional RNP networks.

We have extensively validated the RNP-MaP technology on non-coding RNAs ranging in length from 100 to 20,000 nucleotides. RNP-MaP correctly identifies all major protein binding sites on the U1 small nuclear RNA and highlights interactions between the U1A, 70K, and Sm proteins that together, with the U1 RNA, form an RNP complex. RNP-MaP also reveals the overlapping RNP networks in two structurally-related, but sequence-divergent RNAs: RNase P and RNase MRP. Finally, RNP-MaP highlights how RNP networks define conserved tandem repeat arrays within the XIST long non-coding RNA that are critical for X chromosome dosage compensation. We expect that RNP-MaP will be widely useful for discovery and mechanistic analysis of interactive RNA-protein networks in any RNA of interest.

247 Rapid and Generic Discovery of Small Molecule Ligands Targeting RNA

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The transcriptome, the set of all RNA molecules produced in an organism, represents a highly promising yet under-utilized target for studying and manipulating biological systems. However, it is currently difficult and expensive to screen RNAs of interest for specific binding to small molecule ligands. We have developed a new technology, harnessing the unique benefits of fragment-based ligand discovery and high-throughput RNA structure analysis (SHAPE-MaP), for screening RNA targets against a library of small molecule fragments. We have recently validated this system by detecting binding of fragments known to bind the E. coli thiamine pyrophosphate (TPP) riboswitch, as well as discovering new fragments which bind this RNA target. We have validated the binding of these fragments to RNA by isothermal titration calorimetry (ITC) and obtain binding constants in the low micromolar range. In addition, we have detected secondary fragments which bind to the TPP riboswitch only in the presence of a primary bound fragment, alluding to possible synergistic effects in fragment remodeling of the RNA tertiary structure. Ultimately, this work will result in a broadly applicable framework for developing RNA-structure-specific small molecule ligands for biotechnology applications.

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248 Early life of mitochondrial RNAs in Trypanosoma brucei

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Historically, mitochondrial gene expression in Trypanosoma brucei has been studied in the context of U-insertion/deletion mRNA editing and polyadenylation. However, the upstream transcription and primary RNA processing mechanisms remain unexplored. It is commonly held that both kinetoplast DNA strands are transcribed into polycistronic precursors that undergo endonucleolytic cleavage to produce individual pre-mRNAs. This notion is supported by the 5′ monophosphorylated state of mature mRNA 5′ ends, but the processing endonuclease is unknown. By combining *in vivo* UV-crosslinking, deep sequencing, proteomics and *in vitro* reconstitution, we demonstrate that the 5' end of each mitochondrial RNA is defined by transcription initiation. Furthermore, accumulation of mRNA precursors upon 3′ processome (MPsome, Suematsu et al, Mol Cell, 2016) repression indicates that each mRNA is independently transcribed as a 3′ extended precursor and processed by uridylation-induced 3′-5′ degradation (Zhang et al, EMBO J, 2017). Here, we determined the mechanism by which transcription-defined 5′ terminus is processed from primary triphosphorylated to the mature monophosphorylated state. We show that the mitochondrial edited RNA stability (MERS) complex containing MERS1 NUDIX hydrolase and MERS2 pentatricopeptide-repeat containing (PPR) RNA binding protein is tightly associated with mRNA 5' ends. We provide evidence that MERS complex functions as "protein cap" by physically bridging 5′ end 3′ ends via interactions with RNA editing and polyadenylation complexes. This interaction ultimately protects mitochondrial mRNAs against degradation. Finally, we show that 3′ extended primary transcripts are processed by anti-sense RNA-controlled, MPsome-catalyzed 3′-5′ trimming prior to polyadenylation. In this work, we refute the current view of polycistronic mitochondrial transcription and endonucleolytic cleavage, and present an integrated model connecting mRNA synthesis, 5′ and 3′ processing and stabilization.

249 Methodologies for studying the biological roles of neural-extended 3´UTR isoforms

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Genes often are capable of transcribing more than one 3´UTR variant through the process of alternative cleavage and polyadenylation (APA). It is considered that long 3´UTRs contain more regulatory elements relative to shorter 3´UTRs, which adds a spatiotemporal regulatory layer to gene expression. One of the many genes that express an extended 3´UTR isoform is the Calmodulin1 (Calm1) gene. Calm1, which encodes the Calmodulin (CaM) protein, increases its expression during neuron differentiation and maintains its levels considerably high in mature neurons. Northern blot and RT-qPCR analysis of multiple tissues show that the short 3´UTR isoform of Calm1 (Calm1-S) is ubiquitously expressed, whereas brain tissues are enhanced for expression of the extended 3´UTR of Calm1 (Calm1-L). CaM is one of the conserved calcium signal transducers that has shown to influence molecular aspects of neuron differentiation, development, and maturation in a wide variety of organisms. Upon its activation by intracellular calcium, CaM actuates on downstream effectors to regulate neurite growth, steering, and actin reorganization in growth cones during the early developments of neurons, and also plays role in synapse development and plasticity of neurons. While the importance of CaM during neuronal development has been reported to some extent, what the implication of Calm1-L is in neurons is still nebulous. Here, we show the differential expression of Calm1-L versus Calm1-S in multiple tissues, primary neurons, and immortalized cell lines. Transfection of luciferase reporter constructs harboring the short 3´UTR, extended 3´UTR, or extended 3´UTR with the proximal polyadenylation site mutated, provides a readout of the impact of these sequences on RNA stability and translation in response to RNA binding protein knockdown and overexpression contexts. Public RNA-seq or CLIP-seq data analysis allows preliminary screening of potential trans-regulator of Calm1-L expression. RNA-pulldown coupled to LC/MS provides a method to uncover novel RNA binding partners that interact with the extended 3´UTR in neurons. We present strategies to achieve knockout of extended 3´UTR isoforms using CRISPR to delete distal polyadenylation sites. We demonstrate the effectiveness of such strategies by targeting Calm1 in mice, and *cam* in *Drosophila* to generate "short-only" mutant calmodulin animals.

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250 Mechanism of 3'UTR Dependent CELF2 Expression in T-cells

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More than half of human genes use alternative cleavage and polyadenylation (APA) to generate alternative 3' untranslated regions (3'UTRs). Altered 3'UTR has various functional consequences in cells. However, why the alternative 3'UTRs have evolved and their biological roles in gene regulation, are not entirely understood. CELF2 (CUGBP, Elav-like family member-2) is an RNA binding protein (RBP) that is implicated in playing a major role in shaping the transcriptome of T-cells, and thus tight regulation of CELF2 expression is essential for cellular function. Previous results have shown that 3'UTR of CELF2 undergoes both regulated splicing and APA upon T-cell stimulation. However, the regulation of CELF2 APA, and the relationship between 3'UTR identity and protein expression have not been determined. Here we propose a multipronged approach to uncover the mechanisms and consequences of 3'UTR regulation in controlling CELF2 expression.

We previously identified five different 3'UTR isoforms of CELF2 mRNA in T-cells that result from APA and intron retention. We first show that CELF2 protein binds to the 3' splice site of the regulated 3'UTR intron and controls the stimulation induced intron retention. Moreover, we used a quantitative reporter gene assay to examine whether individual 3'UTR sequences can functionally regulate CELF2 expression. We find that 3'UTRs that exclude the intron promote reporter gene expression in T-cells. The distinct 3'UTR constructs show essentially an identical abundance of mRNA, suggesting that the expression of these CELF2 3'UTRs is regulated at the level of translation**.** Next, we utilized the CRISPR-Cas9 system to interrogate the regulatory activity of the 3'UTR in the native context. We find that CELF2 expression is relatively increased in stimulated T-cells when the 3'UTR intron is deleted. By contrast, CELF2 expression is relatively decreased in both unstimulated and stimulated T-cells when the splice sites were mutated to promote intron retention. Furthermore, CELF2 mRNA half-life in both 3'UTR intron deleted and retained mutant T-cells were similar to wild-type T-cells, which is consistent with our results from the reporter gene assays indicating that CELF2 expression is translationally regulated. Future work will focus on identifying RBPs that control translation of CELF2 in a 3'UTR dependent manner.

251 miRNA-mediated silencing and ARE-mediated silencing intersect at the CNOT1 surface

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The stability of mRNAs and their translation efficiency is regulated *i.a.* by the presence of specific sequences or secondary structure elements in their untranslated regions (UTR), aided by a wide array of RNA-interacting factors. For example, miRNAs usually bind imperfectly complementary sequences present in the 3'UTR, while a protein called tristetraprolin interacts with AU-rich elements (ARE) also present in the 3'UTRs of mRNAs. Both the miRNA-mediated and the ARE-mediated gene silencing pathway employ the CCR4-NOT deadenylase complex to enact translational repression and/or mRNA deadenylation.

One of the crucial steps of miRNA-mediated silencing of mRNAs is the recruitment of the CCR4-NOT deadenylase complex by the GW182 protein. Similarly, one of the crucial steps of ARE-mediated silencing is the recruitment of CCR4-NOT by tristetraprolin.

Here we study how GW182 and tristetraprolin interact with CNOT1, the scaffolding subunit of the CCR4-NOT complex. Hydrogen-deuterium exchange mass spectrometry experiments reveal perturbations of hydrogen bonding networks upon binding GW182 and tristetraprolin-derived peptides. These perturbations are indicative of binding sites and/or conformational changes.

Surprisingly, we found that the GW182 silencing domain interacts with the same CNOT1(800-999) surface region as tristetraprolin, though using a slightly different binding mode. The two proteins share a common motif, RLPXφ, which is proposed to act as a short linear motif. Biochemical experiments excluded the simultaneous binding of GW182 and tristetraprolin to CNOT1.

Thus, GW182 and tristetraprolin compete for the same CNOT1 binding surface suggesting a possible interplay between the miRNA-mediated and ARE-mediated gene silencing pathways in different physiological processes.

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252 Characterization of the AtCPSF30-YTH protein - linking a novel RNA-binding domain, polyadenylation, and plant growth & development

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The *Arabidopsis* gene encoding the orthologue of the 30-kD subunit of the mammalian cleavage and polyadenylation specificity factor (CPSF) encodes both a small peptide (approximately 28 kD, AtCPSF30) and a large peptide (approximately 68 kD, AtCPSF30-YTH). Here we describe AtCPSF30-YTH, which is predominantly nuclear and contains a YTH domain. YTH domains are poorly characterized in plants, but have been extensively linked to m6A-modified RNAs in mammals. In order to investigate the role of AtCPSF30-YTH in plant development and polyadenylation we used knockout plants as well as recombinantly expressed protein to investigate its biological function and RNA binding preferences. Using plants deficient in one or both of the CPSF30 isoforms, we show that plants expressing only AtCPSF30-YTH are deficient in lateral root development and their responses to ethylene. However, plants expressing only AtCPSF30 exhibit wildtype behavior in both of these characteristics. Furthermore, we have observed global changes in poly(A) site choice in plants expressing only AtCPSF30-YTH, indicating this protein does not exhibit wildtype functionality in mRNA polyadenylation. We have also biochemically assessed the preference of the YTH domain of AtCPSF30-YTH for RNA and found this protein to be a novel RNA binding protein with a preference for a canonical m6A-methylation target sequence (AGACU). However, this domain showed no preference for the m6A-modified sequence (AG[m6A]CU) over the unmodified RNA (AGACU). Interestingly, this domain had a much lower affinity for another m6A target sequence (AAACU) than for AGACU, and showed no increased preference for AA[m6A]CU over AAACU. We propose that AtCPSF30-YTH is a conceptual link between polyadenylation and m6A-modified RNAs that acts in an unusual fashion.

253 mRNA Decay Induces Transcriptional Repression During Gammaherpesviral Infection

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The canonical view of gene expression is unidirectional, beginning with transcription and ending with RNA decay. However, we recently described a novel signaling pathway that reverses this information flow. Accelerating cytoplasmic mRNA degradation by exonucleases such as Xrn1 results in impaired RNA polymerase II (RNAPII) recruitment and transcriptional repression of the majority of mammalian genes. This mRNA decay-induced transcriptional repression is activated during infection with gammaherpesviruses including Kaposi's sarcoma-associated herpesvirus (KSHV), as they encode an mRNA endonuclease termed SOX that initiates widespread RNA decay. Notably, viral genes escape transcriptional repression despite the fact that they similarly require RNAPII, providing a system to dissect how susceptibility to this pathway is controlled. We now demonstrate that both the chromatin environment of promoters and their sequence composition can influence their susceptibility to mRNA decay-induced transcriptional repression. Furthermore, we made the surprising discovery that SOX itself is a key determinant in the differential response of host and viral promoters to this pathway, as it selectively protects viral promoters through an additional DNA binding activity. Structure-guided mutation of SOX residues critical for DNA binding but dispensable for mRNA cleavage leads to transcriptional repression of both host and viral promoters. These data suggest a model whereby SOX causes transcriptional repression of mammalian genes through its RNA endonuclease activity that activates cytoplasmic mRNA decay, and simultaneously protects viral promoters through its DNA binding activity. Collectively, our findings reveal surprising interplay between mRNA decay and RNAPII transcription in mammalian cells, which is actively manipulated by gammaherpesviral endonucleases.

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254 A splicing network regulates protein transport efficiency at all stages of the secretory pathway

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We have recently described a splicing switch in SEC16A, a protein that defines endoplasmic reticulum exit sites (ERES) and has an essential role in COPII vesicle formation. Exon 29 inclusion upon T cell stimulation leads to an increase in the number of ERES and enhances COPII dynamics as well as ER-to-Golgi transport efficiency. This is the first connection of alternative splicing with the early secretory pathway and an example, how ER export efficiency is adapted to a changing cellular environment.

Building on this initial observation, we now globally analyzed splicing events that regulate the efficiency of protein secretion. Using a published genome-wide siRNA screen for effectors of protein secretion, we identified four RNA-binding proteins (RBPs) involved in this process. For these RBPs we analyzed publicly-available knock-down RNA sequencing datasets. We focussed on events regulated by at least three of these four RBPs and obtained over 200 splicing events within genes highly enriched for effectors of membrane traffic.

We further investigated selected splicing events regarding their effect on secretion and their functional characteristics by generating genome-engineered cell lines lacking the respective exons. One of these targets is SEC22C, a SNARE protein with a splicing controlled alternative C terminus. Alternative splicing changes SEC22C localization from the ER to the cis-Golgi and exclusive expression of one isoform in our genome-engineered cell line indeed led to impaired protein export capability. Other events in OCRL and IP6K2 regulate phosphorylation levels of phosphoinositides and can thereby modulate later stages of protein transport. Our data furthermore indicate that these and other secretion-associated splicing events are regulated in a tissue specific manner. We thus suggest that a few RNA-binding proteins coordinate an alternative splicing network to adapt protein transport efficiency to tissue specific requirements.

255 Transcription factor regulation of a druggable translational regulon in prostate cancer

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The recent advent of highly potent inhibitors of the androgen receptor and androgen biosynthesis has had the unfortunate iatrogenic effect of fueling new lethal prostate cancer phenotypes in patients. In particular, non-neuroendocrine androgen receptor-low castration resistant prostate cancer (CRPC), an aggressive form of this disease, is increasing in occurrence amongst patients and is uniformly fatal. The main barriers against therapeutic advances are a paucity of relevant disease models and a very poor understanding of the mechanisms that give rise to this phenotype. The process of protein synthesis has long been considered subordinate to alterations at the levels of DNA and RNA in cancer etiology. However, work from our laboratory and others have revealed that protein synthesis control is a dynamic process that coordinates not only bulk mRNA translation, but also the specialized translation of distinct mRNA networks important for cancer phenotypes. Recently, our laboratory has developed and characterized a new *in vitro* and *in vivo* toolkit of both human and murine androgen receptor-low CRPC. We have used these models to discover a critical link between androgen receptor mediated transcription regulation and the process of mRNA translation initiation. In particular, the androgen receptor positively regulates eIF4E binding protein 1 (4EBP1) expression through an androgen response element encoded within its gene body. In an androgen receptor low state, 4EBP1 levels significantly decrease leading to de-repression of eIF4E-mediated translation. Importantly, using state-of-the-art mouse genetics to temporally and spatially control translation initiation complex assembly, we demonstrate that aberrant eIF4E activity is necessary for both the initiation and progression of androgen receptor low prostate cancer. To delineate the downstream effectors of this phenotype we conducted *in vivo* ribosome profiling and discovered a new regulon of pro-proliferation genes exquisitely sensitive to fluctuations in eIF4E function. Lastly, therapeutic trials reveal that physical disruption of the eIF4E translation initiation complex leads to a preferential inhibition of cell proliferation in androgen receptor low prostate cancer. These findings reveal a new previously unrecognized druggable interaction which functionally link the processes of mRNA transcription and translation initiation in human malignancies.

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256 Functional impact of alternative splicing coupled to nonsense-mediated decay in developing neurons

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Differentiation of precursor cells into mature neurons relies on transcriptome-wide changes in gene expression that have to be coordinated in a precise spatiotemporal fashion. Alternative pre-mRNA splicing coupled to nonsense-mediated decay (AS-NMD) is a widespread post-transcriptional mechanism known to orchestrate gene expression dynamics in developmental contexts. Earlier studies identified several neural targets of this pathway; however, in most cases, the extent to which AS-NMD contributes to the overall gene expression dynamics and biological significance of this regulation is poorly understood. Moreover, whether AS-NMD target repertoire undergoes considerable changes in developing brain and how this might fit to the global regulation network underlying neuronal differentiation remains unclear. To address these questions, we analysed transcriptome-wide changes in mouse neuroblastoma cells treated with siRNA against Ptbp1, and/or the translational inhibitor CHX also known to repress NMD. This uncovered a number of putative AS-NMD targets containing PTBP1-repressible "poison" exons predicted to dampen steady-state levels of the corresponding mRNAs including several actin regulator genes. mRNA levels of these newly identified PTBP1/AS-NMD targets are normally downregulated during neuronal differentiation with kinetics similar to that of the Ptbp1 mRNA. We are currently exploring the extent of AS-NMD regulation of these genes by modulating the inclusion of the NMD-promoting exons with corresponding antisense oligonucleotides in mouse embryonic stem cells undergoing neuronal differentiation and primary neural stem cells and neurons. We are additionally analysing how NMD contributes to different stages of neuronal development by acutely inhibiting this pathway in a time-resolved manner. Results of these studies should provide important insights into AS-NMD functions in developing nervous system.

257 PRP40 is involved in co-transcriptional processing of miRNA precursors in plants

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MicroRNAs (miRNAs) are small noncoding RNAs of about 21 nt in length, which regulate gene expression by cleavage or translation inhibition of target mRNAs. Plant miRNA biogenesis takes place in the nucleus. Three proteins: DCL1 (a ribonuclease), HYL1 (a double-stranded RNA binding protein) and SERRATE (SE; a zinc-finger protein) are the key factors responsible for miRNA production in the plant cell nucleus. Interestingly, SE in addition to its involvement in miRNA biogenesis, is also engaged in pre-mRNA splicing.

Many miRNA genes (*MIR*s) contain introns that have to be spliced from primary miRNA precursors (pri-miRNAs) by the spliceosome. We have already shown that splicing of intron-containing pri-miRNAs influences the expression levels of miRNAs. Moreover, we have found that the communication between the spliceosome and the miRNA biogenesis machinery is facilitated by SE and U1 snRNP. Our results show that one of auxiliary U1 snRNP proteins, PRP40, interacts with SE. In addition, PRP40 interacts also with the CTD domain of RNA polymerase II (RNAPII). SE is localized in RNAPII-containing nuclear foci. This co-localization of SE and RNAPII is disturbed in the *prp40a prp40b* double mutant, indicating that PRP40 plays a role in the communication between SE and RNAPII. We also show that co-localization of DCL1 and RNAPII is also significantly reduced in the absence of PRP40, suggesting that PRP40 controls co-transcriptional miRNA biogenesis. Thus, the interplay between RNAPII, PRP40 and SE is important for the co-transcriptional processing of plant *MIR* primary transcripts. In addition, we observe the accumulation of RNAPII at the end of *MIR* genes in the *prp40a prp40b* mutant. This suggests that termination of *MIR*s transcription is connected with PRP40 and the proper processing of miRNA primary precursors. The molecular mechanism of co-transcriptional miRNA biogenesis will be discussed.

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258 Different mammalian introns enhance gene expression at different steps

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Introns enhance the expression of genes in which they are contained, a phenomenon termed intron-mediated enhancement (IME). The presence of an intron has been shown to influence many stages of gene expression, from promoting transcription or 3' end processing, to increasing stability, nuclear export, or translational efficiency of the spliced message.[1] However, not all introns are equal: curiously, different constitutive introns tested in the same reporter construct in *Arabidopsis thalania* have been reported to exhibit large differences in their ability to enhance gene expression, suggesting that intronic sequence elements are at least partly responsible for this effect.[2] To investigate the variability in degree and mechanism of IME among different introns in mammalian cells, we have created reporter cell lines with single-copy site-specific integration of EGFP under the control of either the Ubiquitin C (UbC) promoter or the Elongation Factor 1 Alpha (EF1a) promoter, in both HeLa and HEK293T cells.[3] We deleted the endogenous intron in the 5' UTR of each promoter and performed qRT-PCR to assay mRNA levels and FACS to measure protein levels. While the UbC intron increased gene expression predominantly by increasing steady-state EGFP mRNA (~10-fold), the EF1a intron had little effect on mRNA levels but dramatically increased EGFP protein levels (~200-fold). This observation suggests that IME may be highly sensitive to the intron sequence in mammals. To better understand the intronic sequence elements that mediate different aspects of IME, we are generating additional reporter cell lines in which these introns are replaced with a set of exogenous introns varying in length, GC content, position in host gene, expression of host gene, and other parameters. These experiments should help to classify introns by their mode of enhancement of gene expression and will hopefully elucidate the sequence elements and mechanisms underlying these effects.

[1]PMID: 28673892

[2]PMID: 12458797

[3]PMID: 21768390

259 An investigation of the role of the NuA4 histone acetyltransferase in coordination of transcription and RNA splicing

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RNA splicing occurs co-transcriptionally and there is strong evidence that transcription and RNA splicing are highly coordinated, however the mechanistic details underlying this coordination are still being elucidated. Transcription requires chromatin modification and remodeling enzymes, which alter the structure of chromatin to allow RNA polymerase access to the DNA. Given that co-transcriptional RNA splicing takes place in a dynamic chromatin environment, chromatin modification and remodeling enzymes have the potential to coordinate RNA splicing with transcription. Recently the variant histone H2A.Z was implicated in RNA splicing in *Saccharomyces cerevisiae.* H2A.Z deposition, which promotes transcription, requires the activity of the NuA4 histone acetyltransferase as well as Swr1, an ATP-dependent chromatin remodeling complex. NuA4 acetylates both H2A and H4, which function to recruit the Swr1 remodeling complex that exchanges H2A for H2A.Z. NuA4 then acetylates H2A.Z. Using directed genetic screens we identified unique interactions between splicing factor gene mutations and mutations that alter either the catalytic activity of NuA4 or mutations that interfere with acetylation of specific lysine residues in histone H4. These data suggest that Nua4 may have functions in RNA splicing that are independent of H2A.Z deposition. Furthermore, the mutations that interfere with acetylation of specific histone H4 residues alter the splicing of subsets of pre-mRNAs. We are currently completing experiments to determine the mechanism(s) by which NuA4 and histone acetylation impacts co-transcriptional splicing. In addition, we have initiated experiments to test whether RNA splicing can impact transcription by altering the association of NuA4 with chromatin. Together, our data support a model in which the NuA4 interacts with the splicing machinery to coordinate transcription and splicing.

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260 hnRNPDL extensively regulates transcription and alternative splicing

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RNA binding proteins (RBPs) are key players of genome regulation. Here we report the transcriptome study of HnRNP D-Like protein, which belongs to the hnRNP family. We used RNA-seq to analyze the global transcript level and alternative splicing on *hnRNPDL* shRNA-treated cells and control. Sh-*hnRNPDL* extensively increased in the expression of genes involved in female pregnancy, cell apoptosis, cell proliferation and cell migration. HnRNPDL regulated alternative splicing of hundreds of genes enriched in transcription regulation and signaling pathways. This study provides the first transcriptome-wide analysis of hnRNPDL regulation of gene expression, which adds to the understanding of critical hnRNPDL functions.

261 Short Poly(A) Tails are a Conserved Feature of Highly Expressed Genes

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The poly(A) tails appended to the 3' ends of most eukaryotic mRNAs play important roles in translation and stability. However, recent genome-wide studies concluded that poly(A) tail length was generally not associated with translational efficiency in non-embryonic cells. To investigate if poly(A) tail size might be coupled to gene expression in an intact organism, we used an adapted TAIL-seq protocol to measure poly(A) tails in larval stage *Caenorhabditis elegans*. Surprisingly, we found that well-expressed transcripts contain relatively short, well-defined tails that would likely accommodate only 1-2 poly(A) binding proteins (PABPs). This attribute appears dependent on translational efficiency, as transcripts enriched for optimal codons and ribosome association had the shortest tail sizes, while non-coding RNAs retained long tails. Across eukaryotes, short tails were a feature of abundant and well-translated mRNAs. However, for these genes and almost all others, we were still able to detect transcripts with tail lengths consistent with the very long (>200 nt) poly(A) tails synthesized on nascent mRNAs. The finding that genes with the highest frequencies of optimal codons were represented by mRNAs that spanned the entire range of detectable tail sizes, but were strongly biased for short tailed species, suggests that well-expressed mRNAs undergo poly(A) tail shortening to an optimal length, which we refer to as pruning. The hallmarks of pruning are that poly(A) tails are well-defined and relatively short, while tails on mRNAs enriched for suboptimal codons are more heterogeneous and less defined, showing a spread across the range of possible sizes. Although this seems to contradict the dogma that deadenylation induces translational inhibition and mRNA decay, it instead suggests that well-expressed mRNAs accumulate with pruned tails that accommodate a minimal number of PABPs, which may be ideal for protective and translational functions.

262 UPF1-dependent microRNA-mediated gene regulation

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The stability and quality of metazoan mRNAs are under microRNA (miRNA)-mediated control and nonsense-mediated control, respectively. Although UPF1, a core mediator of nonsense-mediated mRNA decay (NMD), mediates the decay of a target mRNA in 3′ untranslated region (UTR)-length-dependent manner, the involvement of NMD and the detailed mechanism remain unclear. Here, we suggest that 3′UTR-length-dependent mRNA decay is not mediated by nonsense mRNAs but rather by endogenous miRNAs (endo-miRNAs) that downregulate target mRNAs via Ago-associated UPF1/SMG7. Global analyses of mRNAs in response to UPF1 RNA interference in miRNA-deficient cells reveal that 3′UTR-length-dependent mRNA decay by UPF1 requires canonical miRNA targeting via the CUG motif. The repression of miRNA targets is additively or synergistically accomplished by the combination of Ago2 and UPF1 through UPF1-associated SMG7, which may recruit the CCR4-NOT deadenylase complex in a TNRC6-independent manner, indicating the presence of a novel miRNA targeting pathway. This new miRNA-mediated mRNA decay pathway may enable miRNA targeting to become more predictable and expand the miRNA-mRNA regulatory network.

263 Heat Shock Remodels the Non-coding RNA Transcriptome

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In the natural world, organisms experience various environmental insults that, if not properly addressed, can have lethal consequences. A well-conserved Heat Shock Response (HSR) provides a cellular defense against the potentially lethal effects of increased temperature. While the expression and function of heat shock and other proteins that mitigate the damaging effects of HS are well established, little is yet understood about the role of post-transcriptional regulation by non-coding RNAs (ncRNAs) during adverse conditions. To fill this knowledge gap, we are investigating the expression and function of microRNAs (miRNAs) and other ncRNAs during the HSR in *C. elegans*. Using small and standard RNA sequencing, we identified specific miRNAs, long ncRNAs (lincRNAs) and repeat RNAs that are strongly induced by heat stress. Deletion of some of these ncRNA genes affects the survival of animals subjected to heat shock, suggesting that ncRNAs may be important new regulators of the HSR. Furthermore, specific ncRNA genes may be immediate and direct targets of the HSR. A short episode of heat shock in larval stage *C. elegans* triggers the up-regulation of less than 20 genes. Within this limited set of acute heat-induced genes are a miRNA and a class of repetitive RNAs called Helitrons. Similar to canonical heat shock protein genes, we found that the promoter sequences of the miRNA and Helitron genes contain Heat Shock Elements (HSEs) that are bound by the Heat Shock Factor 1 (HSF-1) transcription factor in response to heat shock. In summary, we have determined that heat shock causes extensive remodeling of the ncRNA landscape, providing new candidates for regulation of the HSR.

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264 The splicing factor Mud2 functions in transcription and nuclear mRNP packaging

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Different steps in gene expression are intimately linked to coordinate and regulate this complex process. During transcription, numerous RNA-binding proteins are already loaded onto the nascent mRNA and package the mRNA into a messenger ribonucleoprotein particle (mRNP). Accordingly, these RNA-binding proteins are often involved in more than one step of gene expression. For example, TREX functions in transcription, mRNP packaging and nuclear mRNA export. Previously, we showed that the Prp19 splicing complex (Prp19C) is needed for efficient transcription as well as TREX occupancy at transcribed genes. Here, we show that the splicing factor Mud2 interacts with Prp19C and is needed for Prp19C occupancy at transcribed genes in S. cerevisiae. Interestingly, Mud2 is not only recruited to intron-containing but also intronless genes indicating a role in transcription. Importantly, we show for the first time that Mud2 functions in transcription. Furthermore, these functions of Mud2 are likely evolutionarily conserved as Mud2 is also recruited to an intronless gene and interacts with Prp19C in D. melanogaster. Taken together, we classify Mud2 as a novel transcription factor that is necessary for the recruitment of mRNA-binding proteins to the transcription machinery. Thus, Mud2 is a multifunctional protein important for transcription, splicing and mRNP packaging.

265 Multiple functions of SRSF3 in RNA splicing, export and surveillance

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RNA-binding proteins (RBPs) generally have roles in multiple steps of RNA metabolism from transcription and translation. Serine/arginine splicing factor 3 (SRSF3), a member of a family of RBPs, binds specific RNA and regulates alternative splicing of pre-mRNA. As a shuttling protein between the nucleus and the cytoplasm, it was also shown to be a potent adaptor for RNA export. In addition, RNA splicing and export occur cooperatively with mRNA quality control process, probably thru proper mRNA discrimination machinery. However, the significance of SRSF3-mediated RNA alternative splicing and RNA export have not been clearly understood and the molecular mechanism are not investigated with specific target transcripts. Herein, we will demonstrate the role of SRSF3 in coordinating alternative splicing, export and surveillance of pre-mature termination codon (PTC) containing exons. SRSF3 not only binds PTC-containing transcripts, but also modulates their mRNA stability by recruiting the mRNA decay factors. Furthermore, using MS2-tethering reporter system, we will show that the tethering of SRSF3 on the retained intron near PTC-harboring exons facilitates RNA surveillance. In summary, our data suggest that the SRSF3 can promote the decay of a PTC-containing mRNA, implying it as a target identifier or as a mediator of mRNA decay. Our findings illustrate the multiple roles of SR proteins in post-transcription coupled RNA processing.

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266 Rate-limiting LIN28B levels balance opposing post-transcriptional effects

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RNA binding proteins (RBPs) are often vastly outnumbered by their target RNAs. Variation in RBP expression may therefore play a key, yet currently unstudied role, in defining the extent and character of post-transcriptional regulation. Here we show these considerations are essential for understanding the molecular biology of LIN28B, an RBP whose dynamic expression patterns play instructive roles during mammalian development and disease. By systematically varying LIN28B protein levels in human cells, we discovered a dose-dependent divergence in transcriptome-wide ribosome occupancy that was mediated by: (1) the redistribution of Argonaute-bound microRNAs from families that are regulated by LIN28B to those that are not; and (2) the inhibition of translationally repressive messenger RNA sequences through direct LIN28B binding. Changes in LIN28B expression independently regulated the strength of these opposing post-transcriptional effects and their relative contribution to target regulation. These studies also uncovered a remarkable co-evolution of independent post-transcriptional responses, whose signatures are physically encoded in the mammalian genome, which guarantee that every gene is regulated. We find that messenger RNAs directly bound by LIN28B are depleted in *let-7*/miR-98 sites and are enriched for all other microRNA sites. These correlations and anti-correlations are ultimately predictive of post-transcriptional control. Together, these data show that RBPs can encode much of their regulation through changes in expression.

267 Regulation of the chromatin remodeler Snf2 during meiosis in *S. cerevisiae*

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Eukaryotic organisms have evolved complex gene regulatory networks to launch coordinated responses to external conditions and stimuli. Upon nutrient starvation in *Saccharomyces cerevisiae*, the energetically expensive process of ribosome biogenesis decreases substantially and cells undergo meiosis (sporulation in yeast). Resources are reallocated at the onset of meiosis to ensure survival and viability of the resulting spores. We have previously shown that, upon sporulation, the catalytic component of the SWI/SNF chromatin remodeling complex, Snf2, is responsible for shifting gene expression away from intron-rich ribosomal protein genes (RPGs) to enhance splicing of meiotic intron-containing genes (ICGs). While Snf2 is required early in sporulation to allow expression of critical transcripts such as the meiotic splicing activator *MER1*, it is subsequently downregulated, which dampens RPG expression. Intriguingly, *SNF2* RNA levels remain relatively constant. Although critical to its activity, the mechanism by which Snf2 protein levels change remains unknown.

In order to understand how levels of Snf2 protein are regulated during meiosis, we have examined *SNF2* post-transcriptional control. Here we describe temporally-regulated mRNA isoform switching and m^6A modification under sporulation conditions. Moreover, our preliminary data suggests that these mechanisms converge to control Snf2 protein expression during meiosis. In light of the conservation of Snf2, investigating its dynamic regulation in response to environmental changes in *S. cerevisiae* may carry important implications for Snf2 chromatin remodeling activity in higher eukaryotes, including its role in cancer, fertility and nutrient stress.

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268 Functional coupling of pre-mRNA processes via intron branch-site recognition in budding yeast

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Steps of the gene expression pathway, such as transcription, messenger RNA (mRNA) processing, and nuclear export are extensively coupled in metazoans, primarily via the C-terminal domain (CTD) of the largest subunit of the RNA polymerase II (pol II), which serves as a platform for recruiting RNA processing and export factors. However, the precise mechanisms by which coupling occurs remain enigmatic. Furthermore, despite extensive conservation of the gene expression machineries, the nature and the benefit of co-transcriptional splicing in the yeast *Saccharomyces cerevisiae* remain contentious, hinting at novel modes of action. Here we demonstrate a novel avenue through which transcription impacts on splicing by enforcing proper recruitment of intron branch-site binding protein (BBP/Msl5) and, hence, branch-site recognition. Specifically, we show that, by using a battery of transcriptional perturbations, such as mutational alterations of the transcription initiation factor TFIIB and Rpb2, 6-azauracil treatment, and eliminating several transcription elongation factors can circumvent the need of Sub2, an otherwise essential DExD/H-box splicing factor. Detailed chromatin IP (ChIP) analysis reveals that these perturbations significantly reduce co-transcriptional recruitment of the BBP and the related U1-snRNP binding, thus providing a plausible explanation as to how Sub2 loss can be tolerated. On the other hand, we experimentally evolved yeast cells harboring an *msl5* allele capable of bypassing the loss of Sub2 to explore how cellular fitness can be restored when branch site recognition is deficient. Intriguingly, adaptive mutations on transcription factors, nuclear RNA surveillance and export factors can compensate for splicing deficiency. Taken together, our data suggest a scenario in which the early splicing events at the intron branch site can be modulated by the status of the transcription and export machinery. These findings suggest a potential selective advantage that allows yeast to exploit compensatory changes in coupled pathways to offset the catastrophic loss of key components, a strategy that may well be conserved in evolution, and that naturally occurring polymorphisms may provide "back-up circuitries" that permit survival upon inactivation of essential genes.

269 The Rrp6 Subunit of the Nuclear Exosome and its Role in Promoting Cell Survival During Cellular Stress

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The nuclear exosome plays a central role in RNA processing and degradation. Although biochemical studies have shed light on the activity and functions of the exosome, its role in the cellular response to stress is poorly understood. Here, we show that a genetic interaction between the nuclear exosome and the mitogen-activated protein kinase (MAPK) cascade pathway is essential for survival of the yeast S.cerevisiae in heat stress. The absence of both Rrp6 and the MAP kinase Mpk1 results in a synthetically lethal phenotype at elevated temperatures. We detected a similar synthetic lethal interaction between Rrp6p and the transcription elongation factor Paf1, which is involved in the transcriptional activation of genes in response to heat stress. These synthetic lethal phenotypes can be rescued by the addition of osmotic support, indicating that they result from a compromised cell wall integrity at elevated temperatures. The lethality of mpk1Δrrp6Δ at high temperatures can also be rescued by a catalytically inactive mutant form of Mpk1, suggesting that the preservation of cell wall integrity does not depend on the phosphorylation of Paf1 by Mpk1. We further show that strains defective in other components of the nuclear exosome partially suppress the growth phenotypes of mpk1Δ and that overexpression of Rrp6p shows a similar effect. These observations suggest that the role of Rrp6p in controlling the stress response is mediated by Rrp6 independently from its function as a nuclear specific subunit of the exosome. We are currently investigating the molecular mechanisms by which Rrp6 contributes to controlling the heat stress response in cooperation with Mpk1. Using RNA-Seq, we have identified two candidate genes, HSP150 and NCW2, that are substantially repressed in the mpk1Δrrp6Δ mutant. This repression may in turn be responsible for the observed lethal phenotype, which would indicate that Rrp6 or the nuclear exosome must closely modulate the expression of specific genes to promote cell survival during stress. Our efforts to determine the molecular basis connecting Rrp6 to the MAPK-mediated stress response pathway will provide new insights into the role of one of the nuclear subunits of the exosome in the cell's ability to survive under stress.

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270 mRNA processing in oxygen sensing and hypoxia response

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The aggressive proliferation of cancer cells often result in a microenvironment of low oxygen levels or hypoxia. In response to hypoxia conditions, cancer cells reprogram their gene expression and metabolism to promote malignant transformation and metastasis. Therefore, in order to develop new therapeutic strategies for cancer, it is critical to understand the cellular mechanisms for sensing oxygen levels and responding to hypoxia conditions.

It has been well established that the oxygen-dependent proline hydroxylation of HIF1a is a key mechanism for oxygen-sensing and HIF1a-mediated transcriptional regulation plays a critical role in cellular response to hypoxia. However, it remains unclear whether and how gene expression is regulated by hypoxia at the post-transcriptional level. Here we report that the prolyl 4-hydroxylase P4H is associated with many mRNA processing factors, including splicing and 3' processing factors. Detailed analyses of P4H-associated mRNA 3' processing factors revealed that a number of these proteins contain hydroxylated prolines. Inhibiting P4H enzymatic activity by small molecules or by genetic ablation of the P4HA1 gene resulted in loss of proline hydroxylation and a significantly decrease in the protein levels of mRNA 3' processing factors. Importantly, similar depletion of mRNA 3' processing factors was also observed under hypoxia conditions. Finally we provide evidence that hypoxia-induced depletion of mRNA processing factors leads to mRNA processing alterations. Together our data suggest that P4H-mediated prolyl hydroxylation of mRNA processing factors plays a role in oxygen-sensing and post-transcriptional gene regulation in response to hypoxia. Therefore cellular response to hypoxia is more complicated than previously thought and involves both transcriptional and post-transcriptional mechanisms.

271 Dissecting the (patho)physiological functions of NEAT1 isoforms

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The long non-coding RNA *NEAT1* exists in two nuclear retained isoforms. The short (v1) isoform (3.7 kb) overlaps completely with the *NEAT1* long transcript (v2; 22.7 kb) at its 5' end. Genetic studies in mice established that loss of both isoforms leads to reduced female fertility and defective mammary gland development during pregnancy and lactation. *Neat1* loss also led to impaired oncogene-induced cell proliferation, accumulation of DNA damage and decreased skin cancer development. In order to dissect the contribution of each of the *Neat1* isoforms to these phenotypes, we used various knockdown (KD) and knockout (KO) approaches. Normal mouse embryonic fibroblasts (MEFs) deficient for *Neat1_v1* grew similarly to WT or MEFs KO for both isoforms, indicating that non-transformed cells do not depend on either *Neat1* transcripts for proliferation and survival. Importantly, specific silencing of *NEAT1_v1* in various cancer cell lines did not recapitulate the increased DNA damage and cell cycle arrest phenotypes seen upon KD of either *NEAT1_v2* or both isoforms. Consistently, whereas loss of both *Neat1* isoforms impaired chemically-induced skin hyperplasia, loss of *NEAT1_v1* did not. Furthermore, *Neat1_v1* KO females did not exhibit fertility defects and were able to nurture their pups, just like WT females, indicating that mammary gland development and lactation occurs normally on this genetic background. Together, these data indicate that the long *NEAT1* isoform, but not *Neat1_v1*, is required for mammary gland development and oncogene-induced cell proliferation *in vivo*. Given the well-established role of *NEAT1_v2* in the assembly of nuclear bodies termed paraspeckles (PS), these data establish a possible link between these phenotypes and PS biology. The pathophysiological function of *Neat1_v1*, which is a highly evolutionarily conserved transcript, if any, remains to be elucidated.

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272 Molybdenum-dependent riboswitch: hunting for the missing metabolite

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A potential molybdenum-dependent riboswitch has been identified more than 10 years ago by an extended comparative genomic annotation pipeline. The Moco RNA *motif* presents classical riboswitch characteristics, such as sequence conservation, nucleotide covariation and spare conserved nucleotides alternate to structured base-paired elements. Moreover, studies on the *moaA* riboswitch, a Moco RNA *motif* from *E. coli*, that is located upstream of genes involved in the molybdenum metabolism, proved its implication in their regulation.[1] All these elements make the Moco RNA *motif* a very promising riboswitch candidate. However, no evidence of direct interactions between the *moaA* riboswitch and any metabolite have ever been observed. Moco (Molybdenum cofactor) or one of its biosynthetic precursors are the most accredited metabolite candidates to interact specifically with Moco RNA *motifs*. These molecules are extremely unstable and dioxygen sensitive. In fact, they cannot be synthesized by any chemical reaction and they are only available by biosynthesis.[2] Especially, Moco has never been isolated but has solely been extracted within molybdo-dependent enzymes or Moco Carrier Proteins (MCP).[2] The scarce availability and dioxygen sensitivity of Moco and its precursor make the study of their interaction with the Moco RNA *motifs* not possible with the commonly used biochemical methods to investigate long RNAs. Thus, we have optimized, new procedures for different footprinting assays including in-line probing and Terbium(III) cleavage, in order to perform them under rigorous exclusion of dioxygen.

Our aim is to prove if the *moaA* riboswitch from *E.coli* is able to specifically bind a small cellular metabolite *in vitro* and to identify which molecule along the Moco biosynthetic pathway it would be. This would provide the crucial evidence that the *moaA* riboswitch is actually a riboswitch and that the molybdenum metabolism is regulated by a new family of these regulatory RNA elements. The new methods, which we have developed for anaerobic assays, prove that some metabolites along the Moco biosynthetic pathway do not cause any change in the RNA conformation, so they can be excluded as specific RNA binding partner.

[1] Regulski E.E, et al, *Mol Microbiol*, **2008**, 68: 918.

[2] Schwarz, G, et al, *Nature*, **2009,** 460: 839.

273 Perturbing Your Neighbor: Systematic evaluation of lncRNA-targeted antisense oligonucleotides on lncRNA knockdown and impact on neighboring gene expression

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Long non-coding RNAs (lncRNAs) have received considerable attention for their roles in regulating gene expression. This regulation occurs through complex and multifaceted mechanisms including recruitment of epigenetic factors that influence the transcription of neighboring protein-coding genes. Numerous publications demonstrate that lncRNA knockdown by either siRNA or antisense oligonucleotides (ASOs) elicits expression changes in proximal genes in various systems. These vignettes suggest that lncRNA targeted oligos can be used to specifically modulate endogenous gene expression as a novel therapeutic strategy to correct expression imbalances associated with or driving disease states. However, a systematic evaluation of this approach has not been shown.

We have now conducted, to our knowledge, the broadest and most thorough screening of lncRNA-targeted oligos in a single system to date. In the first phase we surveyed the lncRNA landscape in liver - the tissue most amenable to in vivo oligo delivery- and hepatocyte-derived cell lines using RNA-Seq to identify novel conserved lncRNAs from chromatin/nuclear/cytoplasmic fractions of human, mouse, and non-human primate samples. Next, in the sequenced cell lines we evaluated the effects of both oligo and lncRNA properties on extent and likelihood of knockdown for each exon of 17 human and 10 mouse lncRNA targets. The most optimal parameters found to produce robust knockdown were, in turn, used to screen 100 additional liver-expressed lncRNAs representing a diverse target set that balances hypothesis exploration (e.g. are divergent or overlapping antisense-oriented lncRNAs more active?) with pragmatic drug discovery (e.g. targets have disease relevance and are conserved in model species). Using microfluidic high-throughput qPCR, we measured expression changes, or lack thereof, in ~200 proximal or eQTL-linked protein-coding genes resulting from oligo treatment while controlling for both on and off target effects. We will present an overview of our approach, and showcase our data by summarizing trends, highlighting interesting findings, and discussing important considerations and guidelines for this field of inquiry.

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274 Differential Composition and Localization of Chromatin-Associated RNAs in the Estrogen Response

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The advent of high-throughput sequencing technologies has revealed that the human transcriptome comprises mostly non-protein-coding RNAs. A large class of these transcripts, the long noncoding RNAs (lncRNAs) have been implicated in a diverse array of cellular processes, but their many functions are only beginning to be appreciated. How these molecules operate mechanistically is even less well-understood, but many are believed to act in direct proximity to the chromatin. We are using a recently-developed technique called chromatin-associated RNA sequencing (ChAR-seq) to map RNA-chromatin contacts globally. To understand mechanisms of chromatin-associated lncRNA function, we are treating MCF-7 breast cancer cells with 17β-estradiol and will be presenting progress toward applying ChAR-seq to this well-characterized system. Our results will reveal how the RNA composition and localization of the chromatin correlates with a disease-relevant remodeling of gene expression.

275 Identification orthologous long noncoding RNAs with transcriptomic evidence

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Long non-coding RNAs (lncRNAs) are a large and diverse class of transcribed RNA molecules with a length of more than 200 nucleotides and devoid of protein coding potential. Multiple studies show they are engaged in a plethora of molecular functions and contribute considerably to the observed diversification of eukaryotic transcriptomes. However, our knowledge on evolution of these molecules is still very limited.

To address this issue we have developed a pipeline for evolutionary analyses of lncRNAs. It consists of two parts. The first part enables ab initio transcriptome assembly from RNA-Seq data, followed by identification of lncRNAs, using a number of commonly accepted criteria. The second module serves as a tool for identification of orthologous lncRNAs, utilizing both synthenic and sequence level of comparisons across species of interest. Using the pipeline, we have identified 8994 lncRNAs expressed in human cell line HEK293 and looked for their orthologs across 11 primate species as well as in mouse and zebrafish. These data provided us with a unique opportunity to track evolutionary dynamics of selected lncRNAs and also characterize them on multiple levels, including their genomic context, expression-related phenomena and evolution of Alu elements within transcripts.

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276 Circular RNA Immunity

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The discovery of novel RNAs challenges the simplistic central dogma that RNA is merely an intermediary between DNA and proteins. Circular RNAs (circRNAs) are single-stranded RNAs that are covalently joined head to tail. This is a newly identified class of ubiquitous RNAs in essentially all eukaryotes. Conservation across the eukaryotic tree of life strongly suggests that circRNAs have a functional role in the cell. However, whether and how circular RNAs are sensed or evade the immune system is completely unknown. We unveil a self-nonself discrimination system for circRNAs in human and mouse cells, and demonstrate that foreign circular RNAs potently stimulate innate immune signaling. Our research further identifies that the intron that programs the circular RNA biogenesis dictates its self-nonself status. In addition, we discover that delivery of circRNA confers protection against viral infection, presenting the potential that circRNAs can be developed into an immunotherapy and/or vaccine adjuvant. Collectively, this work reveals a fundamentally new system of RNA self-nonself discrimination, and show how circRNAs dictate RNA immunity.

277 A Cytoplasmically-Localized Long Noncoding RNA Regulates The Switch Between Macrophage Differentiation And Inflammation

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Macrophages are critical effector cells of the innate immune system essential for controlling infection and maintaining tissue homeostasis. At the cellular level, pathogen-response involves recognition by Toll-like receptors (TLRs) and complex intracellular signaling cascades that result in induction of an inflammatory program. Perturbations to these signaling pathways can have devastating consequences, leading to diseases, such as Rheumatoid Arthritis and Cancer. Macrophages arise from monocytes in a differentiation process that is tightly regulated, involving many microRNAs, proteins and stage-specific expression of transcription factors. Long non-coding RNAs (lncRNAs) represent the largest group of RNA produced from the genome and are described as transcripts greater than 200 nucleotides in length that lack protein-coding ability. LncRNAs are rapidly emerging as critical regulators of a broad range of biological processes including genomic imprinting, development, and cancer. We sought to identify novel lncRNAs involved in monocyte to macrophage differentiation. We generated comprehensive RNA-sequencing data sets from primary healthy human monocytes, differentiated macrophages and identified hundreds of lncRNAs differentially expressed during differentiation. We characterized one lncRNA, called GAPLINC, which is dramatically induced over one thousand fold transitioning from monocyte to macrophages. GAPLINC is localized to the cytoplasm, but does not associate with polysomes. Interestingly, this lncRNA is rapidly downregulated upon TLR stimulation suggesting a connection to inflammatory pathways. Knocking down GAPLINC in primary human macrophages results in up-regulation of inflammation-related genes, suggesting this lncRNA may negatively regulate inflammatory pathways. Overexpression of GAPLINC suppresses the inflammatory response and promotes proliferation. Here we reveal an interesting role for a lncRNA in regulating the switch between macrophage differentiation/proliferation and the downstream inflammation pathways.

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278 The role of long non-coding RNAs in mediating human steroidogenesis

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The adrenal cortex synthesizes steroid hormones including glucocorticoids, mineralocorticoids and androgens. Steroidogenesis is tightly regulated process stimulated by Angiotensin II (AngII), potassium, and adrenocorticotropic hormone (ACTH). Research has traditionally focused on the signal transduction pathways and transcription factors regulating the steroidogenic gene expression response. However, very little is known about the role of long non-coding RNA (lncRNA), an important class of RNA regulatory factors, in modulating steroidogenesis. Indeed, steroid receptor activator (SRA1), a lncRNA, has been shown to regulate steroidogenesis. This prompted us to identify novel lncRNAs that regulated the steroidogenic response. Here, we performed RNA-seq on adrenocortical carcinoma cells (H295R) treated with either AngII or forskolin for ¼, 1, 2, 4, 8, 24, and 48 hours. We identified 49 lncRNAs, including MALAT1, exhibiting statistically significant differential expression upon stimulation in human adrenocortical cells. We find that a number of these lncRNAs show a rapid induction in response stimulation. Our current efforts focus on characterizing the precise cis- and/or trans-regulatory mechanisms and targets of these lncRNAs. Due to their robust induction in response to both AngII and forskolin stimulation, these lncRNAs may be strong candidate biomarkers for adrenal disorders or may serve as targets for antisense oligo therapy in patients. Ultimately, our studies will provide insight into adrenal disease management as well as novel regulators of human steroidogenesis.

279 LncRNA MEG3 functions as a tumor suppressor in prostate cancer

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Long noncoding RNAs (lncRNAs) have been implicated in many biological processes and mainly function through epigenetic mechanisms. Our previous study, comparing prostate basal and luminal cells via RNA-Seq analysis, found that over 20% of the differentially expressed transcripts are lncRNAs. However, the biological function and molecular mechanism of these lncRNAs in prostate cancer (PCa) remain unclear. Here, we show that Maternal Expressed Gene 3 (MEG3) is predominantly expressed in human prostate basal cells when compared to prostate luminal cells and PCa tissues. Additionally, ONCOMINE datasets suggested that loss of expression of MEG3 significantly correlated with metastasis, higher clinical stage, and poor overall survival of PCa patients. Using in vitro and in vivo systems, we demonstrated that MEG3 overexpression suppressed PCa cell proliferation, colony and sphere formation, and metastasis. Furthermore, loss of MEG3 enhanced EZH2 function, leading to a stronger epigenetic suppression of target gene DAB2IP. Overall, these observations highlight MEG3 as a potential diagnostic and prognostic biomarker that may be exploited therapeutically.

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280 Mirror-Image In vitro Selection of Modified L-DNA Aptamer Targeting MicroRNA-155

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MicroRNAs (miRs) are small, noncoding RNAs which can act as post-transcriptional regulators of gene expression and are involved in various biological processes. In particular, miR-155 plays a key role in various physiological and pathological processes, and the overexpression of miR-155 is implicated in the development and aggressiveness of various cancers. Thus, the development of a novel class of specific and high affinity nucleic acid aptamers capable of inhibiting miR-155 biogenesis could be attractive therapeutic approach. We have shown previously that aptamers comprised of L-RNA, the nuclease resistant enantiomer of native D-RNA, are ideally suited for targeting structured RNAs. Because L-aptamers are incapable of forming contiguous WC base pairs with native D-nucleic acids, they instead bind their RNA targets through tertiary interactions (or shape). This unique binding modality reduces the probability of off target effects. Here, we report the in vitro selection of the first L-DNA aptamers capable of binding a native D-RNA target, precursor miR-155. L-DNA aptamers are more stable, easier to handle, and less expensive to prepare than their L-RNA counterparts. Additionally, we introduced a cationic amino modification in one of the nucleobases, which facilitates additional salt bridge or H-bond interaction with the target D-RNA. We show that modified L-DNA aptamers bind precursor miR-155 with high affinity (< 50 nM) and specificity and are completely dependent on the cationic modification. The in vitro selection methodology, chemical synthesis, and functional analysis of L-DNA aptamers will be discussed.

281 Regulation of Hepatic Glycogen by a Long Noncoding RNA that Determines Total Serum Cholesterol in Man

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Nearly two dozen genome-wide association studies (GWAS) have pointed out a well-known coding gene PPP1R3B (the regulatory liver protein phosphatase PP1 subunit) as a candidate causal gene controlling metabolic phenotypes. We tested the hypothesis that the true causal genetic variants lie in a linked long noncoding RNA gene (LOC157273), 173 kb from PPP1R3B. The GWAS single-nucleotide-polymorphisms (SNPs) that associate with a wide variety of metabolic disease phenotypes map over the long noncoding RNA gene LOC157273. The lncRNA gene is unusual. It is confined to primates (absent from rodents); it is expressed only in liver hepatocytes. The lncRNA is unusual compared to other lncRNAs in subcellular localization assessed by "Stellaris" fluorescent *in situ* hybridization (FISH), confined to cytoplasmic "bodies" of 0.5-1.2 μ lying just outside the nucleus. Using cultured primary human hepatocytes we knock down the gene (loss-of-function) with small-interfering RNA (siRNA); for LOC157273 gain-of-function, we use insulin. The siRNA knockdown (KD) leads to an upregulation of the coding gene PPP1R3B. Insulin also upregulates PPP1R3B mRNA, assessed using TaqMan qRT-PCR. Screening 16 human hepatocyte donors we found one donor heterozygous for the LOC157273 SNP rs4841132 where major allele is G and minor allele is A. Compared to G/G donors, this A/G donor exhibited reduced (75%) LOC157273 lncRNA and elevated (4- to 5-fold) PPP1R3B mRNA. In accordance with the elevated PPP1R3B mRNA, the A/G donor displays 3-fold elevated levels of glycogen. To learn if both major and minor copies of LOC157273 in the A/G donor are equally expressed, we developed two different methods to assess allele-specific transcription; the minor allele shows 60-85% transcriptional crippling. To learn additional genes dependent on LOC157273 transcript level, we performed deep RNA-seq after siRNA-mediated KD of LOC, confirming the effect on PPP1R3B but revealing another several dozen genes influenced by the lncRNA. We have cloned 5.6 kb of the major and minor versions of LOC157273 and linked fragments to luciferase reporter to identify the *cis*-acting element in the minor haplotype that harbors the transcriptional deficit. Together, our findings support a direct role of an lncRNA operating in human liver regulating hepatic glycogen content and total serum cholesterol.

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282 Characterization of R-loop structures in budding yeast

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R-loops are nucleic-acid structures composed of an RNA:DNA hybrid and a displaced single-stranded DNA. R-loops occur more frequently in the genome and have greater physiological importance than was previously predicted. They play vital roles in regulating gene expression (1), DNA replication (2), and DNA and histone modifications (3). Paradoxically, while they do play essential positive functions, they also contribute to DNA damage and genome instability (4). Recent evidence shows that R-loops are involved in a number of human diseases, including neurological disorders (5) and cancer (4). Despite the growing interest in R-loop regulation, there are currently few techniques available to identify and characterize R-loop structures *in vivo*. We are developing new strategies for mapping R-loops genome wide. We will present progress on establishing these techniques to map R-loops in *Saccharomyces cerevisiae*. We are also using reporter assay and the budding yeast genetic system to identify factors that may promote formation of R-loops formed by lncRNAs (an abundant class of non-coding RNAs that have been implicated in gene regulation (6)). Taken together, we hope to provide a detailed understanding of molecular interactions that promote formation of biologically beneficial R-loops.

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283 Long noncoding RNA in vascular smooth muscle cells regulates vascular calcification

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Vascular calcification (VC) occurs in response to misregulated calcium and phosphate metabolism and is characterized as the damage of vascular smooth muscle cells (VSMCs). It declines vessel elasticity resulting in impaired cardiovascular hemodynamics and increased morbidity and mortality associated with hypertension, cardiac hypertrophy, and other cardiovascular diseases. Long noncoding RNAs (lncRNAs) are transcripts longer than 200 nucleotides without protein-coding potential. The majority of lncRNAs share features of protein-coding mRNAs because they are capped, polyadenylated, and spliced. While the function of their smaller counterparts such as small nuclear RNAs or microRNAs has been well-established, the role of lncRNAs in many biological phenomena are much less understood, especially during VC. Using RNA sequencing, we identified >1,200 novel lncRNAs and also many lncRNAs whose expression was altered in rat VSMCs treated with inorganic phosphate (Pi), which mimics VC. We validated the expression of a subset of the most differentially expressed lncRNAs. They are transcribed nearby protein-coding genes which have pivotal roles in VSMCs. We further selected four candidates lncRNAs and defined their gene structures by performing 5' and 3' rapid amplification of cDNA ends (RACE). By overexpressing the lncRNAs, we found that calcium deposition substantially diminished by these lncRNAs. Now we are investigating the working mechanism of these lncRNA candidates. The observation from this study will help us to find a novel therapeutic strategy for the prevention or treatment of a variety of diseases associated with vascular calcification.

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284 *LINK-A* interacts with PtdIns(3,4,5)P_3 to hyperactivate AKT and confer resistance to AKT inhibitors

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Background: Phosphatidylinositol-3,4,5-trisphosphate (PIP3), serving as a second messenger, is an indispensable mediator in many signaling pathways to regulate fundamental cellular responses to extracellular signals. Although primordial functions of phospholipids and RNAs have been hypothesized in the prebiotic "RNA world", the existence of physiological RNA-phospholipid interactions and further involvement of these events in essential cellular processes remain a mystery. Methods: RNA-lipid interaction was examined using RNA-lipid overlay assay, lipid-RNA pulldown assay, fluorescence resonance energy transfer (FRET) assay, and phospholipid-containing DOPC giant vesicles binding assay. Alpha binding assay was used to determine Kd for RNA-lipid interaction. The AKT kinase activity was measured by a non-radioactive AKT kinase activity kit. RNA PIP3-binding motif knockout cell lines were generated using the CRISPR/Cas9 genome editing system. Spheroid growth and 3-D invasion of parental MDA-MB-231 and its genomically edited derivative cells were conducted using Cultrex® 3-D Spheroid Fluorometric Proliferation/Viability Assay and 3D Spheroid BME Cell Invasion Assay kits. In vivo tumorigenesis and glucose uptake study were investigated with [^{18}F]-FDG PET/CT imaging. In addition, the somatic copy number variations (CNVs) of *LINK-A* gene loci were detected from whole genome and whole-exome sequencing from matched tumor and normal samples based on TCGA database. Results: *L*ong *I*ntergenic *N*oncoding RNA for *K*inase *A*ctivation (*LINK-A*) directly and specifically interacts with AKT and PIP3 at single nucleotide level, which facilitates the binding of AKT to PIP3 and consequent enzymatic activation. The *LINK-A*-dependent AKT hyperactivation leads to tumorigenesis and resistance to the pharmacological AKT inhibitors. Genomic deletions of the *LINK-A* PIP3-binding motif sensitized breast cancer cells 20,000 fold to AKT inhibitors. Furthermore, meta-analysis uncovered the association between expression of *LINK-A* and the high incidence rate of an SNP (rs12095274:A>G), which further correlated with AKT phosphorylation status, and poor outcomes for breast and lung cancer patients. Conclusion: Our data reveal a PIP3-dependent role of lncRNA in meditating AKT activation and conferring resistance to AKT inhibitors in cancer cells. From the clinical point of view these findings provide a rationale for the development of *LINK-A* -targeting RNA medicine, either alone or in combination with AKT inhibitors, for treating breast tumors.

285 The Long Noncoding RNA PLUTO-201 is Associated with Prostate Cancer Progression

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Background: Long noncoding RNAs (lncRNAs) have recently been shown to serve as drivers of malignancy and represent novel therapeutic targets. We have recently identified over 46,000 novel lncRNAs through *in silico* analyses of nearly 8,000 tumor or normal tissue specimens. In this study, we utilize this compendium of lncRNAs to identify those associated with metastatic progression of prostate cancer (PCa), and functionally investigate our top nominated candidate, PLUTO-201, in preclinical models of disease.

Methods: Transcriptional profiling of a cohort of prostatectomy patients was used to identify new PCa-associated lncRNAs. The gene most strongly associated with subsequent metastatic progression, PLUTO-201, was further analyzed. Rapid Amplification of cDNA Ends (RACE) was used to define its gene structure, and Fluorescence In-Situ Hybridization (FISH) was used to localize PLUTO-201 transcripts. Knockdown and over-expression studies were performed *in vitro* and *in vivo* to elucidate the functions of PLUTO-201. Finally, transcriptional analysis was performed to identify gene sets associated with this candidate.

Results: Of all annotated protein-coding genes and lncRNAs, PLUTO-201 was the most strongly associated with metastatic progression, via Area Under the Curve (AUC) assessment on a Receiver Operating Characteristic (ROC) analysis. For multivariable analyses, PLUTO-201 remained prognostic while accounting for standard clinicopathologic variables. Additionally, PLUTO-201 expression is relatively specific for PCa. Knockdown of PLUTO-201 significantly impaired proliferation and invasion of PCa cells *in vitro,* and tumor growth and metastasis *in vivo*. Conversely, PLUTO-201 over-expression increases the invasion potential of the prostate epithelial cell line 22Rv1.

Conclusions: We have identified a novel, prostate-specific lncRNA (PLUTO-201) that promotes PCa proliferation and metastasis, and is associated with poor clinical prognosis. Future investigations regarding its mechanisms of action are warranted to further elucidate its role in PCa progression.

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286 Withdrawn

287 Investigations into a long non-coding RNA critical for cancer cell survival and proliferation

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BC200 is a 200 nt primate-specific long non-coding RNA expressed at high levels in brain. In addition to brain, BC200 is expressed at moderate levels in testes and is over-expressed in wide variety of tumours and tumour-derived cell lines. BC200 is postulated to play a role in translational regulation of a subset of mRNAs, possibly acting as a key regulator of site specific protein translation. Data concerning the role of BC200 in neural tissue as well as tumour cells is preliminary and to date no mRNAs directly regulated by BC200 have been elucidated. Here we present our work to characterize the structure and function of BC200. Knock-down of BC200 expression results in a dramatic loss of viability through growth arrest and induction of apoptosis; whereas BC200 overexpression had no discernible impact on cell growth or viability. A substantial decrease in BC200 expression was observed upon cell confluence, as well as drug induced cell cycle arrest in G1 or G2 but not S- or M-phases. Protein binding partners of BC200 have been identified through mass spectrometry approaches, as have potential cancer-specific RNA targets via qPCR arrays. Together the work presents compelling evidence that BC200 plays a central role in cancer cell survival and proliferation.

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288 Long noncoding RNA complementarity and target transcripts abundance.

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Eukaryotic mRNA metabolism regulates its stability, localization, and translation using complementarity with counter-part RNAs. To modulate their stability, small and long noncoding RNAs can establish complementarity with their target mRNAs. Although complementarity of small interfering RNAs and microRNAs with target mRNAs has been studied thoroughly, partial complementarity of long noncoding RNAs (lncRNAs) with their target mRNAs has not been investigated clearly. To address that research gap, our lab investigated whether the sequence complementarity of two lncRNAs, *lincRNA-p21* and *OIP5-AS1*, influenced the quantity of target RNA expression. We predicted a positive correlation between lncRNA complementarity and target mRNA quantity. We confirmed this prediction using RNA affinity pull down, microarray, and RNA-sequencing analysis. In addition, we utilized the information from this analysis to compare the quantity of target mRNAs when two lncRNAs, *lincRNA-p21* and *OIP5-AS1*, are depleted by siRNAs. We observed that human and mouse *lincRNA-p21* regulated target mRNA abundance in complementarity-dependent and independent manners. In contrast, affinity pull down of *OIP5-AS1* revealed that changes in *OIP5-AS1* expression influenced the amount of some *OIP5-AS1* target mRNAs and miRNAs, as we predicted from our sequence complementarity assay. Altogether**,** the current study demonstrates that partial complementarity of lncRNAs and mRNAs (even miRNAs) assist in determining target RNA expression and quantity.

289 Pof8 regulates Sm to Lsm2-8 complex transition in *S. pombe* during telomerase RNA biogenesis

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Telomeres are protective caps at the ends of linear chromosomes that protect natural DNA ends from being recognized as double-strand breaks. Deficiency to maintain proper telomere structure results in premature aging or chromosome instability. As part of telomere maintenance, telomerase, a specialized reverse transcriptase, extends shortened telomeres. The telomerase consists at its core of a protein catalytic subunit (TERT) with a reverse transcriptase domain, and a non-coding RNA subunit (TR), functioning as the template and a scaffold for the holoenzyme. The biogenesis pathway of TR (as TER1 in fission yeast *S. pombe*) ensures correct folding of the RNA and proper assembly of the ribonucleoprotein complex. In *S. pombe*, TER1 is transcribed as a longer precursor that is bound by the Sm protein complex. Sm complex binding promotes spliceosomal cleavage, the first catalytic step of a splicing reaction. After spliceosomal cleavage, the Sm complex is replaced by the Lsm2-8 complex which stabilizes and loads mature TER1 onto Trt1, TERT in *S. pombe*. Currently, the mechanism of Sm to Lsm2-8 complex transition is unknown. Here we report that Pof8, structurally similar to the ciliated telomerase subunits p43 and p65, functions in a hierarchical assembly pathway by promoting the binding of the Lsm2-8 complex to telomerase RNA, which in turn promotes binding of the catalytic subunit. Loss of Pof8 reduces TER1 stability, causes a severe assembly defect, and results in critically short telomeres. We also show that Pof8 is a constitutive component of active telomerase in fission yeast, making Pof8 next to TERT the most widely conserved telomerase subunit identified to date.

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290 Cell cycle-regulated lncRNA promotes cell proliferation by controlling HIPPO/YAP signaling.

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Human genome encodes a large number of non-protein coding RNA (ncRNA) genes, including ~16,000 long ncRNA (lncRNA) genes. Few of these lncRNAs have thus far been assigned a specific role; yet recent observations suggest that they participate in vital cellular functions. LncRNAs control the expression of protein-coding genes, either by altering the chromatin structure or by influencing the recruitment of proteins to specific genomic loci. Recent studies have reported that lncRNAs contribute to cell proliferation and survival, and their aberrant expression is linked to several diseases, including cancer. However, the molecular mechanism/s underlying the role of lncRNAs in cell cycle progression is yet to be elucidated. By performing genome-wide transcriptome analyses in cell cycle-synchronized cells, we identified ~400 lncRNAs, which show enhanced expression (>2-fold) during specific stages of cell cycle. Functional studies revealed that SUNO1, one such S phase-upregulated lncRNA, plays crucial roles in cell cycle progression and DNA damage response. Cells depleted of SUNO1 shows defects in cell proliferation and DNA damage response. SUNO1 promotes cell proliferation by regulating the expression of genes controlling cell proliferation networks such as HIPPO/YAP signaling pathway. At the molecular level, SUNO1 associates with protein factors with transcriptional activating roles, including DDX5, and facilitates DDX5-mediated loading RNA polymerase II to target gene promoters. Finally, the elevated expression of SUNO1 in colon adenocarcinoma patients is associated with poor prognosis. Depletion of SUNO1 decreased tumorigenic cell properties, including anchorage-independent growth and ability to form tumors in nude mice. Based on this, we conclude that cell cycle-regulated lncRNAs participate in cell proliferation by controlling the activity of cell cycle genes.

291 DDX5/p68 associated lncRNA LOC284454 is differentially expressed in human cancers and modulates gene expression

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Long non-coding RNAs (lncRNAs) are emerging as important players in regulation of gene expression in higher eukaryotes. LncRNAs in association with RNA binding proteins impart specific molecular functions by targeting RNA binding proteins to specific sites as guides or titrating away specific proteins as decoy or by acting as a scaffold wherein the single RNA binds distinct effector molecules that modulate diverse biological processes. DDX5/p68, a well-known RNA helicase, in addition to its established role in mRNA splicing, influences various cellular processes. Earlier studies depicts that DDX5/p68 also interacts with lncRNAs like, SRA and mrhl, to modulate gene expression. With this background and considering the role of p68-lncRNA complex in diverse cellular processes, we are interested to identify the novel p68 interacting non-coding RNAs in human cells. We performed RIP-seq analysis in HEK293T cells to identify the complete repertoire of DDX5/p68 interacting transcripts including 73 single exonic lncRNAs. The LOC284454 lncRNA is the second top hit of the list of single exonic lncRNAs which we have characterized in detail for its molecular features and cellular functions. The RNA is located in the same primary transcript harboring miR-23a~27a~24-2 cluster. LOC284454 is a stable, nuclear restricted and chromatin associated lncRNA. The sequence is conserved only in primates among 26 different species and is expressed in multiple human tissues. Expression of LOC284454 is significantly reduced in breast, prostate, uterus and kidney cancer and also in breast cancer cell lines (MCF-7 and T47D). Global gene expression studies upon loss and gain of function of LOC284454 revealed perturbation of genes related to cancer-related pathways. Focal adhesion and cell migration pathway genes are downregulated under overexpression condition, and these genes are significantly upregulated in breast cancer cell lines as well as breast cancer tissue samples suggesting a functional role of LOC284454 lncRNA in breast cancer pathobiology.

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292 Analysis of functional features of SINEUPs, antisense lncRNAs for targeted translation enhancement

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The SINEUP lncRNA class harbors unique regulatory antisense lncRNAs which partially overlap sense protein-coding mRNAs at 5′ end and specifically enhance their protein expression without altering the target transcript level [Carrieri *et al.*, Nature 2012, 491 (7424); Zucchelli *et al.*, Front Cell Neurosci. 2015, 9:174]. The representative member of this class was initially reported in a mouse lncRNA antisense (AS) to *Uchl1* (Ubiquitin C-Terminal Hydrolase L1) gene which upon binding to sense mRNA facilitated its association with polysomes and enhanced UCHL1 protein production [Carrieri *et al.*, Nature 2012, 491 (7424)]. This translation up-regulatory function was found to be due to an inverted SINEB2 (short interspersed nuclear element) repeat of B3 sub-family embedded at the 3′ end of AS *Uchl1*. Thus, SINEUPs consist of two regions- *i)* Binding domain (BD), the region overlapping to sequence within 5′ UTR and few bases of CDS of target mRNA; and *ii)* Effector domain (ED), an inverted repeat of SINEB2 indispensable for SINEUP function [Zucchelli *et al.*, Front Cell Neurosci. 2015, 9:174]. In recent years, SINEUPs had proven their efficacy against a variety of exogenous and endogenous targets in a wide range of human and mouse cell lines and also in *in-vivo* system, still the functional features of SINEUPs are not well studied [Carrieri *et al.*, Nature 2012, 491 (7424); Zucchelli *et al.*, Front Cell Neurosci. 2015, 9:174; Indrieri *et al.*, Sci Rep. 2016, 6:27315]. In current study, we investigated key sequence and structural features of SINEUPs to get a better understanding of SINEUP biology.

Previously, we identified more than 30 natural antisense transcripts (NATs) in mouse FANTOM3 cDNA dataset with similar genomic features to that of AS *Uchl1* [Carrieri *et al.*, Nature 2012, 491 (7424)]. Here, we report the SINEUP effect of a number of diverse SINEB2 sequences isolated from some of these NATs which were tested in synthetic SINEUP-GFP in human cells. We found that not only the B3 sub-family but other sub-families of mouse SINEB2 also display the SINEUP effect suggesting vastness of SINEUP class. Moreover, we report crucial functional features of SINEUPs to guide future biological and therapeutic studies.

293 SINE1/2 elements of *Entamoeba histolytica* down regulate mRNA transcription of neighboring genes and contain conserved RNA Pol III promoter motifs

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Messenger RNA transcription is prone to regulation at various levels, many of which remain to be discovered. The retrotransposable element, EhSINE (Short Interspersed Nuclear Element) is present in hundreds of copies dispersed throughout the genome of *Entamoeba histolytica*, a protozoan parasite of humans. We have interrogated whether the presence of SINE copy in the vicinity of a protein-coding gene has any effect on its transcription. To extract syntenic loci polymorphic only for EhSINE occupancy we have compared the genomic location of SINEs in two strains of *E. histolytica* (HM-1:IMSS and Rahman) and selected all loci where SINE was present in HM-1:IMSS but absent in Rahman. Four such loci were obtained. Expression of the neighboring genes was determined by northern blot analysis and found to be comparable in both strains for two loci, while in the other two loci (locus 19, EhSINE1; locus 50, EhSINE2) there was downregulation of downstream genes. To further confirm the role of SINE in differential expression of these genes, we made luciferase reporter constructs containing the upstream sequences with- or without- the SINE element. Luciferase expression, checked both by enzyme assay and northern analysis, confirmed downregulation in the EhSINE-containing constructs. These data have implications in the possible role of EhSINE genomic transposition on virulence-related functions in different *E. histolytica* strains.

SINEs in model organisms are transcribed by RNA Pol III using an internal promoter. Pol III promoters have not yet been studied in *E. histolytica*. To characterize the EhSINE promoter we obtained RNA-Seq data to identify the transcribed versus silent copies of EhSINE1. Of the full-length EhSINE1 copies we found 151 to be expressed while 121 were silent. Sequence analysis of 5'-end using MEME revealed the presence of Pol III-promoter consensus A and B box motifs (similar to model organisms) in all the expressed EhSINE1 copies, while these were absent in the silent copies. Experimental validation of the role of these motifs in EhSINE1 transcription is under way.

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294 Regulation of endothelial cell metabolism by the lncRNA RENCEM

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RENCEM is a nuclear associated lncRNA that is ubiquitously expressed and shows a high degree of conservation between humans and mice but nothing is known about its function. In cultured human endothelial cells the knockdown of RENCEM (CRISPR/Cas9) resulted in increased sprouting in a modified spheroid model, while its overexpression elicited the opposite effect. As RENCEM is localized in the nucleus, the expression of chromatin modifiers and remodelers were measured using qPCR after partial knock down of RENCEM. This resulted in significant upregulation of methyltransferases (EZH2, CARM1, PRMT1 and PRMT8) and a downregulation of proteins involved in ubiquitination (UBE2A, USP16 and RING1), indicating a role for RENCEM in the modulation of the epigenetic landscape. In addition RNA-seq and metabolomic analyses revealed changes in nucleotide metabolism. Importantly, the nucleotide pathway is known to be dysregulated in atherosclerotic plaques and the expression of RENCEM was increased in atherosclerotic plaque samples compared to control samples from healthy humans. The potential relevance of RENCEM in atherosclerosis was further supported by its presence in macrophages. Indeed, RENCEM overexpression in polarized macrophages increased the M2 marker IL-10 in M1 macrophages, while simultaneously resulting in a decrease of IL-10 in M2 macrophages after polarization. Thus, these first results indicate a role for RENCEM in endothelial cell proliferation and macrophage polarization.

295 Withdrawn

296 Rewiring of the RNA-binding protein network by the oncogenic lncRNA *SAMMSON* isrequired for synchronised increase in mitochondrial and cytosolic translation

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A fully functional OXPHOS chain requires proteins translated by mitochondrial and by cellular machinery, thus offering a unique opportunity to coordinate cell and mitochondrial activities and efficiently respond to environmental cues. Importantly, while the mitochondria can adjust their protein synthesis rate to finely balance the income of cytosolic proteins, modifications of mitochondrial translation will invariably induce an adaptive response that, depending on the duration and type of stress, can result in cell cycle arrest or cell death. *SAMMSON* is a melanoma-specific lncRNA that was shown to be essential for melanoma cell survival by interacting with p32, a protein required for ribosome biogenesis and protein synthesis in the mitochondria.

Here we identified a key role for lncRNA *SAMMSON* in nuclear-mitochondrial communication by means of its ability to rewire the RNA-binding protein network to maintain the equilibrium between mitochondrial and cellular translation rate. Aberrant expression of *SAMMSON* actively participate to cell transformation by coordinately boosting translation in the cytosol and in the mitochondria to avoid proteotoxic stress.

297 *SAMMSON* as a lineage addiction oncogenic event

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In the past few years, the identification and characterization of lncRNAs important for human diseases has incredibly expanded both diagnostic and therapeutic opportunities.

Amplification of the region containing the melanoma-specific oncogene *MITF* occurs in 10% of melanomas and is associated with poor prognosis. However, whether other genes in this region also contribute to melanomagenesis is unknown. The long non-coding RNA gene *SAMMSON* is adjacent to and invariably co-gained with *MITF*. Furthermore, *SAMMSON* is a direct target of the lineage-specific transcription factor SOX10 and, as a consequence, it is expressed in the vast majority (90%) of human melanomas but undetectable in normal adult tissues, including primary melanocytes. *SAMMSON* knock-down drastically decreases the viability of melanoma cells irrespective of their mutational status or of the tumor phenotype and increases sensitivity to MAPK-targeting therapeutics both *in vitro* and in patient-derived melanoma xenograft models. Intriguingly, *SAMMSON* is upregulated at the transition between the radial growth phase to the vertical growth phase, when melanoma cells become fully malignant and its exogenous expression is sufficient to increase their tumorigenic potential *in vitro* and *in vivo*.

We identified the mitochondria protein p32 as a *SAMMSON* protein interactor. p32 is a protein required for mitochondrial protein synthesis. By physically interacting with p32, *SAMMSON* participates in the reprogramming of the mitochondrial metabolism that allows melanoma cells to cope with their elevated demands in energy and protein synthesis. Inhibition of *SAMMSON* resulted in severe defects in oxidative phosphorylation and in the induction of the mitochondrial Precursor Over-accumulation Stress (mPOS) in the cytoplasm, ultimately leading to a dramatic decrease in cell viability. Mechanistically, *SAMMSON* can hijack the RBP network to promote a balanced increase in protein synthesis both in the cytosol and in the mitochondria, in order to satisfy the increased energy demand of tumor cells.

Our results indicate that targeting *SAMMSON* disrupts mitochondrial homeostasis in a melanoma-specific manner and is therefore expected to deliver highly effective and tissue-restricted therapeutic responses. Thus, we speculate that *SAMMSON* could be used both as a diagnostic marker of early melanoma transformation and as a therapeutic target, either alone or in combination with MAPK-inhibitors.

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298 The function and mechanism of long non-coding RNA ARHGAP5-AS1 in breast cancer metastasis

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Breast cancer is the most common malignant cancer in women. Metastasis is the leading cause of high mortality of breast cancer. Recent studies have shown that non-coding RNAs play an essential role in the 6 hallmarks of cancer. Our study focus on the non-coding RNAs involved in breast cancer metastasis. We use RNA high-throughput sequencing to detect the transcriptome of triple-negative breast cancer cell line MDA-MB-231 and its high pulmonary metastasis subtype MDA-MB-231-LM2. According to the different selective methods, 80 down-regulated lncRNAs and 48 up-regulated lncRNAs were found in LM2 cells. Then, by using quantitative real-time PCR, we successfully identified 7 down-regulated lncRNAs and 8 up-regulated lncRNAs for the following in vitro functional detection. The cell migration assay was used to detect the effect of the above 15 lncRNAs on the cell motility. The down-regulated lncRNA-ARHGAP5-AS1 (NR_027263) inhibited cell migration, so we studied the molecular mechanisms underlying the effects of ARHGAP5-AS1 on cell motility. We detected diverse cellular function of Lnc-ARHGAP5-AS1, it inhibited cell migration and the formation of stress fibers in breast cancer cells. However, it had no effect on cell proliferation and colony formation. Then, we used HuprotTM human protein array to identify the protein SMAD7, interacting with LNC-ARHGAP5-AS1. The interaction between Lnc-ARHGAP5-AS1 and SMAD7 was once again verified by RNA pull down assay and RNA immunoprecipitation assay. Whether knockdown or over-expression of LNC-ARHGAP5-AS1 has no effect on the cellular distribution of SMAD7, but in the experiment of the separation of nuclear and cytoplasmic protein, we found that knockdown of LNC-ARHGAP5-AS1 reduced the expression of SMAD7. Knockdown or over-expression of LNC-ARHGAP5-AS1 had no effect on the mRNA level of SMAD7. However, the protein level of SMAD7 was influenced by LNC-ARHGAP5-AS1, suggesting that Lnc-ARHGAP5-AS1 could affect the degradation of SMAD7. Then, we used protein synthesis inhibitor CHX and protease inhibitor MG132 treated with cells to prove that LNC-ARHGAP5-AS1 stabilizes SMAD7. Knockdown of SMAD7 could simulate the function of knockdown of Lnc-ARHGAP5-AS1, which inhibits cell migration and the formation of stress fibers, suggesting that Lnc-ARHGAP5-AS1 might present its biological function via SMAD7.

299 Genome-wide screening of *NEAT1* regulators reveals mito-paraspeckle communication

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The long non-coding RNA *NEAT1* (nuclear enriched abundant transcript 1) nucleates the formation of paraspeckles, which constitute a type of nuclear body that has multiple roles in gene expression. How the *NEAT1* gene itself is regulated and how paraspeckles communicate with other cell compartments remains incompletely understood. Here we identify regulators of *NEAT1* transcription using an endogenous *NEAT1* promoter-driven EGFP reporter in human cells coupled with genome-wide RNAi screens. In addition to transcription factors and chromatin modulators, the screens unexpectedly yielded gene candidates involved in mitochondrial functions as essential regulators of *NEAT1* expression and paraspeckle formation. Mitochondrial defects altered *NEAT1* transcription via ATF2 and uncoupled 3' end processing of the short *NEAT1_1* from its long isoform *NEAT1_2* isofrom, resulting in generation of elongated paraspeckles that have different features from regular, globular bodies. Correspondingly, *NEAT1* depletion has profound effects on mitochondrial dynamics and function by altering sequestration of mRNAs of mitochondrial genes enriched in paraspeckles. Overall, our data provide a rich resource for understanding *NEAT1* and paraspeckle regulation, and reveal an unexpected crosstalk between cytoplasmic organelles and nuclear bodies.

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300 Long non-coding RNAs as modulators of splicing in trans

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Long non-coding RNAs (lncRNAs) constitute an abundant class of transcripts widely observed across eukaryotes and involved in a plethora of cellular processes. Functions played by lncRNAs are closely related to their subcellular localization; in spite of the fact that a number of lncRNAs display nuclear localization, only a handful of them have been functionally characterized. In particular, little is known about their engagement in gene expression regulation by coming into lncRNA:RNA interactions. By hybridizing with other transcripts, lncRNAs could be involved in at least several regulatory mechanisms.

Our goal is to investigate splicing-related functions of lncRNAs that are exerted in the context of RNA:RNA duplexes. Recently, based on *in silico* predictions of lncRNA:RNA base-pairings across the human transcriptome, we discovered that there is a great potential for lncRNAs to play a role of splicing modulators by means of masking splice sites and/or other splicing signals. In order to select most promising and biologically relevant candidates for experimental tests, we decided to focus on cases where lncRNA lead to a shift in protein coding capacity of transcripts. We also required that the lncRNAs of interest also show higher expression level in the nucleus than in the cytoplasm (from RNA-Seq data) and they represent relatively stable transcripts (established with 4SU-Seq data).

At the moment, we are performing laboratory tests on a subset of candidates. One of them is *OIP5-AS1* (*OIP5 antisense RNA 1*), which functions as oncogene in certain types of cancers. Subcellular fractionation of HEK293 cell line and Real-Time PCR have confirmed the bioinformatics predictions about nuclear localization of the lncRNAs. Furthermore, knockdown of lncRNAs with antisense LNA gapmers results in changes in splicing pattern, as expected. Now we are going to perform RPA (Ribonuclease Protection Assay) experiments to check whether the observed phenomenon results from direct lncRNA:RNA interactions.

301 Ultrastructure of the FC/DFC organization and nascent pre-rRNA sorting in the nucleolus

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Found in all eukaryotes, the nucleolus is one of the most important nuclear bodies and best known for its role in ribosomal RNA (rRNA) biogenesis. The nucleolus forms around rDNA tandem repeats, which spread out on five pairs of chromosomes in human cells, and only part of rDNAs are active at any given time. Previous studies, largely supported by electron microscope (EM), have found that nucleoli in mammalian cells have three morphologically distinct sub-regions named fibrillar centers (FCs), dense fibrillar component (DFC) and granular component (GC). It's believed that the nucleolar ultrastructure is a product of the function it performs that allows the continuous Pol I transcription within the FC and the subsequent radial flux of rRNAs through the DFC into the GC and finally into the nucleoplasm. However, how the efficient coordination between continuous Pol I transcription and pre-rRNA processing is achieved and how the directionality of nascent pre-rRNA sorting is controlled remain largely elusive. Here, we have applied a number of super-resolution microscopy technologies, single molecule RNA FISH as well as mathematic simulations to re-visit sub-nucleolar arrangements at an unprecedented resolution. We present a detailed model of the FC/DFC ultrastructure for Pol I transcription and pre-rRNA processing as well as the mechanism controls the directionality of nascent pre-rRNA trafficking in human nucleolus.

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302 Role of lncRNA OIP5-AS1 in myogenesis

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The human population worldwide is aging rapidly. Sarcopenia is a major cause of disability in the elderly, leading to progressive loss of muscle mass, quality, and strength. Skeletal muscle contains functional muscle fibers with long multinucleated and contractile structures named myotubes that arise through differentiation of myoblasts through a process termed *myogenesis*. Long noncoding (lnc)RNAs are emerging as critical regulators of myogenesis and muscle regeneration. However, the specific mechanisms whereby lncRNAs regulate myogenesis are largely unexplored. To study myogenesis in humans, we characterized human myoblasts undergoing differentiation into myotubes by examining changes in cell morphology, patterns of expression of myogenic genes, and creatine kinase (CK) activity. In this investigation, we focused on the recent discovery that *OIP5-AS1*, a lncRNA highly abundant in muscle, is upregulated early during myogenesis. Interestingly, silencing *OIP5-AS1* impaired differentiation of cultured myoblasts, as determined by monitoring myotube formation and several parameters of differentiation, including the levels of myogenic proteins MYOG, MEF2C, and MYH. We found that *OIP5-AS1* interacts with the RNA-binding proteins HuR and AUF1, which are known to regulate myogenesis and regulate the expression of myogenic factors transcriptionally and post-transcriptionally. Our ongoing investigation is aimed at establishing the functional role of *OIP5-AS1* lncRNPs in myogenesis. We anticipate that this study will elucidate how *OIP5-AS1* enables myogenesis and muscle regeneration, and will pave the way for possible interventions to delay sarcopenia and age-associated muscle loss.

303 Effects of deregulation of growth-phase-dependent RyeB expression on growth phenotypes of *Escherichia coli*

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Most of small noncoding RNAs (sRNAs) are known to base pair with their target mRNAs and regulate mRNA stability or translation with the help of Hfq, resulting in various changes in cell metabolism. One of sRNAs, RyeB (also known as SdsR) is expressed after the exponential phase and represses *tolC* and *mutS* expression. However, the importance of the growth-phase-dependent regulation of RyeB in cell growth is not known. We investigated how cell growth or survival would be affected if RyeB is ectopically expressed in the exponential phase. We found that the ectopic expression of RyeB led to significant cell death in an Hfq-dependent manner. The RyeB-driven cell death was alleviated by overexpression of RyeA, an sRNA transcribed on the opposite DNA strand, suggesting that RyeB/RyeA is a novel type of toxin-antitoxin system where both toxin and antitoxin are sRNA. We defined the minimal region required for the RyeB-driven cell death. RNA-seq analysis identified 209 genes whose expression was altered more than 2-fold by the RyeB overexpression. Many (about 30%) of them were related to energy production and conversion. Our data suggest that the RyeB-driven cell death might be caused by accumulation of altered expression of multiple genes.

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304 Cis-regulation of the ribosomal protein L21 operon in Bacillus subtilis

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The ribosome is the cellular machine responsible for synthesis of cellular proteins across all branches of life. Ribosomes are structurally complex, containing numerous RNA and protein components. Thus ribosomal building blocks must be produced in precise stoichiometric ratios to facilitate proper assembly, and make the best use of cellular resources. To control the production of ribosome components bacterial ribosomal protein operons are frequently regulated by cis-regulatory RNAs that occur in 5' untranslated regions of the genes they regulate. These cis-regulatory RNAs, or leader sequences, form secondary structures that bind a ribosomal protein - often from the same operon- leading to repression of translation. This phenomenon is well established in E. coli, a gram-negative bacterium, but little work has been done to assess how regulation may occur in other bacteria. In the model gram-positive organism B. subtilis several leader sequences have been identified computationally, but few have verified regulatory action. One such leader is that preceding the L21 operon. This operon consists of ribosomal proteins L21 and L27 as well as a protease involved in the maturation of L27. An RNA structure preceding L21 was computationally identified in in organisms across the phylum Firmicutes. Using beta-galactosidase activity assays and qPCR, L21 was found to inhibit translation of the L21 operon, resulting in a 2-fold repression in protein production. Future in vitro studies aim to determine whether directing binding between the L21 protein and the L21 leader structure occurs.

305 Discover of a highly conserved RNA thermometer in two *Clostridioides difficile* hypervirulent strains using RNA-seq

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An RNA thermometer (RNAT) which controls a putative erythromycin efflux pump was identified in two *Clostridioides difficile* hypervirulent strains (QCD-32g58 and R20291) from our previous RNA-seq [1]. RNAT is a temperature-sensitive non-coding RNA molecule, which usually locates at 5′untranslated region (5′UTR) of an mRNA and regulates gene translation by changing their secondary structure to hide the ribosome binding site (RBS) and the translation initiation codon. A cloned RNAT from *C. difficile* fused with a green fluorescent protein (GFP) showed that GFP fluorescence intensity was significantly inhibited ($P<0.01$) at 42°C but was not affected at 30°C. This indicated that the discovered RNAT functioned as an "OFF" switch when environmental temperature increased. Thus, we assumed *C.difficile* would be more sensitive to erythromycin at high temperatures. Our preliminary test on *C. difficile* hypervirulent strains R20291 agreed with our hypothesis, a statistically significant ($P<0.01$) slower growth under erythromycin stress was found at 42°C comparing to no difference in growth at 37°C. Blast result of the RNAT sequence against the Nucleotide Collection database revealed it is highly a conserved sequence with among 40 *Clostridioides* strains. Temperature controlled antibiotic resistance maybe common in *Clostridioides*.

1. Scaria, J., et al., Differential Stress Transcriptome Landscape of Historic and Recently Emerged Hypervirulent Strains of Clostridium difficile Strains Determined Using RNA-seq. PLoS One, 2013. 8(11).

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306 Ecologically Guided Searches for New Riboswitch Functions

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Riboswitches are structured RNA elements that regulate gene expression by directly binding ligands. Bioinformatics approaches have greatly accelerated the discovery of candidate riboswitches by searching for conserved RNA structures. However, discovering the ligand that binds to a conserved element remains challenging. Traditional approaches search for clues to the binding partners of riboswitches by analyzing the function of downstream genes. This approach has proven highly successful, but poses challenges in poorly annotated genomes, such as from metagenomic samples, and may miss important environmentally delivered ligands. Here we propose to use ecological interactions to search for riboswitch functions. For our model system we will use herbivores which feed on sagebrush, highly defended by toxic chemicals known as plant secondary metabolites (PSM). We hypothesize that the microbial communities from these gut environments will contain riboswitches responding to the plant chemicals. Our collaborative research will produce shotgun metagenomics data from the fecal samples collected from animals ingesting sagebrush, bioinformatics searches for candidate riboswitch variants in the samples, and biochemical and biophysical validation of riboswitches using plant extracted chemicals. If successful, the research will produce new antibiotic candidates and targets, and will advance our understanding of how microbial communities contribute to the plant-herbivore arms race that drives biodiversity across the planet.

307 Hfq-independent post-transcriptional regulation by DsrA in *Escherichia coli*

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Many small RNAs (sRNAs) carry out regulation of gene expression by base-pairing with their target mRNA with the help of Hfq in Escherichia coli. It has been known that DsrA activates translation of *rpoS* mRNA and this activation requires Hfq. DsrA was also reported to be able to partially bypass Hfq when overproduced. However, the precise mechanism by which DsrA bypasses Hfq is unknown. In this study, we constructed strains lacking all three *rpoS*-activating sRNAs, ArcZ, DsrA, and RprA, in hfq+ and hfq- backgrounds, and could control cellular DsrA concentrations by ectopic expression in those strains. Then we determined how the expression level of *rpoS* would be changed according to the concentration of DsrA. We found that both *rpoS* mRNA translation and stability are enhanced by DsrA regardless of the presence or absence of Hfq although the Hfq depletion causes a rapid degradation of DsrA and decreases stability of *rpoS* mRNA. These results suggest that the observation of the Hfq-dependency of *rpoS* activation by DsrA mainly results from destabilization of DsrA in the absence of Hfq, but that DsrA itself can participate in translational activation and stability of *rpoS* mRNA in an Hfq-independent manner.

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308 Allosteric Modulation of a Bacterial HDV-like Ribozyme by Glucosamine 6-Phosphate: The Substrate of the Adjacent Gene Product

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Ribozymes are RNA molecules that catalyze a variety of chemical transformations. Self-cleaving ribozymes were discovered 30 years ago and have been found throughout nature, from bacteria to animals, but little is known about their biological functions and regulation, particularly how cofactors and metabolites alter their activity. A hepatitis delta virus-like self-cleaving ribozyme maps upstream of a phosphoglucosamine mutase (*glmM*) open reading frame in the genome of the human gut bacterium *Faecalibacterium prausnitzii*. The presence of a ribozyme in the untranslated region of *glmM* suggests a regulation mechanism of gene expression. In the bacterial hexosamine biosynthesis pathway, the enzyme glmM catalyzes the isomerization of glucosamine 6-phosphate into glucosamine 1-phosphate. In this study, we investigated the effect of these metabolites on the co-transcriptional self-cleavage rate of the ribozyme. Our results suggest that glucosamine 6-phosphate, but not glucosamine 1-phosphate, is an allosteric ligand that increases the self-cleavage rate of drz-Fpra-1, providing the first known example of allosteric modulation of a self-cleaving ribozyme by the substrate of the adjacent gene product. Given that the ribozyme is activated by the glmM substrate, but not the product, this allosteric modulation may represent a potential feed-forward mechanism of gene expression regulation in bacteria. We also hypothesize that the HDV-like ribozyme scaffold is robust enough to incorporate mechanistic dependency on selected metabolites, like glucosamine 6-phosphate. Utilizing *in vitro* selection technique, we plan to isolate and characterize metabolite-dependent HDV-like ribozymes.

309 Comprehensive sequence analysis of group II introns and their evolution in bacteria

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Group II introns are the mobile self-splicing introns found in genomes of bacteria, archaea, and eukaryotic organelles. The group II introns are composed of six domains from domain I to VI based on RNA secondary structure. Especially domains V and VI are essential for self-splicing and evolutionarily highly conserved. In some cases, domain IV contains Intron Encoded Protein (IEP) that contributes to splicing and mobility reaction. Group II introns are classified in approximately 10 types according to domain structures and IEP types. In order to elucidate molecular evolution of group II introns, studies for the exact identification of the sequences from each genome have been attempted. However, the established pipeline is limited mainly to those for full-length IEP-containing introns.

We aim to establish a pipeline that can detect all types of group II introns from genome sequence data. Currently, our computer program extracted any sequences containing domains V and VI based on homology of RNA secondary structures, and determined the presence or absence of IEP in domain IV. Thus, we can classify the IEP-containing and some of non-IEP-containing group II introns. We applied this program to representative 2,896 bacterial genomes, and obtained a set of sequences including 1,192 IEP-containing types and 360 non-IEP-containing types of introns. By mapping them on a 16S rRNA phylogenetic tree, it was shown that group II introns are widely distributed in 30 out of 31 bacterial phylums. This result suggests that group II introns were already present in bacterial genomes at the early stages of bacterial evolution. In addition, it was revealed that at least three clusters of genus or species level with particularly high number of the introns (more than 10 per genome) independently existed on the phylogenetic tree. We concluded that group II introns may have proliferated to high copy number in certain species.

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310 Structure-guided discovery of small molecules targeting the PreQ1 riboswitch

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Riboswitches are structured RNA elements that typically reside in the 5'-untranstated regions of mRNAs, and regulate gene expression by recognizing their cognate ligand. The preQ1 (7-aminomethyl-7-deazaguanine) riboswitch modulates the expression of genes responsible for queuosine (Q) biosynthesis in response to preQ1. Q is a modified nucleotide at the wobble position of certain bacterial and eukaryal tRNAs that is crucial for translational fidelity. Bacteria synthesize the free base preQ1 that is incorporated into the tRNA wobble position by transglycosylation and is subsequently elaborated into Q. Importantly, eukaryotes lack the Q biosynthetic pathway, and obtain the modified nucleobase from diet. Here, we report the discovery of synthetic small molecules that bind the class I preQ1 riboswitch and co-crystal structures with the small molecules. A fluorescently labeled preQ1 riboswitch was screened against a small-molecule microarray. Several hits were detected, and the interactions between the riboswitch and these compounds were confirmed by waterLOGSY NMR and SPR experiments. The co-crystal structure of the riboswitch with one of the hits showed a dibenzofuran-derivative binding the preQ1 binding cleft. The heterocycle of the synthetic ligand is sandwiched between G11 and the G5•C16 pair, and its ring oxygen hydrogen-bonds with A29. These nucleotides are conserved among preQ1 riboswitches. Based on this co-crystal structure, we developed second-generation dibenzofurans. A co-crystal structure with a dimethylamine-containing derivative showed that the nitrogen atom of this functional group is recognized by the riboswitch. A carbazole derivative, which has a ring nitrogen instead of the oxygen of dibenzofuran, binds similarly to dibenzofurans, but the hydrogen bonding network with the RNA is different. We are now testing the effects of these compounds on gene expression through *in vitro* transcription termination assays. Preliminary results show that the dibenzofuran-containing compound increases transcription termination efficiency, suggesting that the compound has the potential to downregulate gene expression. Our co-crystal structures are the starting point for developing additional compounds that target the bacteria-specific Q biosynthetic pathway. This work was supported in part by the Japan Society for the Promotion of Science, and the Intramural Programs of the National Heart, Lung and Blood Institute and the National Cancer Institute, NIH.

311 An Inducible Glycine-Cleavage System for the Study of Glycine Riboswitches

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Studies on glycine riboswitches have primarily focused on *in vitro* structural and biochemical analyses describing how they bind their small-molecule ligand. *In vivo* research to determine how they modulate the expression of genes is of great interest but is difficult to perform because it is challenging to change the concentration of glycine inside bacterial cells. Glycine is involved in several important metabolic pathways, and its concentration is tightly regulated via feedback mechanisms. Here, we report the construction of an *E. coli* strain with inducible glycine degradation for the modulation of glycine concentration inside bacteria. In normal bacterial cells, the glycine cleavage system degrades excess glycine to produce methylene-tetrahydrofolate, which is used in other pathways involving one-carbon metabolism. By modulating the transcription factors that normally control the *gcv* operon, we are able to over-express the glycine cleavage system and degrade glycine, even when it is not in excess. We demonstrate this decrease in glycine concentration with a glycine riboswitch reporter assay. In addition, an independent HPLC assay is being developed to confirm the decrease in glycine concentration in the modified *E. coli* strain.

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312 RNA processing and activation of type IIIA CRISPR-Cas systems

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The clustered regularly interspaced palindromic repeats/CRISPR-associated (CRISPR/Cas) surveillance complexes are adaptive immune systems crucial in defence of bacteria and archaea against foreign genetic elements such as viruses. CRISPR cassettes contain short conserved repeats interspaced by variable spacers. The spacers are integrated during viral infection and act as memory of the infection. Upon infection, the CRISPR loci are transcribed, and processed within the repeats, to produce small CRISPR RNAs (crRNAs). crRNAs are bound by Cas proteins to form CRISPR/Cas effector complexes that cleave foreign nucleic acids that are complementary to the crRNA guide. Main CRISPR/Cas systems are types I, II or III. While there is substantial data on the DNA recognition mechanisms of types I and II, the mechanism of RNA-dependent DNA cleavage by type III Csm/Cmr effector complexes is poorly understood. It is unknown how the Csm/Cmr complexes recognize RNA targets, and how this target binding activates DNA cleavage. RNA binding is immediately followed by RNA cleavage; leaving open the question on how RNA recognition temporally regulates DNA cleavage. DNA cleavage continues for long times after cleavage of the activating RNA. The verification mechanism of target from non-target RNAs (e.g. RNAs with mismatches) is also unknown. Since effector complexes of type I (Cascade) and Csm/Cmr share strong structural similarity signifying deep evolutionary relationship, we hypothesized that type III CRISPR/Cas systems apply target recognition mechanisms similar to type I; which include a directional RNA binding and verification of the integrity of bound RNA. At this conference, I will present single molecule fluorescence results on RNA recognition by type IIIA Csm from Streptococcus thermophilus.

313 Small RNA OxyS from *Escherichia coli* triggers cephalothin resistance by modulating the expression of CRP-associated genes

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The emergence of antibiotic resistance among pathogens requires the development of novel strategies. Small-noncoding RNAs (sRNAs) modulate gene expression by base-pairing with target mRNAs. Cellular levels of Hfq-dependent sRNAs influence antibiotic resistance; therefore, such sRNAs could be used to identify target mRNAs that modulate antibiotic resistance and may themselves be used as druggable molecules. Here, we report the identification of genes and regulatory pathways involved in OxyS sRNA-mediated cephalothin resistance using genome-wide RNA sequencing, literature mining, and phenotypic analyses. OxyS regulated the expression of >2,000 genes in both *hfq*+ and *hfq*- strains, and the differential expression of 25 OxyS-regulated genes was involved in cephalothin resistance. $OxyS_{48\text{-}63}$ and $OxyS_{91\text{-}109}$ were required for the phenotype in *hfq*+ and *hfq*- strains, respectively. Levels of OxyS and OxyS-modulated genes (*cirA, cycA,* and *fes*) were also altered in multidrug-resistant *E. coli* strains. Together, these data suggest that OxyS extensively modulates sRNA-mRNA interactions involved in the development of cephalothin resistance. OxyS-regulated targets, either individually or in combination, could be used as biomarkers for the identification of cephalothin-resistant strains.

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314 Highly sensitive sequence requirements for a glycine riboswitch singlet revealed by high-throughput mutational analysis

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Riboswitches alter gene expression in response to changing levels of specific effector molecules. They typically contain a structured aptamer domain that, upon ligand-binding, stabilizes one of two competing helices in a downstream expression platform. However, the sequence requirements for effective ligand-induced modulation are not well understood. Therefore, we set out to probe the fitness landscape of a transcriptionally controlled glycine riboswitch type-1 singlet to elucidate how mutations throughout the aptamer and expression platform domains affect termination efficiency. We developed a sequencing-based high-throughput assay for measuring transcription termination. Using this assay, we quantitatively characterized all 522 single point mutants of a prototypical type-1 singlet simultaneously. Deleterious mutations clustered in highly conserved and functionally relevant regions of the riboswitch. A comprehensive analysis of the expression platform revealed that ligand binding destabilizes the terminator hairpin by just ~3 kcal/mol in this construct—comparable to a single GC-to-GU mutation. These results demonstrate that functional modulation of gene expression is highly sensitive to minor perturbations in large areas of the riboswitch.

315 A small RNA mediates stress response network in *Deinococcus radiodurans* by regulating multiple pathways associated with oxidative stress

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Small RNA (sRNA) networks frequently regulate bacterial stress responses; however, these networks are largely uncharacterized in nonmodel organisms that may exhibit unique responses and resistances to environmental stresses. One such bacterium is *Deinococcus radiodurans*, a gram-positive bacterium capable of extreme survival to environmental stresses including oxidative damage, desiccation, and ionizing radiation (up to 20 kGy). Despite recent discoveries of radioresistance mechanisms, including transcriptional regulatory pathways and proteome protection mechanisms, the role of regulatory RNAs such as sRNAs has largely remained unexplored. Previously, using novel integrated bioinformatics approaches, we discovered 8 functional sRNAs that are differentially expressed under oxidative stress in *D. radiodurans*. We have characterized one of these differentially expressed sRNAs by determining its mRNA targets and its phenotypic impact on *D. radiodurans* survival under ionizing radiation. Interestingly, deletion of this sRNA results in a decrease in *D. radiodurans* survival under ionizing radiation, suggesting its importance in radioresistance mechanisms. Utilizing a combination of high-throughout techniques (including MS2 affinity purification coupled with RNA sequencing (MAPS) and transcriptomics) we determined a set of potential mRNA targets to validate utilizing Electrophoretic Mobility Shift Assays (EMSAs). Our results indicate that this sRNA regulates several mRNA targets involved in other transcriptional radiation response networks including the Radiation and Desiccation Response (RDR) and DrRRA pathway. This suggests a hierarchy of radiation resistance pathways to be controlled by sRNAs under ionizing radiation in *D. radiodurans*.

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316 In Vivo Expression Studies of the V. cholerae Glycine Riboswitch including Mutational Analysis of Ligand-Binding and Dimerization Regions

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The glycine riboswitch isolated from the V. cholerae (VC) genome is a tandem riboswitch with two aptamer sites specific to glycine. In its native context, the glycine riboswitch controls the translation of an amino acid transporter. Previous biochemical studies on the riboswitch have shown that the two binding sites behave independently in ligand-binding assays and that dimerization is important for ligand binding. Here we report the creation of a reporter construct for monitoring how the VC glycine riboswitch modulates gene expression inside E. coli. The promoter and 5'-untranslated-region containing the riboswitch was inserted into a vector, pRS414, in-frame with a beta-galactosidase reporter gene. Mutational analysis was conducted to analyze ligand-binding site and tertiary interactions within the glycine riboswitch to determine their importance for translational control. Mutation sites were chosen based on published biochemical analysis, and gene expression was monitored via beta-galactosidase activity assays. This in vivo analysis allowed for the interaction of natural polymerase and transcription and translation factors to affect riboswitch function. We demonstrate that the VC glycine riboswitch is OFF under normal cellular conditions, and that mutations which disrupt the structure of the riboswitch turn the gene ON. We further show that mutations to single ligand-binding sites have minor effects on gene expression under normal cellular conditions, but that mutating both binding sites turns the gene ON. Based on the results of this in vivo study, the model for the tandem VC glycine riboswitch can be updated. The VC glycine riboswitch is an OFF switch, turning the downstream gene off in normal glycine. The two binding sites behave relatively independently in normal glycine, but the overall structure is necessary to maintain gene control.

317 Revealing the hidden transcriptome: Analysis of nonsense-mediated mRNA decay target reveals mechanistic insights

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The nonsense mediated mRNA decay (NMD) pathway prevents accumulation of erroneous transcripts with premature termination codons. It also regulates gene expression when coupled with alternative splicing. The "50nt rule" is the prevailing model for how premature termination codons are defined in mammals and some other eukaryotes, and requires a splice junction downstream of the stop codon. There is also evidence that a longer 3' UTR triggers NMD in yeast, plants, flies, and mammals. Often, the expression of splicing factors is regulated through alternative splicing leading to NMD, although little is known about the extent and nature of this regulation.

After inhibiting NMD in multiple eukaryotes (human, mouse, frog, zebrafish, fly, *S. pombe*, and *Arabidopsis*), we identified hundreds to thousands of transcripts with increased expression. This includes 20-40% of the genes alternatively spliced in a given species. We also gained insight into what defines NMD targets from our RNA-Seq data. We found that the 50nt rule is a strong predictor of NMD degradation in human cells, and also seems to play a role in the other species tested, with the exclusion of *S. pombe*. In contrast, we found little to no correlation between the likelihood of degradation by NMD and 3' UTR length in any of the species, independent of the 50nt rule.

To further explore the features of NMD-targeted transcripts in human cells, we analyzed RNA-Seq data from a polysome fractionation experiment (TrIP-Seq). This method does not require the NMD pathway to be inhibited, and thus allows for testing the physiological transcriptome. NMD targets are known to be enriched in the monosome fraction (bound by a single ribosome). In the monosome fraction, we find enrichment of transcripts with stop codons ≥50 nt upstream of the final exon-exon junction, relative to transcripts with normal stop codons. In contrast, transcripts with longer 3'UTRs are not associated with higher abundance in the monosome fraction.

Overall, we found that the mechanism of regulation via alternative splicing coupled with NMD is prevalent in each tested species.

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318 MiRNA expression quantitative trait loci (miR-eQTL) regions identified in bovine species

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Fat deposition and composition can influence the sensory characteristics and nutritional value of beef. It is an important trait that is influenced by many genes that participate directly or indirectly in biological processes such as adipogenesis and lipid metabolism. These biological processes are very complex and are dependent of several regulatory mechanisms, for example, post-transcriptional regulation of gene expression. One of these post-transcriptional processes is gene expression regulation and/or modulation by miRNA, by blocking the translation of target mRNAs. These miRNA molecules can post-transcriptionally modulate gene expression by blocking the translation of target mRNAs. The main aim of this study was to identify genomic regions across the bovine genome that can regulate the expression level of miRNAs (eQTL-miRNA). Genotyping (BovineHD BeadChip) and miRNA sequencing data of 178 samples of *Longissimus dorsi* muscle from Nellore steers were obtained by Illumina technology. The Matrix eQTL statistical package of R program was used to identify regions in Nellore genome that were associated with miRNA expression level. Herein, 257 *trans*-miR-eQTLs were identified across autosomal chromosomes. In addition, miR-eQTLs hotspots were identified on BTA4 (88 Mb), BTA12 (61 Mb) and BTA19 (49 Mb) chromosomes. More than 50 different genomic regions were associated with the miRNAs, bta-miR-338 and bta-miR 182. Among the biological processes enriched by DAVID v6.8 from the list of target genes were identified: regulation of centrosome duplication and nucleic acid binding (adj-pvalue < 0.05). These identified processes agree with the previous knowledge, which reported that small RNAs correspond to centromere heterochromatic repeats and are responsible for the regulation of gene expression. This regulation occurs by miRNA binding to sequence-specific locations on transcribed mRNA down-regulating their stability or translation. These findings add additional evidence, which helps to better understand the role of the miRNAs in muscle gene expression regulation and provide new insights into the complex gene networks and genetic architecture in beef cattle.

319 Annotation and cluster analysis of aging-related circRNA expression in rhesus monkey brain

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Circular RNAs (circRNAs), as a novel endogenous class of transcripts, their abundance and function in mammalian brain has been reported, but alterations in circRNA expression and function in rhesus macaque brain during post-embryonic development and aging remain elusive. Here, using deep RNA profiling with linear RNA digestion by RNase R we described a comprehensive map of changes in circRNA expression in rhesus macaque (macaca mulatta) brain during ageing, total 17,050 well-expressed, stable circRNAs were identified. Cluster analysis reveals that dynamic changes in circRNA expression show the spatial-, sex- and age-biased specificities. On the basis of separate profiling of the RNAs, the diversity of correlation between circRNA and host mRNA expression was found in macaque brain during post-embryonic development and aging. Our results demonstrate the power of changes in circRNA expression which reveals insights into a potentially circRNA-mediated regulatory mechanism underlying the biology of brain ageing.

320 EZH2 binding of transcripts and active chromatin regulates alternative splicing

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The emerging oncogenic activity of EZH2, the catalytic component of polycomb repressive complex 2 (PRC2) and its binding of nascent RNAs in embryonic stem cells and in vitro with limited selection suggest unknown functions of EZH2 regulation. Here we show that EZH2 binds much less to lncRNAs but more extensively to both spliced and primary mRNA transcripts in prostate cancer cells. EZH2 binds to transcriptionally active chromatin, which positively correlates with the gene transcription activity and EZH2-RNA interaction. EZH2-RNA interaction negatively correlates with H3K27me3 deposition but positively with H3K36me3 deposition. EZH2 physically contacts H3K36me3 and U2AF65/35, and inhibits exon skipping in cancer cells. These findings support a model in which EZH2 globally associates mRNA transcripts and transcribing chromatin, which promotes H3K36me3 deposition and influences alternative splicing. This model suggests a general PRC2-independent function of EZH2 in tumorigenesis.

321 Selective export into exosomes and function of tRNA fragments during T cell activation

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The discovery of miRNA sorting into exosomes revealed a novel mode of intercellular communication, and uncovered a link between cellular endomembrane compartments and small RNAs in miRNA-secreting cells. T cells are a robust source of exosomes containing small RNAs, and T cell miRNAs increase in the serum of immunized mice and human patients. To determine the mechanisms by which RNAs are selectively exported into exosomes, we set out to study changes in the RNA profiles in exosomes during T cell activation. We used differential sedimentation followed by gradient centrifugation to separate exosomes from the contaminated RNA/protein aggregates in the cell culture supernatant of stimulated T cells. RNA in exosome fractions contained intact discrete RNA species, whereas aggregates fractions were dominated by apparently randomly fragmented RNAs. Fewer than 1% of miRNAs expressed by stimulated T cells were increased in exosomes compared to the corresponding cellular RNA. Strikingly, 45% of tRNA fragments (tRFs) derived from different regions of tRNAs were increased significantly in exosomes. To determine whether immune response induces selective release of tRFs, we also isolated exosomes secreted from resting T cells. T cell activation increased the exosome enrichment of tRFs derived from the 5'-portion of tRNAs (5'tRFs), and decreased the exosome enrichment of 3'tRFs with a hairpin structure. The nSMase inhibitor GW4869 disrupted exosome enrichment of both of these classes of tRFs regardless of stimulation. However, cell fractionation studies indicated distinct secretion pathways for stimulation-dependent and stimulation-independent tRFs. Exosome-associated tRFs were enriched in multivesicular bodies (MVBs) only in activated T cells, and only in the absence of GW4869. Moreover, only those tRFs that are enriched in exosomes in a stimulation-dependent manner were preferentially associated with MVBs, indicating that select tRFs are release via MVB sorting in activated T cells, whereas stimulation-independent tRF release occurs in an MVB-independent fashion. Transfection of secreted tRFs into T cells led to aberrant T cell activation, with increased CD62L down-regulation but reduced proliferation and IL-2 receptor induction. These results indicate that activated T cells sequester tRFs into membranous organelles and release them within exosomes, possibly to thwart their ability to induce aberrant T cell activation.

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322 A read-through Argonaute1 isoform promotes double stranded RNA processing and regulates cell growth.

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Members of the highly conserved Argonaute family of proteins play essential roles in small RNA-mediated gene regulation. Guided by different classes of small RNAs, Argonautes bind to and silence targets primarily at post-transcriptional level. Although they localize primarily to cytoplasmic structures known as processing bodies (P bodies), some studies using biochemical fractionation and immunofluorescence microscopy have reported the presence of endogenous Argonaute (Ago) proteins in the nuclei of mouse and human cells 1,2.

In this study we demonstrate that an isoform of Ago1, generated through translational readthrough, regulates the growth of breast cancer cells. The region downstream of the Ago1 stop codon is highly conserved and undergoes translation, as revealed by ribosome profiling. We found that the translational readthrough Ago1 isoform (Ago1x) localizes in the nucleus, its expression strongly correlating with proliferation markers in human breast cancer tissue sections. CRISPR/Cas9-induced mutations in the readthrough region lead to strong impairment in the overall growth of a breast cancer cell line measured in real time. The molecular analysis of these cells uncovered a unique role of Ago1x in the processing of dsRNAs. Our work reveals a novel function of Ago1x outside of the miRNA effector pathway and may explain seemingly paradoxical results regarding the localization and function of Ago1 in mammalian cells.

1. Huang, V. et al. Ago1 Interacts with RNA polymerase II and binds to the promoters of actively transcribed genes in human cancer cells. PLoS Genet. 9, e1003821 (2013).

2. Alló, M. et al. Argonaute-1 binds transcriptional enhancers and controls constitutive and alternative splicing in human cells. Proc. Natl. Acad. Sci. U. S. A. 111, 15622–15629 (2014).

323 The communication between the plant miRNA biogenesis machinery and RNA Polymerase II

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MicroRNA (miRNAs) are short, non-coding RNA engaged in the regulation of gene expression. In plants, genes encoding miRNAs (MIRs) are transcribed as long primary precursors (pri-miRNAs) containing stem-loop structures in which the miRNA sequence is embeded. Many pri-miRNAs contain introns. It has been previously shown in our laboratory that splicing of intron-containing miRNAs affects the levels of mature miRNA originated from such precursors.

Plant pri-miRNAs are processed in the nucleus to mature miRNAs by the multi-subunits complex called Microprocessor, which contains the endoribonuclease DCL1 (Dicer-like 1), SERRATE (SE) and the HYPONASTIC LEAVES I protein (HYL1). The results obtained by us have suggested that both SE and U1snRNP are involved in the communication between the plant miRNA biogenesis machinery and the spliceosome. We have shown that SE can interact witch four auxiliary proteins of U1snRNP: PRP39, PRP40a, PRP40b and LUC7rl. The interaction between SE with PRP40 is important for plant development because of lethality of the triple *Arabidopsis thaliana* mutant *se/prp40a/prp40b*. Interesingly, PRP40 directly interacts with the CTD domain of RNA polymerase II (RNAPII). Loss of PRP40a and PRP40b in *Arabidopsis* plants leads to the decreased level of pri-miRNAs, suggesting that PRP40 is involved in the regulation of miRNA biogenesis

We show that SE and PRP40 partially colocalize with RNAPII in the plant cell. This communication between RNAPII and SE was also proved using the proximity ligation assay (PLA). Moreover, the communication between RNAIIP and SE is significantly reduced in the prp40 mutant that suggests the involvement of PRP40 in the crosstalk between SE and RNAPII. The SE protein colocalizes with RNAPII that has the CTD domain phosphorylated at serine 5 and serine 2. We show also that the PRP40 is important for colocalization of RNAPII with DCL, syggesting the involvement of PRP40 in cotranscriprional processing of pri-miRNAs.

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324 An abundant miRNA aids the dysregulation of p21 by RNA Binding protein FXR1 in Oral Cancer cells

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RNA-binding proteins (RBPs) regulate numerous aspects of co- and post-transcriptional gene expression. RBP fragile X-related protein 1 (FXR1) belongs to a family of RNA-binding proteins that includes functionally similar proteins FMR1 and FXR2. FXR1 protein is highly expressed in multiple cancers including lung and oral cancers. Previously, we demonstrated that RBP FXR1 plays an essential role in the growth of head and neck squamous cell carcinomas (HNSCC) by blocking cellular senescence. During this process, FXR1 promotes the stability of Telomerase RNA Component (TERC), a non-coding RNA and simultaneously, destabilizes p21 mRNA. Here, we show that the expression of certain mature miRNAs is dependent on FXR1 in HNSCC cells. Also, our data indicate that absence of FXR1 in multiple HNSCC cells significantly reduces the expression of miRNA 301a-3p. Interestingly, inhibition of miR301a-3p increases both p21 mRNA and protein levels, thus, miR301a-3p negatively regulates p21. Our published data indicate that FXR1 binds and destabilizes p21 mRNA both in vitro and in vivo. Our new results demonstrate that FXR1 also binds to miR-301a-3p in vivo. Therefore, we propose a model where FXR1 helps stabilize miR301a-3p to target the 3'UTR of p21 and consequently controls cell cycle and cellular senescence in HNSCC.

325 A Novel Small RNA Pathway Targets the 3' UTRs of mRNAs in C. elegans

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We have identified a novel small RNA pathway which specifically targets the 3' UTR of hundreds of germline genes in C. elegans. To our knowledge, this is the first evidence that a specific small RNA pathway only targets the 3' UTR of genes, further diversifying the function of non-coding small RNAs. In C. elegans, endogenous small RNAs, 22Gs, bind Argonautes to regulate almost all germline genes. There are two major types of 22Gs in *C. elegans* germline: one group binds Argonaute CSR-1, playing important roles in chromosome segregation and embryonic development; the other binds multiple Argonautes, WAGOs, playing critical roles in silencing nonfunctional genes, mostly transposons and viruses, and some functional genes. In C. elegans, 22Gs are generated by nonprocessive RNA-dependent RNA polymerases (RdRPs) using mRNAs and other RNAs as templates. Usually these 22Gs are generated from both coding regions and UTRs of RNAs. Here we have identified a novel small RNA pathway which specifically targets the 3' UTRs of hundreds of genes, many of which play important roles in germline and embryonic development. Our preliminary results indicated that these genes are targeted both by CSR-1 and WAGO Argonautes. However, CSR-1 majorly targets the 5' UTR and coding regions, while the WAGO Argonautes only target the 3' UTRs. We have dissected the biogenesis pathway of this 3'UTR-targeting 22Gs and analyzed the functions. In all, we are reporting a novel small RNA pathway which specifically targeting the 3'UTR of hundreds of functional genes, which are also regulated by another small RNA pathway (CSR-1).

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326 Real time imaging and analysis of synthetic RNA riboswitch activity in mammalian cells via Fluorescent Cross Correlation Spectroscopy (FCCS)

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Synthetic RNA riboswitches have emerged as promising candidates for the realization of externally controlled genetic networks. The modular nature and portability of riboswitches offers unique opportunities for their rational design and application. However, previous studies regarding the efficacy of riboswitches have yielded inconsistent results. A fundamental understanding of what sets apart high and low performers is still needed for successful applications. This information has been difficult to obtain because investigating the behavior of RNA inside living cells is challenging, due to the fact that they are transient, unstable molecules that must fold into secondary and tertiary structures necessary for their action. Existing methods to study RNA such as qRTPCR, FISH, and SHAPEseq fall short as they alter the behavior of the RNA molecules being investigated or only quantify the presence and structure of RNA. This loses sight of important temporal and mechanistic contexts inside of living cells in which they function. I will overcome these challenges by implementing FCCS to measure single molecule riboswitch dynamics directly at the site of transcription in living cells. Data will be collected using a state of the art laser scanning fluorescence microscope capable of 2-photon excitation and 3D orbital tracking. This enables the collection of fluorescence time course data on a millisecond timescale for hours with minimal photo-bleaching. A previously developed β-globin RNA reporter system contains PP7 and MS2 bacteriophage hairpins in the first or second intron and final exon. Upon transcription, these hairpins are bound by fluorescently tagged phage coat proteins and enables the simultaneous quantification of transcription and splicing kinetics inside of living cells. Specifically, cross correlation analysis of intron and exon signals originating from active transcription sites and subsequent modeling will quantify the number of nascent RNA transcripts, rate of transcription, timing of transcript release and degrees of co-transcriptional splicing. FCCS data obtained across several RNA constructs will provide critical insight into the temporal and mechanistic behaviors that contribute to riboswitch performance. This project will not only aid in the design of improved synthetic RNA riboswitches, but will also introduce FCCS methodology as a tool to study synthetic RNA in-vivo.

327 Developing a method to identify and study the transcriptome of microRNAs important in myogenesis

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microRNAs (miRNAs) play major roles in controlling gene expression, thereby regulating many cellular processes. Defects in miRNA expression have been implicated in many diseases. miRNAs bind specific mRNA targets and trigger mRNA degradation or prevent translation. In order to understand the specific function of a miRNA, the direct mRNA targets of the miRNA must be identified. A means to concretely identify the "transcriptome" (i.e. complete set of mRNA targets) for a given miRNA remains elusive. Current methods to identify the specific mRNA targets of miRNAs have mixed success. Computational programs that predict miRNA/mRNA interactions often result in many false positives and/or negatives. Experimental approaches often use cell-altering tactics, like overexpression or knockdown of a miRNA, followed by measuring global mRNA changes, which can identify indirect targets.

This study aims to develop a novel experimental method for identifying the endogenous mRNA targets of a select miRNA. We are developing an oligo-affinity based purification method called crosslinking oligo purification (xOP). xOP is being optimized targeting miR-206 and miR-26a in mouse skeletal muscle C2C12 cells. C2C12 cells upregulate miR-206 and miR-26a during myogenesis. Cells are crosslinked with formaldehyde to generate a reversible network of miRNA, mRNA, and protein crosslinks. Cellular extracts are made, and miRNA-containing complexes are captured and purified using a biotinylated DNA antisense oligo specific to the miRNA of interest (miR-206 or miR-26a). The mRNAs that co-purify with a select miRNA after xOP are identified via Illumina sequencing (xOP-seq).

Extensive optimization of xOP has been performed. xOP specifically and efficiently captures the miRNA of interest in C2C12 cells. We validated recovery of mRNA targets using qRT-PCR to detect the small handful of known targets for each miRNA. We recently sequenced samples for xOP against miR-206 before and after differentiation. We are currently developing a bioinformatics workflow to identify the most enriched mRNAs, which we will then validate as targets of miR-206. We will also investigate the role that downregulation of these mRNA targets plays in myogenesis. This novel methodology will allow for identification of *bona fide* mRNA targets of miRNAs, which could then be applied to any system.

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328 Genome-wide identification of natural RNA aptamers in prokaryotes and eukaryotes

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RNAs are well-suited to act as cellular sensors that detect and respond to metabolite changes in the environment, due to their ability to fold into complex structures. Here, we introduce a genome-wide strategy called PARCEL that experimentally identifies RNA aptamers in vitro, in a high-throughput manner. By applying PARCEL to a collection of prokaryotic and eukaryotic organisms, we have revealed 58 new RNA aptamers to three key metabolites, greatly expanding the list of natural RNA aptamers. The newly identified RNA aptamers exhibit significant sequence conservation, are highly structured and show an unexpected prevalence in coding regions. We identified a prokaryotic precursor tmRNA that binds vitamin B2 (FMN) to facilitate its maturation, as well as eukaryotic mRNAs that bind and respond to FMN, highlighting FMN as the second RNA-binding ligand to affect eukaryotic expression. PARCEL results show that RNA-based sensing and gene regulation is more widespread than previously appreciated in different organisms.

329 The RPAP3 C-terminal domain identifies R2TP-like quaternary chaperones

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The R2TP complex is a co-chaperone for HSP90 that is known for its role in the assembly of key RNPs. It is composed of four proteins: RPAP3, which directly binds HSP90; PIH1D1, which interacts with RPAP3 and some client proteins; and RUVBL1/2, essential AAA+ ATPases whose function remain poorly characterized. The HSP90/R2TP system has been shown to be required for assembly of : box C/D and H/ACA snoRNPs, the telomerase RNP, and more recently, the U4 and U5 snRNPs. In addition, the HSP90/R2TP system also works on protein-only complexes, such as the nuclear RNA polymerases and mTORC1/mTORC2. The HSP90/R2TP system is conserved from *S. cerevisiae* to human but with differences in RPAP3 which contains a C-terminal domain (RPAP3-Cter) in human, absent from the yeast protein. Here, we performed structure-function analysis of the human RPAP3-Cter domain.

We solved the structure of RPAP3-Cter by NMR and show it adopts a novel helix-bundle fold. Using quantitative proteomics and in vitro binding assays, we show that RPAP3Cter directly binds RUVBL1/2 hexamers, thus playing an essential role in the assembly of the R2TP. Interestingly, SILAC-IP of RPAP3-Cter mutants that no longer interact with RUVBL1/2 also leads to the loss of client protein recruitment, such as NOP58, a protein of snoRNP and PRPF8 and EFTUD2, proteins of snRNP U5. This indicates that RUVBL1/2 are also involved in recruiting client proteins to R2TP.

The human genome encodes two other proteins bearing RPAP3-Cter-like domains, named SPAG1 and CCD103. We show that SPAG1 binds a paralog of PIH1D1 called PIH1D2, and that it further binds RUVBL1/2 to form an R2TP-like complex, which we termed R2SP. Using Y2H and proteomics, we found 68 potential R2SP clients. These are involved in a variety of function and some are known for their role in RNA metabolism. One client that we validated is liprin-a2, a signaling protein. We show that R2SP enhances liprin-a2 expression and association to its partners, indicating that R2SP functions in quaternary protein folding, like the canonical R2TP. Interestingly, expression data indicates that R2SP is highly enriched in testis, suggesting that it plays a specific role in this organ.

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330 Pervasive Overlapping Transcription in the Human Genome

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Many classes of non-coding RNA have been discovered in the last few decades, several of which overlap with gene promoters, termed Promoter Associated Non-Coding RNAs (PANC RNAs). PANC RNAs occur near transcriptional start sites (TSSs) and can arise from a downstream antisense TSS (daTSS), upstream sense-oriented TSS (usTSS), or an upstream antisense oriented TSS (uaTSS). The RNA produced from these promoter proximal TSSs are short-lived and can only be accurately measured in nascent RNA or after RNA degradation machinery is inhibited. When utilizing nascent RNA sequencing datasets ≈28% of human protein coding genes have a daTSS converging on the sense TSS, and ≈20% have convergent upstream transcription. Many of the transcriptional signatures of PANC RNAs suggest that the transcriptional overlaps could be functional since it is known that RNA polymerases cannot transcribe the same or opposite strands simultaneously. It is unknown, however, to what extent these transcriptional signatures conflict or influence each other, if they are occurring contemporaneously in the same nucleus, or if they are influential for the local chromatin environment.

We are investigating the regulatory capacity of PANC RNAs and the Promoter Associated Convergent Transcription (PACT) that spawns them using nascent RNA sequencing, CRISPR-Cas9 mediated knock outs, and novel imaging and sequencing techniques. We characterize this type of transcription and describe its effect on host genes and the genome at large. We find certain types of PACT regions are enriched in genes with high transcriptional activity, overrepresented in human genetic diseases, respond to environmental stimuli, and have transcription dynamics independent of host-gene TSSs.

331 The HYL1 protein, a key component of the machinery of miRNA biogenesis in plants: a study of the three-dimensional structure in solution using small-angle X-ray scattering (SAXS)

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The *Arabidopsis* HYPONASTIC LEAVES1 (HYL1) is a double-stranded RNA-binding protein that forms a complex with DICER-LIKE1 (DCL1) and SERRATE to facilitate processing of primary miRNA gene transcripts (pri-miRNAs) into miRNAs. HYL1 has two double-stranded RNA-binding domains (dsRBD), and its C-terminal end includes six almost perfect repeats of a 28-amino acid sequence. HYL1 binds double-stranded RNAs *via* its first dsRBD domain and exhibits a binding preference to the miRNA/miRNA* region of miRNA precursors. It has been shown that binding of HYL1 to RNA *in vitro* induces its dimerization, which is crucial for the activity of HYL1 *in planta*. The dimerization interface is provided by the second dsRBD domain. It has been reported that the function of HYL1 is disrupted by phosphorylation-mimicking mutations of Ser42 and Ser159. Based on the crystal structure of dsRBD domains, we hypothesize that the phosphorylation of Ser42 and Ser159 affects RNA binding by HYL1 and its dimerization, respectively. A phosphate group attached to Ser42 could potentially interfere with the minor groove interaction, thus disrupting binding of HYL1 to dsRNA. Using a filter binding assay we have shown that the double (Ser42 and Ser159) phosphomimic HYL1 mutant exhibits decreased binding to pre-miRNA164-C in comparison to the wild-type protein.

The small-angle X-ray scattering (SAXS) is a method that can provide information about the low-resolution structure (three-dimensional shape, oligomeric state) of macromolecules and their complexes in solution. Using SAXS we found that the HYL1 protein exists in solution mainly as a dimer and showed that HYL1 has an elongated shape . Moreover, SAXS data and *ab-initio* modeling indicate that both the full-length protein and the fragment covering two dsRBD domains exhibit a significant conformational flexibility. The source of this flexibility are most probably the linker between dsRBD domains and the C-terminal tail.

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332 Nuclear Ago2 Participates MicroRNA Regulation of Viral RNA Splicing by Interacting with RNA C*is*-elements

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MicroRNAs (miRNAs) are noncoding, post-transcriptional regulators that bind to complementary sequences on target mRNAs for translational repression and gene silencing. Recent studies indicate that a group of mature miRNAs and AGO2 coexist in the nucleus, implying possible roles of certain nuclear miRNAs in post-transcripitonal processing. Our recent report also indicates that nuclear or cytoplasmic AGO2 distribution varies from cell types and tissues. We found human AGO2 in cervical and skin tissues and in primary keratinocytes is primarily nuclear. In contrast, human AGO2 in all examined cancer cell lines and in laryngeal tissues, is primarily cytoplasmic (Sharma NR, Zheng ZM, et al. J. Biol. Chem.291:2302-2309, 2016). Data suggest different functions of AGO2 in different cell type and tissues. To investigate the role of miRNAs in native immunity against human papillomavirus (HPV) infection, we conducted a genome-wide prediction of potential miRNA targets in four HPV types and found several dozens of potential miRNA binding sites in each HPV genome. We found that HPV-16 L1 and E6 expression dramatically increased in Dicer$^{-/-}$ knockout cells over the Dicer wild-type cells, suggesting that cellular miRNAs might function as a form of native immunity against HPV gene expression. 15 miRNA seed matches were found in the coding regions of HPV-16 L1. A miR-10a binding site in an exonic splicing suppressor (ESS) of the HPV-16 L1 was identified for its response to endogenous nuclear miR-10a detectable by Northern blot. This suppressive effect by miR-10a on L1 splicing and expression was greatly reduced in highly differentiated keratinocytes where miR-10a expression is dramatically decreased and HPV-16 becomes productive. Disruption of the miR-10a binding site by point mutation was found to promote L1 RNA splicing and L1 protein expression and reduce association of L1 RNA with SRSF1 and AGO2, a major component of RISC (RNA-induced silencing complex) and a nuclear-cytoplasmic shuttling protein in keratinocytes. Consistently, using a miR-10a with compensatory mutations to the mutated miR-10a binding site inhibited L1 RNA splicing. This study provides the first evidence of miRNA involvement in regulation of RNA splicing by direct interaction with RNA cis-elements.

333 Low oxygen influences the composition of human ribosomes and alternative splicing of ribosomal protein genes

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Ribosomes are often considered tightly regulated and static in composition due to their essential role of catalyzing protein synthesis. This view is changing, as mutations in certain ribosomal proteins are tolerated by cells, albeit with disease phenotypes known as "ribosomopathies". Additionally, specialized ribosomes have been observed in stressed bacteria and yeast cells which possess transcript specificity during translation. Here, we show that the ribosomal protein complement of human ribosomes is influenced by low oxygen (hypoxia), a key feature of the tumor microenvironment. We quantified ribosomal protein levels in actively translating ribosomes by Tandem Mass Tags mass spectrometry. Our data suggest that human ribosomes are heterogeneous, and that select ribosomal proteins are more likely to be incorporated into hypoxic ribosomes than their normoxic counterparts. Furthermore, hypoxia affected the expression of over a third of ribosomal protein genes and induced five alternative splicing events within a subset of these genes. We propose that alternative splicing events within RPL17 and RPS24 could act as predictive tumor biomarkers based on splicing trends observed in spheroids, in vitro models of tumor hypoxia. The splicing of RPS24 switches from a short isoform in a cell monolayer to a long isoform in spheroids of four cell lines: HEK293, U87MG glioblastoma, HCT116 colorectal cancer, and PC3 prostate cancer. Both endogenous RPS24 isoforms are actively translated into protein, and exogenously expressed FLAG-tagged versions of the two isoforms generate functional proteins that incorporate into ribosomes. We are currently investigating the regulation of RPS24 splicing and whether alternative RPS24 protein isoforms provide transcript specificity to hypoxic ribosomes. Given that rapidly dividing eukaryotic cells contain millions of ribosomes, any alterations in ribosome composition can have a profound effect on translation. This study highlights the adaptability of a fundamental biological process in human cells under low oxygen and other cellular stressors present in tumors.

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334 Assembly and early maturation of large subunit precursors

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The eukaryotic ribosome is assembled through a complex process involving more than 200 factors. Ribosome assembly starts in the nucleolus where RNA polymerase I transcribes a long polycistronic containing 3 of the 4 ribosomal RNAs: the 35S pre-ribosomal RNA in yeast. As this RNA is transcribed, assembly factors bind the nascent pre-rRNA and guide its adequate folding, modification and cleavage. This leads to the formation of large multi-component complexes sometimes called small subunit (SSU) and large subunit (LSU) processomes. These molecules are later separated by cleavage events in the middle of the pre-rRNA at the so-called sites A2 and A3. While the assembly of the SSU processome has been studied (1,2), assembly of the large subunit precursors, or pre-60S, is less well understood. Recent structures (3,4) of nucleolar intermediates of large subunit assembly have shed light on the role of many early large subunit assembly factors but how these particles emerge is still unknown. Here, we use a partially reconstituted system to examine the initial assembly of the pre-60S. Our data is in good agreement with a previously published study on the formation of early large subunit precursors (5). Using this approach, we also show that RNA processing at the 5' and 3' ends of the 27SA is required but not sufficient for the emergence of the nucleolar pre-60S.

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335 Thallium(I) impacts protein synthesis via blocking ribosome biogenesis pathway.

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Thallium is a metal classified in the group III A, distributed in the environment as monovalent Tl(I) and trivalent Tl(III) state. Thallium is considered as an emergent contamination because of its potential use in superconducting alloys, with increasing demand from high-technology industry. Tl(I) is highly toxic to the animals since it can affect numerous metabolic processes. We dissect how Tl(I) impacts physiological functions of the cells. We observed that protein synthesis was highly decreased and eIF2α is phosphorylated after thallium treatment. It has been proposed that Tl(I) can interact with sulfhydryl groups to inactivate the protein functions. Although accumulation of denature proteins would activate ER stress, it was not observed in our experimental conditions. Notably, the ratio of ribosomal large subunit (60S) to small subunit (40S) was decreased with increasing amount of thallium. Due to Tl(I) shares similarities with potassium (K) in ionic charge and atomic radius, it has been proposed that Tl(I) occupies certain K^+-binding sites and inactivates ribosome function. Thallium may impact 60S levels from triggering degradation of inactive 60S. However, activation of autophagy or proteasomal degradation of large subunits was not observed. Interestingly, while the activity of RNA polymerase I was not altered, rRNA processing was severely blocked. Therefore, we proposed that Tl(I) decreased protein synthesis from abnormal ribosome synthesis, resulting cell growth inhibition and lethality.

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336 Analysis of RNA helicases in human ribosome biogenesis

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Ribosomes are essential ribonucleoprotein (RNP) complexes composed of 4 ribosomal RNAs (rRNAs) and approximately 80 ribosomal proteins, which are responsible for the production of all cellular proteins. In eukaryotes, the process of ribosome assembly involves in excess of 200 *trans*-acting factors and is one of the most energy consuming processes in the cell. During their maturation, pre-ribosomal complexes undergo many structural transitions before they achieve the final architecture present in mature complexes. RNA helicases, which are best known for their functions in the NTP-dependent unwinding of RNA duplexes, but can also displace proteins from RNAs and act as RNA clamps, have emerged as important regulators of pre-ribosome remodelling events. In yeast, 21 RNA helicases are required for ribosome biogenesis and diverse functions, such as mediating the release of small nucleolar RNAs (snoRNAs) and enabling the pre-ribosomal access of RNA methyltransferases and endonucleases, have been described for these proteins. While many yeast RNA helicases are evolutionarily conserved, RNAi-based screens suggest that some of these proteins may have different or additional functions during human ribosome assembly. Furthermore, several metazoan-specific RNA helicases have recently been implicated in this pathway and many human RNA helicases remain largely uncharacterised. To gain further insight into the pre-ribosome remodelling events mediated by RNA helicases in human cells, we are combining *in vitro* techniques to dissect the molecular functions of specific RNA helicases with *in vivo* approaches to analyse the pre-ribosomal binding sites of these proteins and elucidate their roles during ribosome assembly.

337 Crosstalk between ribosomal RNA processing and pre-mRNA splicing machineries in ribosome biogenesis

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Ribosome biogenesis requires transcription by RNA polymerases (Pol) I, II, and III. Ribosomal RNAs (rRNAs) are transcribed by Pol I and III in the nucleolus and processed by multiple exo- and endonucleases to produce mature 18S, 5.8S, 25S, and 5S rRNAs. The genes that encode ribosomal proteins are transcribed in the nucleoplasm by Pol II. In the yeast *Saccharomyces cerevisiae*, these genes are enriched with introns, which are removed by the spliceosome to produce mature ribosomal protein gene transcripts. There has been an emerging understanding that the production of rRNA and ribosomal proteins is coordinated to ensure synthesis of functional ribosomes. We have recently uncovered evidence that this coordination occurs, in part, by tuning rRNA biogenesis to pre-mRNA splicing of ribosomal protein transcripts. In a genetic screen to identify genes that regulate the activity of the essential splicing factor Prp2, we uncovered numerous suppressors containing disruptions within the ribosomal DNA locus that suppress the lethal temperature sensitivity of the *prp2-1* allele. Specific disruptions within the ribosomal DNA locus were found to suppress both the rRNA processing defect and the global splicing defect of the *prp2-1* mutant, suggesting that the activity of pre-mRNA splicing and rRNA processing machineries is tuned to maintain a balance of rRNA and ribosomal protein production that is optimal for proper cellular function.

338 Insights into the interaction of Nop53 with the RNA exosome

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Eukaryotic ribosomal biogenesis is a highly energy demanding process that requires hundreds of trans-acting factors to dynamically build the intriguingly organized subunits 40S and 60S. Both ribonucleoprotein complexes are composed of specific rRNAs and ribosomal proteins which are structured in functional domains. In order to properly fold the ribosomal RNA in coordination with the recruitment of specific ribosomal proteins, the pre-rRNA processing pathway must be orchestrated following a hierarchy of assembly factors. After cleavage at A2 site of pre-rRNA 35S, the pre-40S and pre-60S particles take independent routes of maturation, during which the RNA exosome, a conserved 3'-5' exoribonuclease complex, plays a crucial role as one of the processing factors. The exosome catalytic subunits Rrp6 and Rrp44 degrade the 5'ETS and trim pre-rRNA 7S to 5.8S. Recently, the yeast nucleolar protein Nop53, a 60S assembly factor that has been identified interacting with Nop7 in the nucleoplasmic stage of maturation, was shown to be the adaptor responsible for recruiting the exosome for 7S processing. Interestingly, it has also been shown that Nop53 interacts with the catalytic subunit Rrp6. Here we highlight new interactions, through which Nop53 would possibly modulate the exosome activity in the context of 60S maturation.

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339 New transcription of 18S and 25S ribosomal RNA components by RNA Polymerase II, in Candida albicans during nutritional depletion

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We have recently reported that *Candida albicans,* as it approaches the stationary growth phase, produces 18S and 25S rRNA molecules resistant to a 5′-phosphate-dependent exonuclease (*BMC Molecular Biology* 2018 **19**:1). It appeared that these molecules contained an additional phosphate(s) at the 5'-end and therefore raised the possibility of new transcription. We performed a series of experiments to see whether these molecules also contained a 5'-methylated guanosine cap (m7G).

Total RNA from stationary organisms was isolated and ran through a 1.5% formaldehyde gel and blotted onto nylon membrane. Both 18S and 25S rRNAs were detected by anti-m7G-cap H-20 (Millipore). Total RNA, also from stationary organisms, was precipitated with same anti-m7G-cap antibody and reverse transcribed with 18S and 25S specific primers. After Rnase H digestion 18S and 25S PCR products were amplified with appropriate primers. We also isolated 18S and 25S molecules from total RNA from stationary yeast cells with His-tagged cap binding protein (Biomart) and they were clearly visible on gel electrophoresis. These bands were also detected by anti-cap-m7G antibody. This was again confirmed by reverse transcription and PCR amplification with 18S and 25S specific primers. Antibody to 10 repeats of YSPT[pS]PS corresponding to the carboxyl-terminal domain of RNA polymerase II (Epigentek) was used for chromatin immunoprecipitation (ChIP) on genomic DNA from stationary organisms. Both 18S and 25S sequences were amplified by PCR with appropriate primers.

The current understanding of 18S and 25S molecule production is that they are products of processing from a full-length copy of rDNA and thus should have a single phosphate at their 5'-end. Our newer data indicating the presence of m7G-cap at the 5'-end on some 18S and 25S molecules from stationary organisms strengthens our previous finding of resistance to a 5'-phosphate dependent exonuclease. Furthermore, the presence of m7G-cap, combined with our chromatin immunoprecipitation data, makes them products of RNA polymerase II transcription. The mechanism which allows RNA polymerase II to copy these ribosomal components and what regulates this process remains unknown.

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340 Characterization of the Essential Las1 HEPN Nuclease in Ribosomal RNA Processing

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Ribosome biogenesis is a critical, highly regulated pathway beginning in the nucleolus. The primary RNA transcript requires the removal of internal spacer regions (ITS1, ITS2) and external spacer regions (5' ETS, 3' ETS) to yield the functional 18S, 5.8S and 25S rRNAs. Las1 was recently identified as the essential endoribonuclease that initiates processing of the ITS2 by cleaving its C2 site. Las1 cooperates with the polynucleotide kinase (PNK) Grc3 to process the ITS2 by forming a constitutive super-dimer complex in which two Grc3/Las1 protomers are present. These two enzymes display molecular crosstalk, as mutations in the active site of Las1 result in phosphorylation defects in Grc3 and mutations in the active site of Grc3 cause defects in the nuclease activity of Las1. After C2 cleavage by Las1, Grc3 phosphorylates the 5' hydroxyl of the cleavage product, targeting it for degradation. Mutations in the human homologue of Las1 have been linked to congenital motor neuron disease and X-linked intellectual disability, demonstrating the importance this enzyme. However, aside from being dependent upon Grc3 for coordinated kinase and nuclease function, little is known about the molecular mechanisms governing the nuclease activity of Las1. The N-terminal Higher Eukaryote and Prokaryote Nucleotide Binding domain (HEPN) of Las1 is responsible for nuclease activity and contains a conserved RxxxxH motif (where x is any amino acid) linked to catalysis. By disrupting conserved and non-conserved residues found in this motif, we identified specific residues required for Las1-mediated pre-rRNA cleavage and Las1-dependent Grc3-mediated phosphorylation *in vitro* and *in vivo*. Super-dimer formation and thermal stability of the mutant complexes was also assessed. The crosstalk between Grc3 and Las1 ensures the direct coupling of cleavage and phosphorylation during pre-rRNA processing. Taken together, our studies provide key insight into the nuclease activity of the essential enzyme Las1 and its molecular crosstalk with the polynucleotide kinase Grc3.

341 Microexons alternatively spliced in the transcript of a human ribosomal protein gene.

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We searched extensively for evidence of alternative splicing of mammalian ribosomal protein (RP) genes in RNA-Seq datasets. Amongst the 80 genes encoding human RPs, one, RPS24, uses multiple microexons to produce transcripts that are alternatively spliced in a tissue specific manner. These alternative splice events are conserved across vertebrates.

RPS24 exons 5, 6, and 7 are microexons that are spliced in multiple combinations. Splicing occurs from the upstream exon 4 to any of four exons: exon 5 (3 nt), 6 (18 nt), 7 (22 nt), or 8, the terminal exon. Subsequent splicing can occur from 5 to 6, then to 7, then to 8, or other combinations, except splicing from 6 to 8 is never observed. Microexon 5 is included in 80% of the transcripts in thyroid tissue, 40% in prostate tissue, and only 1% in adipose tissue and skeletal muscle. Other tissues span this range. Exons 6, 7, and 8 contain termination codons. The resulting proteins have C-terminal ends of ...GKK, ...GKKK, ...GKKKK, or ...GKKPKE. The last is a strong candidate for SUMOylation*. The RPS24 C-terminus is external to the body of the ribosome, and such lysines are potential targets for additional modification. Stop codons in microexons 6 and 7 do not activate nonsense-mediated decay (NMD), because of the short distance to the final exon-exon junction. Thus, NMD and the presence of internal stop codons drive evolutionary pressure to keep exons 6 and 7 short.

*Hendriks et al., Nature Struc. & Mol. Biol. 24, 325, 2017

342 Modular assembly of the nucleolar pre-60S ribosomal subunit

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Early co-transcriptional events of eukaryotic ribosome assembly result in the formation of precursors of the small (40S) and large (60S) ribosomal subunit. A multitude of transient assembly factors regulate and chaperone the systematic folding of pre-ribosomal RNA subdomains. However, due to limited structural information, the role of these factors during early nucleolar 60S assembly is not fully understood. Here we have determined cryo-EM reconstructions of the nucleolar pre-60S ribosomal subunit in different conformational states at resolutions up to 3.4 Ångstroms. These reconstructions reveal how steric hindrance and molecular mimicry are used to prevent both premature folding states and binding of later factors. This is accomplished by the concerted activity of 21 ribosome assembly factors that stabilize and remodel pre-ribosomal RNA and ribosomal proteins. Among these factors, three Brix-domain proteins and their binding partners form a ring-like structure at rRNA domain boundaries to support the architecture of the maturing particle. Mutually exclusive conformations of these pre-60S particles suggest that the formation of the polypeptide exit tunnel is achieved through different folding pathways during subsequent stages of ribosome assembly. These structures rationalize previous genetic and biochemical data and highlight the mechanisms driving eukaryotic ribosome assembly in a unidirectional manner.

343 Unraveling novel roles for RNase E and KsgA in ribosome quality control in *E. coli*

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Ribosome assembly is a remarkably complex and multi-faceted process. The post-transcriptional processing and modification of ribosomal RNA (rRNA) is a critical step in the assembly of ribosomal subunits. Several ribonucleases and accessory biogenesis factors participate in *in vivo* assembly to ensure the rapid synthesis of functional ribosomes. Recent studies have shed light on quality control mechanisms that identify and degrade improperly processed rRNA. However, it remains unclear how ribonucleases are differentially recruited for maturation and degradation of rRNA. Importantly, it is poorly understood how the function of ribonucleases is influenced by other members of the ribosome biogenesis pathways.

In this study, we have characterized a novel functional interaction between essential endoribonuclease, RNaseE and a universally conserved ribosome biogenesis factor, KsgA in *E. coli*. Both factors perform known functions during the assembly of the 30S subunit. While RNase E participates in the maturation of the 5' end of 16S rRNA, KsgA is solely responsible for the dimethylation of two adjacent adenosines (A1518 and A1519) on Helix 45 of 16S rRNA. We observed that the overexpression of RNase E results in the accumulation of mature 16S rRNA missing 43 nucleotides (16S(Δ43) rRNA) at its 3' end, in a region that forms Helix 45 in the 30S subunit. Interestingly, the absence of KsgA resulted in reduced amounts of 16S(Δ43) rRNA. Cells containing a catalytically inactive KsgA mutant also accumulated 16S(Δ43) rRNA to a lesser extent, indicating that the methylation of Helix 45 by KsgA is vital for the observed RNaseE-induced cleavage. Additionally, we independently identified a genetic interaction between KsgA and RNase E, further supporting a common functional pathway between the two proteins. *E. coli* lacking KsgA and exoribonuclease, PNPase exhibit severe a growth defect and remarkably, a single point mutation in RNaseE (Ala55Thr) suppresses the growth defect. Further characterization of the suppressor showed alleviation of ribosome biogenesis defects and improved translational fidelity. Understanding the significance of 16S(Δ43) rRNA formation is essential to uncovering a novel ribosome quality control mechanism involving KsgA and RNase E.

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344 Ribosomal RNA levels and Translational Capacity of Articular Chondrocytes are Regulated by BMP-7 via BAPX1-NKX3.2

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The cellular availability of ribosomal RNAs (rRNA) is rate-limiting in the assembly of ribosomes. It is known that BMP-7 increases the anabolic capacity of articular chondrocytes. Previously we found that BMP-7 growth factor treatment of chondrocytes resulted in increased expression levels of bagpipe homeobox homolog 1 (BAPX-1/NKX3.2) expression. We hypothesized that BMP-7 supports the chondrocyte's anabolic response via BAPX-1/NKX3.2-dependent synthesis of rRNAs.

Osteoarthritic (OA) human articular chondrocytes (HACs) were isolated from OA cartilage from total knee arthroplasty. Chondrocytes and SW1353 chondrocytic cells were exposed to BMP-7 (1 nM) and expression of several rRNAs and genes (*18S, 5.8S, 28S*, *47S* intermediates, *RMRP*, *U3*, *UBF-1* and *BAPX-1/NKX3.2*) was determined by RT-qPCR and immunoblotting. BAPX-1/NKX3.2 overexpression and knockdown were achieved via transfection of a FLAG-BAPX-1/NKX3.2 plasmid or a BAPX-1/NKX3.2 siRNA duplex. Translational capacity of the cells was determined via SUnSET assays.

BMP-7 treatment resulted in increased *18S* and *5.8S* rRNA levels as well as increased *UBF-1*, *RMRP* snoRNA and *U3* snoRNA expression, while expression of a pre-18S rRNA intermediate was reduced. This correlated with increased BAPX-1/NKX3.2 mRNA and protein expression. Independently, overexpression of BAPX-1/NKX3.2 resulted in increased rRNA expression levels (*18S, 5.8S*), increased *UBF-1, RMRP* and *U3* levels, and reduced pre-18S rRNA levels. Reciprocally, BAPX-1/NKX3-2 knockdown resulted in opposite *18S, 5.8S, UBF-1, RMRP, U3* and *pre-18S* rRNA expression levels. Finally, BMP-7 induced protein translational capacity in SW1353 chondrocytic cells.

Here we show that BMP-7 growth factor stimulation increases cellular levels of mature rRNAs as well as expression levels of *trans*-acting factors involved in the transcription and maturation of rRNAs. Our data indicate that this action depends on BAPX-1/NKX3.2. In concert with increased rRNA expression, the overall translational capacity of chondrocytic cells increased after BMP-7 exposure. We are now exploring at which level BMP-7 and BAPX-1/NKX3-2 influences rRNA levels and we have preliminary data indicating that BMP-7 supports the transcriptional activity of the 47S rRNA promoter. Together our data indicate that BMP-7 induces an anabolic response in chondrocytes by supporting ribosome biogenesis and translational capacity in a BAPX-1/NKX3.2-dependent manner.

345 Bcp1 and methyltransferase Rkm1 regulate the stability of ribosomal protein Rpl23 (uL14) coordinately

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Ribosomes, highly conserved ribonucleoprotein complexes constituted of rRNAs and ribosomal proteins, are responsible for protein translation. As soon as rRNAs are synthesized, ribosomal proteins and transacting factors are loaded to form pre-ribosomal subunits. More than 200 transacting factors are known to join ribosome biogenesis process in eukaryotes. They are required for processing and editing nascent rRNAs, regulation of ribosome synthesis, and assembly of the correct ribosomal subunits. Some of them are identified as chaperones for specific ribosomal proteins recently. They are important to ensure the stability and correct incorporation of associated ribosomal proteins. In our previous study, Bcp1 was identified as the chaperone of Rpl23 (uL14) in *Saccharomyces cerevisiae*. In this study, Rkm1, the lysine methyltransferase of Rpl23, was also found to form a complex with Rpl23 and Bcp1 *in vitro* and *in vivo*. Interestingly, the activity of Rkm1 is triggered by Bcp1 only when correct conformation was achieved within the complex. Although the stability of Rpl23 was not decreased in the absence of Rkm1, the combination of *rkm1*Δ and *bcp1*^{ts} mutant exacerbated the decrease of Rpl23. The methylation may compete with ubiquitination on the lysine and further protect Rpl23 from degradation.

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346 Processing of the Ubiquitin-like Ribosomal Fusion Protein FUBI-eS30 is Required for 40S Subunit Maturation in Human Cells

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The eukaryotic small and large ribosomal subunits each contain a ribosomal protein (RP) which is synthesized as a fusion to the C-terminus of ubiquitin. Similarly, another RP of metazoan small subunits, eS30, is joined to the ubiquitin-like protein FUBI during translation. In contrast to eS30, FUBI is not part of mature 40S ribosomal subunits and is cleaved off by an as of yet unknown protease. Here, we have used human cell lines inducibly expressing wild-type and non-cleavable mutants of FUBI-eS30 to study the importance of its processing for ribosome biogenesis. A failure in FUBI processing impairs 40S maturation, which manifests in changes in the steady state localization of 40S biogenesis factors and processing defects of 18S rRNA precursors. Currently, we are investigating whether FUBI promotes the incorporation of eS30 into pre-40S particles and how a failure in FUBI removal affects 40S subunit maturation.

347 Exploring a novel mode of gene regulation during C. elegans embryogenesis

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During animal embryogenesis, the foremost task for a fertilized zygote is to replicate their genome. We mapped DNA replication origins at high temporal resolution in developmentally staged *C. elegans* embryos in order to investigate how genome duplication is integrated into the complex program of embryogenesis. Remarkably, we observe that DNA replication origins are associated with chromatin modifications found at gene promoters and enhancers, specifically H3K27ac, H3K4me1, and H3K4me2. An MNase-ChIP analysis of histone modifications at time points spanning embryogenesis reveals that "activating" marks are established prior to zygotic transcription and can be explained by germline transcriptional patterns, which are transgenerationally inherited from the parental chromatin. The zygotic transcriptome changes drastically throughout the course of embryogenesis, yet surprisingly, the "activating" chromatin modifications remain fixed relative to expressed genes: transcripts which are expressed early in embryogenesis are located in close proximity to the "activating" marks while transcripts expressed later in embryogenesis do so at a great distance (>10 kb). Currently, we are exploring a model in which the developmental transcriptome in *C. elegans* is regulated by distinct transcription elongation patterns, rather than enhancer looping interactions and TADs. We hypothesize that the independent evolution of operons in nematodes and the pervasive use of trans-splicing has given rise to a novel mode of gene regulation, which has shaped the landscape of the *C. elegans* genome.

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348 RNA demethylase FTO influences nuclear pre-mRNA processing events

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Eukaryotic RNAs can carry more than 100 different types of chemical modifications. Early studies have been focused on modifications of highly abundant RNAs, such as rRNA or tRNAs. However, the development of new detection methods allows nowadays to study also mRNA modifications. Among them, N6-methyladenosine (m6A) is particularly interesting as it can be 'erased' by the RNA demethylases ALKBH5 and FTO [1,2], although FTO has been recently linked to m6Am demethylation [3], a modification found on the adjacent adenosine of the 5' m7G mRNA cap.

Our recent study of the RNA demethylase FTO revealed that it preferentially binds to intronic regions of pre-mRNAs, suggesting that RNA demethylation could be a co- or early post-transcriptional process [4]. In addition, we performed transcriptome analysis of HEK293 FTO knockout mammalian cell line and uncovered that depletion of FTO leads to changes in alternative pre-mRNA splicing (AS) and 3' ends of mRNAs. FTO depletion leads mostly to AS exon skipping events and the splicing pattern can be rescued by catalytically active FTO. The measurements of mRNA half-life did not reveal differential stability of the two AS isoforms in WT neither in FTO KO cells, further pointing to the role of FTO in pre-mRNA processing. We were able to reproduce the FTO-dependent AS phenotype by using a reporter mini-gene construct, which we subsequently use to characterize the RNA region(s) and cofactors involved in the AS regulation. Altogether, our data indicate, that FTO and its activity plays a role in determining the mature form of subset of mRNAs via affecting the pre-mRNA processing steps.

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349 Genome-wide analyses reveal the coordination between alternative splicing and DNA methylation for light response in plants

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Alternative splicing (AS) is a widespread mechanism in eukaryotes that generates multiple mRNA isoforms from a single gene. It contributes largely to the complexity and environmental fitness of higher eukaryotes. As sessile organisms, plants constantly face fluctuating environmental conditions. Recent global analysis of AS with RNA sequencing (RNA-seq) has revealed that AS is highly responsive to environmental changes. In the case of light, one of the most important environmental factors for plant growth and development, accumulating data has suggested that intensive AS occurs in response to light. Detailed regulation still requires further investigation.

DNA methylation is known as an epigenetic mechanism to control transcription activities, however recent studies have shown that it also regulates AS co-transcriptionally. In this study, we investigated the possibility that light regulates AS through modulating DNA methylation of specific gene loci. We performed bisulfite sequencing and RNA-seq for *Arabidopsis* seedlings grown in the dark or irradiated by light. Cytosine methylation over whole genome and the regions adjacent to the splice sites where show light-regulated intron retention (IR) were analyzed. The changes of DNA methylation and AS are shown and correlated thru stepwise comparisons. The results suggest that DNA methylation at the IR regions associates with splicing efficiency. In conclusion, we propose that light promotes DNA de-methylation on specific sites near light-responsive AS regions in *Arabidopsis*. A co-transcriptional mechanism of light coordinating DNA methylation and AS are suggested. Detailed results will be discussed.

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350 Control of RNA structure and RNP assembly by N^6-methylation of adenine in sheared basepairs

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N^6-methyladenine is the most widespread RNA modification. Using X-ray crystallography we have shown that N^6mA is tolerated in Watson-Crick A-U and A-G basepairs, but completely prevents the formation of *trans* Hoogsteen-sugar A•G basepairs. Sheared A•G basepairs are widespread in RNA structure, and would be logical targets for control of conformation by methylation.

Using bioinformatics we have found that a sub-set of human box C/D snoRNA species have target GAC sequences that result in the formation of N^6-methyladenine at a key *trans* Hoogsteen-sugar A•G basepair, of which half are methylated *in vivo*. The GAC target is conserved only in those that are methylated. Methylation prevents binding of the 15.5 kDa protein and the induced folding of the RNA. Thus the assembly of the box C/D snoRNP can be regulated by RNA methylation at its critical first stage. More generally, we show that N^6-methylation of adenine occurs at sheared A•G basepairs involved in tertiary contacts in the human signal recognition particle RNA and related Alu retrotransposon RNA species.

N^6-methylation at A•G basepairs is probably a general method of controlling conformation and RNP assembly in cellular RNA.

L. Huang, S. Ashraf, J. Wang and D. M. J. Lilley Control of box C/D snoRNP assembly by N^6-methylation of adenine. *EMBO rep*. **18**, 1631–1645 (2017).

351 RNA binding to a chromatin modifier - Polycomb Repressive Complex 2

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Polycomb repressive complex 2 (PRC2) is a key chromatin modifier responsible for methylation of lysine 27 in histone H3, and a large number of lncRNAs and pre-mRNAs have been found to be associated with PRC2 in mammalian cells. The physiological functions of the RNA-PRC2 interaction remain largely unknown, hampered by the lack of a separation-of-function mutant defective in RNA binding. We have reported that PRC2 exemplifies a novel mode of RNA binding conserved over vast evolutionary distance, and we have now been able to make a separation-of-function mutant to investigate how RNA binding regulates recruitment and methyltransferase activity of PRC2 genome-wide. This system could provide a paradigm for an emerging list of other epigenetic modifiers that bind RNA without canonical RNA-binding motifs.

352 RNA-mediated chromatin alterations in cellular dormancy

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In eukaryotes, constitutive heterochromatin is demarcated by trimethylated lysine 9 of histone H3 (H3K9me) and packages repetitive DNA elements into a highly condensed chromatin structure, refractory to transcription and recombination. Heterochromatin formation at chromosomal structures such as centromeres and telomeres is critical for their proper functioning and its loss leads to genomic instability and chromosome missegregation. This is also true in the fission yeast in which (ncRNAs) and RNAi machinery specify the recruitment of H3K9 methyltransferase Clr4/Suv39h to heterochromatic regions. Loss of RNAi leads to defects in Clr4/Suv39h recruitment, H3K9 methylation and heterochromatin function. Most of what is known about constitutive heterochromatin proteins is in the context of their activities at heterochromatic domains. But recently, using the fission yeast, we reported a new function for heterochromatin proteins – the establishment of the global transcriptional program of quiescent (G0) cells. We found as cells enter G0, they coopt the existing heterochromatin machinery to regulate the expression of a set of metabolic, cell cycle and highly transcribed genes, required for the establishment of the G0 state. The *de novo* targeting of heterochromatin factors to euchromatic parts of the genome requires Ago1-associated sRNAs, resulting in H3K9 methylation of several euchromatic gene clusters. We call this mechanism Quiescent-Induced Transcriptional Silencing (QuieTS) and propose a model in which stress-induced Argonaute-associated nuclear sRNAs can deploy heterochromatin factors globally, forming transcriptionally co-regulated gene clusters critical for the establishment of the adaptive transcriptional program of G0 cells. Overall this work describes another function for Ago1-associated small RNAs and constitutive heterochromatin proteins, which in response to certain environmental conditions can be repurposed to adjust/establish the global transcriptional program of cells. I will discuss some of our latest data testing the predictions of this model.

353 Identification and characterization of RNAs that interact directly with the human LSD1 complex.

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Histone Lysine Specific Demethylase 1 (LSD1) and the corepressor of RE1 silencing transcription factor (CoREST) function as nucleic acid binding proteins and are part of a multi-domain complex that performs shape-based molecular recognition on nucleosomes. Previous studies have shown that LSD1 can specifically recognize a stacked, parallel-stranded intramolecular G-quadruplex (GQ) ($K_d \approx 96$ nM) and can distinguish RNA from DNA-GQ structures *in vitro*. [1,2] Cell-based and *in vitro* cross-linking mass spectrometry has independently identified an RNA binding site on the SWIRM domain of LSD1. [2,3] Further, RNA disrupts LSD1 catalytic activity and acts as a noncompetitive inhibitor [2], likely sequestering LSD1 from nucleosome substrates. To better understand how RNA interacts with chromatin-remodeling enzyme complexes, transcripts preferentially bound to the LSD1-CoREST complex were identified in HEK293 cells using photoactivatable ribonucleoside crosslinking immunoprecipitation (PAR-CLIP). Sequences from overlapping reads that map to the human transcriptome were organized into 1,433 and 1,068 clusters for LSD1 and CoREST, respectively. The composition of the mapped RNA reads compared to the composition of RNA clusters reveal that lncRNAs, 3'-UTRs, and intronic regions of mRNAs are preferentially enriched in LSD1 and/or CoREST. Interestingly, a two-fold enrichment of G-quadruplex motifs exists among the bound transcripts, suggesting that the LSD1/CoREST complex may preferentially interact with G-rich regions of RNAs. Biochemical validation of a subset of these identified transcripts show that these G-rich regions fold into GQ structures and bind to the LSD1-CoREST complex with comparable affinities. These findings provide insight into the potential RNA-mediated transcription regulation of LSD1 and hold implications for the recruitment mechanisms of LSD1 to chromatin.

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354 Determining the effects of mutations in the chromatin remodeler subunit SMARCA4 on alternative splicing in lung adenocarcinoma

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Alternative splicing is a process that is involved in generating much of the genetic diversity in eukaryotic organisms and is misregulated in a number of diseases such as cancer. Regulation of this process via chromatin structure by changing the elongation rate of RNA Pol II remains poorly understood. The gene SMARCA4 is a subunit of the chromatin remodeling SWI/SNF complex and known to be recurrently mutated in lung adenocarcinoma. This project investigates how loss-of-function mutations in SMARCA4 cause changes in alternative splicing and how those changes relate to tumorigenesis. Through analysis of RNA-seq data from patients with lung adenocarcinoma from The Cancer Genome Atlas (TCGA), we found that of the 495 patients samples, 18 had deleterious mutations of SMARCA4 and had 101 significantly associated alternative splicing changes (FDR corrected p-value < 5%, $|\Delta$ percent spliced in $| > 10\%$). Our results indicate that SMARCA4 mutants show a clear preference for exon skipping and alternative acceptor splicing events. Splice site strength was lower in exons showing greater inclusion in the SMARCA4 mutants as compared to the wild-type. We are currently analyzing RNA-seq data from a mouse lung cell line with and without SMARCA4, and comparing those results with our TCGA analysis. This project aims to advance our understanding of how chromatin structure regulates alternative splicing and how misregulation of this process contributes to tumorigenesis.

355 Regulation of alternative splicing by p300-mediated acetylation of splicing factors

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Splicing of precursor mRNA (pre-mRNA) is an important regulatory step in gene expression. Recent evidence points to a regulatory role of chromatin-related proteins in alternative splicing (AS) regulation. Using an unbiased approach, we have identified the acetyltransferase p300 as a key chromatin-related regulator of AS. p300 promotes genome-wide exon inclusion in both a transcription-dependent and -independent manner. Using CD44 as a paradigm, we found that p300 regulates AS by modulating the binding of splicing factors to pre-mRNA. Employing a tethering strategy, we found that binding of p300 to the CD44 promoter region promotes CD44v exon inclusion independently of RNAPII transcriptional elongation rate. Promoter-bound p300 regulates AS by acetylating splicing factors, leading to exclusion of hnRNP M from CD44 pre-mRNA and activation of Sam68. p300-mediated CD44 AS reduces cell motility and promotes epithelial features. Our findings reveal a mechanism through which chromatin-related proteins regulate AS and show the impact of this mechanism on cell function.

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356 Novel Oncogenic Function of ARID1A-L in Ewing Sarcoma

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The AT-rich interactive domain-containing protein 1A (ARID1A, BAF250a) gene encodes a central component of the mammalian BAF complex. The BAF complex is described as either a tumor suppressor or having oncogenic function depending on the context of the specific subunit mutation. Ewing sarcoma (ES) is a highly aggressive cancer of the bone and soft tissue characterized by the EWSR1-FLI1, chromosomal translocation oncogene that translates to oncogenic fusion protein EWS-FLI1. We recently showed that EWS-FLI1 has multiple direct connections with the spliceosomal complex and also drives aberrant splicing which alters many mRNA isoforms; however the role of these specific isoforms in ES oncogenesis remains unknown. Small molecule inhibitor of EWS-FLI1, YK-4-279, blocks protein interactions and reverses EWS-FLI1-associated splicing. We identify ARID1A-L isoform as an EWS-FLI1 regulated isoform required for ES oncogenic growth that connects EWS-FLI1 into the BAF complex. Loss of ARID1A significantly reduced cell proliferation. Re-expression of the *ARID1A-L* isoform restored ES cell proliferation while the short isoform *(ARID1A-S)* did not restore growth. Both EWS-FLI1 reduction and YK-4-279 treatment shifts the ARID1A-L isoform to the ARID1A-S. In addition, we show the chromatin remodeling or epigenetic reprogramming functional connection of EWS-FLI1 through ChIP-seq, ATAC-seq, and a transcriptome analysis. These novel findings also indicate that targeting *ARID1A-L* isoform associated with EWS-FLI1 could support both therapeutic stratification and novel targeting opportunities.

357 Withdrawn

358 Profiling of recurrent 5′ UTR mutations reveals altered regulatory elements and structural stability in cancer genomes

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Somatic mutations in the human genome are the primary cause of oncogenesis. While alterations in protein-coding regions have been historically the focal point in cancer research, the study of mutations in noncoding regions and their relationships to cancer formation are still largely uncharacterized. The 5' untranslated region (UTR) lies upstream of the initiation codon and regulates gene translation through multiple mechanisms, including ribosome recruitment and RNA-binding protein binding sites. Mutations here have a direct effect on translational efficiency of oncogenes and tumor suppressor genes, making them possible therapeutic targets or biomarkers. We selected 5' UTR mutations from sequencing data provided by the Pan-Cancer Analysis of Whole Genomes (PCAWG), an international collaboration featuring whole genomic data from over 2,000 patients across 33 tumor types. Through a series of computational analyses, we identified significant recurrent mutations and assessed the impact of these mutations on sequence motifs and secondary structures in the 5' UTR. We detected significant recurrent mutations in *BCL2*, *TMSB4X*, and 824 other previously reported genes. A recurrent mutation in *TBC1D12* created a new stop codon upstream of the annotated start codon. Mutations in *IGLL5* and *DMD* led to noticeable differences in the number of upstream alternative start and stop codons. We also discovered mutations that cause depletions in the number of G-quadruplexes in several ribosome-related genes. *TMSB4X* and other genes with recurrent mutations also saw declines in the structural stability of their 5' UTR sequences. Our findings suggest that mutations in the 5' UTR may influence translational regulation by modulating the structural stability and sequence-specific elements of these regions and reveal potential candidates for future exploration of function and clinical relevance.

359 Computational Tools for 2D and 3D Structure Modeling of RNA

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A secondary structure prediction approach is presented that is geared towards predicting the base pairing of multiple interacting RNA and DNA strands that potentially contain pseudoknots. It is shown that the approach successfully reproduces the structure of several designed nucleic acid nanostructures. The approach can be used to predict melting temperatures of RNA, DNA and RNA/DNA hybrid nanostructures.

Next, we developed a 3D coarse-grained model for generating RNA tertiary structures from secondary structure models. We demonstrate that this approach can successfully detect portions of designed RNA structures that are under strain in terms of helical geometry.

To aid in the prediction process, we compiled a novel library of RNA 3D motifs. It is shown that this motif library can aid in the prediction of non-canonical base pairs and of RNA 3D structures.

We present several examples where this set of computational tools aided in the design and prediction of RNA complexes.

360 Functional RNA interactions as defined by RNA binding protein data and RNA secondary structure probing under *in vitro* and *in vivo* conditions

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The immensely complex post-transcriptional interactions of many RNA molecules with RNA binding proteins (RBPs) is poorly understood. The role of secondary structure in defining these interactions remains even more elusive, especially for many mRNAs. Experimental chemical probing of RNA, such as with *in vivo* click selective 2'-hydroxyl acylation and profiling experiment (icSHAPE), remains the most accurate approach for measuring RNA secondary structure. By deeply probing the transcriptome from HepG2 and K562 cell lines under both i*n vivo* and *in vitro* (*in vivo* extracted, de-proteinated) conditions, we aim to define the protein-interactive structural features of individual RNA molecules *in vivo*. Numerous enhanced crosslinking and immunoprecipitation (eCLIP) experiments have catalogued the RNA binding landscape of hundreds of RBPs in the same cell lines. By integrating these datasets we observe several results. First, that icSHAPE measurements for a given transcript differ more between *in vitro-in vivo* conditions than they do between cell lines. Second, globally higher icSHAPE measurements in *in vivo* indicate widespread transcript interactions with helicases. However, less variable structural measurements in the coding sequence as well as anomalies at the start and stop codons likely result from the constrained sequence space of the coding sequence, as opposed to interactions with ribosomal proteins, as these patterns occur under both *in vivo* and *in vitro* conditions. Third, sites of large structural differences between *in vitro-in vivo* measurements are enriched in RBP binding sites and suggest patterns of structure probing data that can predict protein binding interactions. Additionally, an R-based web application allows the interactive visualization of individual transcripts' icSHAPE data and RBP binding sites, enabling individualized study of secondary structure-RBP interactions.

361 Divergence of singleton and tandem glycine riboswitch aptamers

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Riboswitches are RNA motifs found within the 5' untranslated region (UTR) of bacterial mRNAs that regulate downstream expression of crucial metabolic genes in response to the presence or absence of a ligand. Canonical riboswitches are comprised of one ligand-binding aptamer motif and one expression platform, which undergoes conformational changes affecting expression in response to aptamer-ligand binding. The glycine riboswitch is unique in that it is commonly found in both the canonical conformation (singleton) and with two aptamers regulating a single expression platform (tandem). While this tandem conformation is well documented, it is not clear what, if any, benefit it provides over the singleton conformation, or how it arose and became conserved across bacterial phyla. To elucidate how tandem glycine riboswitches differ from singletons and across phyla, we need a better understanding of the tandem glycine riboswitch's conservation and role. To address this, we created singleton and tandem riboswitch covariance models and subsequently used them to gather a large set of any concrete singleton and tandem incidences. We then clustered the sequences based on taxonomic distribution and regulatory context.

Our findings indicate that within tandem riboswitches the individual aptamers experience distinct evolutionary conservation. Using consensus structures based on sequence and structural data gathered from 69 Vibrionaceae and 51 Bacillaceae tandem riboswitches, we observe a dichotomy in riboswitch conservation across these two bacterial families. Within Vibrionaceae tandem riboswitch the second aptamer is more highly conserved, while within Bacillaceae the first aptamer is more highly conserved. This correlates with regulatory context, as Vibrionaceae riboswitches were found to regulate transport proteins, while all except 4 Bacillaceae riboswitches were found to regulate proteins related to glycine degradation. Moreover, when all 120 riboswitches were phylogenetically clustered, the 4 riboswitches from Bacillaceae which regulated transport proteins were more closely related to Vibrionaceae riboswitches regulating transport proteins than with riboswitches originating from their bacterial family. This suggests horizontal gene transfer of the glycine riboswitch and aligns with the hypothesis that regulatory context may be predictive of whether a given riboswitch up- or downregulates gene expression in response to glycine.

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362 PyRy3D: software for modeling macromolecular complex structures and its application to the *Trypanosoma brucei* 20S editosome

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One of the major challenges in structural biology is to determine the structures of macromolecular complexes and to understand their function and mechanism of action. However, compared to structure determination of the individual components, structural characterization of macromolecular assemblies is very difficult. To maximize completeness, accuracy and efficiency of structure determination for large macromolecular complexes, a hybrid computational approach is required that will be able to incorporate spatial information from a variety of experimental methods (like X-ray, NMR, cryo-EM, cross-linking and mass spectrometry, etc.) into modeling procedure. For many biological complexes such an approach might become the only possibility to retrieve structural details essential for planning further experiments.

We developed PyRy3D, a method for building and visualizing low-resolution models of large macromolecular complexes. The components can be represented as rigid bodies (e.g. macromolecular structures determined by X-ray crystallography or NMR, theoretical models, or abstract shapes) or as flexible shapes (e.g. disordered regions or parts of protein or nucleic acid sequence with unknown structure). Spatial restraints are used to identify components interacting with each other, and to pack them tightly into contours of the entire complex (e.g. cryo-EM density maps or ab initio reconstructions from SAXS or SANS methods). Such an approach enables creation of low-resolution models even for very large macromolecular complexes with components of unknown 3D structure. Our model building procedure applies Monte Carlo approach to sample the space of solutions fulfilling experimental restraints.

The editosome is a 0.8MDa protein complex that catalyzes the RNA editing process in African trypanosomes. We applied the hybrid modeling approach implemented in PyRy3D software in order to build ensembles of structural models of the *Trypanosoma brucei* 20S editosome that agree with currently available experimental and theoretical data.

363 Understanding the Basis and Evolution of tRNA Gene Regulation with Comparative Epigenomics

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Transfer RNAs (tRNAs), in addition to being a universal and crucial element of life, are frequently duplicated in eukaryotic genomes, hindering our ability to study both tRNA gene regulation and evolution. The presence of multiple identical copies of a large portion of tRNA genes makes RNA-sequencing insufficient to determine how these tRNA genes are controlled by the cell. tRNAs are copied in the genome as parts of retrotransposons (SINE and LINE-1 repetitive elements) and as part of larger segmental duplications of the genome. We used chromatin data produced by the Roadmap Epigenomics Project to build a profile of tRNA expression in over 100 cell types to understand where individual tRNA genes are active and recognize distinct tRNA regulation profiles. We found that there were several modes of tRNA expression, one of which was a group only expressed in stem and embryonic cell types. To understand how expression patterns relate to evolutionary conservation, we built a method for inferring tRNA orthology between genomes using syntenic blocks anchored on neighboring protein-coding genes. Using these orthologs maps and published mouse epigenetic chromatin data, we found that expression profiles are conserved between the human and mouse genomes for 148 of 208 orthologous tRNA loci. Constitutively expressed tRNA genes are most likely to be conserved between these mammalian genomes, but are often also duplicated within genomes. We find that tRNA arrays (genes clustered nearby in the genome) show some similarity in expression patterns, but can vary, suggesting that location in the genome alone does not determine tRNA expression. We also observe other important factors affecting tRNA expression, including the primary sequence of the tRNA (containing the A and B box internal promoters), close proximity to other gene promoters (including protein coding and long non-coding RNAs), and the presence of CpG islands which can affect the methylation state of the tRNA gene. Using these data we propose a model for tRNA expression where tRNA genes are controlled by a complex mixture of both intrinsic (gene sequence) and extrinsic (chromatin neighborhood) factors which allow for identical duplicated genes to take on unique roles in the cell.

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364 Transcriptomic splicing information may leak personal private information by computationally linked to the genome

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Sharing genomes without personal identifiers is common practice. However, recent studies revealed the risk of re-identifying people from their genomes, or attached quasi-identifiers, such as sex, birthdate and zip code. The additional availability of an individual's RNA-seq data has implications for privacy, as it may be linked to the genome, potentially allowing the person's privacy to be breached. For example, sex and ethnicity information may be inferred directly from a genome, and the study may provide zip code. This could be linked to RNA-seq data from a diabetes study with attached birthdates and income. These combined quasi-identifiers may uniquely identify the person, and the study reveals the person's disease status.

RNA-seq reads contain genetic variants, and thus can be directly linked to the genome. To avoid this risk, some researchers now release gene expression, isoform expression and exon read count data instead of raw reads.

However, gene expression can also be linked to the genome based on expression QTLs. Using a Bayesian framework, we found that it is feasible to predict genomic variants from relative isoform expression. Based on GTEx splicing QTLs data, using relative isoform expression from 15 genes, we could identify the target genome within a pool containing hundreds of individuals with >90% accuracy. It is possible to identify the target genome of an RNA-seq dataset from millions of individuals using more splicing QTLs. Researchers have proposed to eliminate the risk of gene-expression-based linking attacks by adding noise to the gene expressions, based on the observation that only a few genes enable linkage. However, we found that there are now many more such genes than previously reported. We find that expression data enables the re-identification of target genome from a pool containing billions of genomes. Our result implies that mitigation of the linking risk by adding noise would severely abrogate biological entity of the data, since the data will no longer be biologically meaningful when over half of gene expressions are modified. Our study also implies that other kinds of "omic" data, including DNA modification and protein metabolite levels, may also leak genome privacy.

365 Comprehensive portrait of canonical and non-canonical splicing in cancer

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Current annotation methods typically classify mutations as disruptors of splicing if they fall on either the consensus intronic dinucleotide splice donor, GT, or the splice acceptor, AG. As a group, splice site mutations have been presumed to be invariably deleterious because of their disruption of the conserved sequences that are used to identify exon-intron boundaries. While this classification method has been useful, increasing evidence suggests that mutations outside of the canonical splice site can lead to transcriptional changes beyond disruption of the canonical junction. In this study, we have developed a bioinformatic pipeline to determine the global and local effects of mutations on splicing factors and genomic sequences, respectively, across 33 cancer types. To evaluate the local effect of mutations on splicing, we systematically identified splice-disrupting mutations (SDMs), splice-creating mutations (SCMs) and SFM (splice-factor mutations), genome-wide. We identified 1,964 novel SCMs, of which 26% and 11% were mis-annotated as missense and silent mutations and validated 10 of 11 genes in a mini-gene splicing assay. SDM identification predicted complex splicing patterns associated with canonical splice site mutations and mutations in proximity to the canonical junction that disrupt splicing factor binding sites. Interestingly, further investigation of the novel neoantigens produced by SCMs and SDMs are likely several folds more immunogenic than missense mutations. To explore mutations that disrupt core splicing factors, HotSpot3D was applied to identify SFMs that are significantly proximal to one another using HotSpot3D. Our anlaysis has classified SFMs that disrupt the spliceosomal complex and globally impact downstream splicing targets creating novel peptide sequences and alter key cancer genes. The current study has greatly extended the insight into the transcriptional ramifications of genomic alterations by integrating DNA and RNA sequencing data and painting the portrait of alternative splicing across cancer genomes.

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366 Rfam: The transition to a genome-centric sequence database

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BACKGROUND

Rfam is a database of non-coding RNA families in which each family is represented by a multiple sequence alignment, a consensus secondary structure, and a covariance model [1]. These models can be used to annotate sequences with RNA families using the Infernal software [2].

RESULTS

Starting with release 13.0 [1], Rfam switched to a new genome-based sequence database, which currently includes a non-redundant set of over 14,000 reference genomes maintained by UniProt [3]. The new database is more scalable and gives a more accurate view of the distribution of Rfam entries. Using complete genomes enables meaningful taxonomic comparisons and identification of a repertoire of RNA families found in a certain species.

The text search functionality of the Rfam website was significantly improved. Users can now more easily search Rfam with the new and more powerful faceted text search. For example, it is possible to explore RNA families or ncRNAs in any annotated genome and compare annotations across genomes. The new summary pages provide a way for users to explore ncRNAs within genomes as well as visualise genes through a genome browser.

Rfam currently contains 2,772 families and continues to grow. In order to speed up the creation of new families, we are developing a new curation pipeline that will be made accessible to external expert users to create new families.

CONCLUSION

The transition of Rfam to a genome-centric sequence database and the new website features make Rfam a more valuable resource for the sequence analysis community. Rfam is available at http://rfam.org.

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367 miRTrace: a tool for quality control and tracing taxonomic origins of microRNA sequencing data

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In forensics, food safety and laboratory quality control it is often crucial to resolve the organismal origins of samples. Previous studies have shown that miRNAs are continuously being added to animal and plant genomes through time and are rarely subsequently lost. These features make them as promising phylogenetic markers. Here we present our newly developed software miRTrace, which is the first method to trace taxonomic origins of microRNA sequencing data (sRNA-Seq) based on the composition of clade-specific miRNAs. We show that miRTrace can accurately resolve origins of low-quantity samples, such as single cells and parasitic RNA in host serum, with high sensitivity. Surveying more than 700 sRNA-Seq public data sets from model organisms, we find that > 7% contain lowly abundant cross-species contaminations, suggesting that researcher-induced contaminations are rare. When applying miRTrace to carefully controlled in-house data, we discover that Illumina index mis-assignment during sample demultiplexing is an important source of contaminations. Last, using an *in silico* simulation framework, we estimate the impact of cross-clade contamination on miRNA expression analysis and novel miRNA gene prediction. miRNAs from close-related species are more likely to detrimentally affect analyses, increasing the false positive rate and lowering the accuracy. miRTrace is implemented in java for portability and can survey dozens of sRNA-seq samples in minutes. It will be a useful quality control tool for both sequencing platforms and research groups.

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368 A human-specific switch of alternatively spliced AFMID isoforms contributes to TP53 mutations and tumor recurrence in hepatocellular carcinoma

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Pre-mRNA splicing can contribute to the switch of cell identity that occurs in carcinogenesis. Here, we analyze a large collection of RNA-seq data sets and report that splicing changes in hepatocyte-specific enzymes, such as *AFMID* and *KHK*, are associated with HCC patients' survival and relapse. The switch of *AFMID* isoforms is an early event in HCC development and is associated with driver mutations in *TP53* and *ARID1A* The switch of *AFMID* isoforms is human-specific and not detectable in other species, including primates. Finally, we show that overexpression of the full-length *AFMID* isoform leads to a higher NAD+ level, lower DNA-damage response, and slower cell growth in HepG2 cells. The integrative analysis uncovered a mechanistic link between splicing switches, de novo NAD+biosynthesis, driver mutations, and HCC recurrence.

369 rna-pdb-tools: a toolbox to analyze structures and simulations of RNA & dataset of curated structures submitted to the RNA-Puzzles experiment

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Structural RNA bioinformatics is a relatively young field of science struggling with a lack of bioinformatic tools to facilitate the daily work of the researcher. To fill this gap, we developed a software package called **rna-pdb-tools** (https://github.com/mmagnus/rna-pdb-tools). rna-pdb-tools is a Python library and a set of tools dedicated to RNA structural file handling and manipulation, like (1) rebuilding of missing atoms in RNA structures, (2) structural clustering, (3) standardization of PDB format to comply with the format required by RNA-Puzzles, (4) visualization of secondary RNA structures and drawing RNA arch diagrams of secondary structure, and much more. The package can be promptly used from the command line, integrated into a script or executed on Jupiter Notebooks.

The package consists of both Python wrappers to existing tools, such as ClaRNA, SimRNA, Rosetta, Infernal, 3DNA and new, original scripts such as Clanstix (RNA clustering with CLANS), diffpdb (text-based diff utility for PDB files), and Python classes, e.g, SimRNATrajectory (for manipulation of SimRNA trajectory files), RNAalignment (RNA alignments manipulation, e.g. slicing columns or sequences). The software has been used in various tools developed in the laboratory: NPDock (RNA/DNA-protein docking method), SimRNAweb (RNA 3D structure prediction method), mqapRNA (model quality assessment for RNA 3D) and RNArchitecture (structure-based classification of RNA families).

Furthermore, rna-pdb-tools was used to curate structures submitted to RNA-Puzzles, https://github.com/mmagnus/RNA-Puzzles-Normalized-submissions) facilitating the comparison of structural models to track the progress in computational methods for RNA 3D structure prediction.

The rna-pdb-tools code is open, free, modular and well documented which should encourage developers to build new applications on top of it. The documentation can be found at the link http://rna-pdb-tools.rtfd.io. The ability to predict and design RNA 3D structures opens great opportunities for the new developments in biotechnology and basic science. However, it is not possible to take advantage of these opportunities without an efficient toolbox for analysis of RNA structural models and computational simulations.

rna-pdb-tools seems to be an important piece of this toolbox. In particular, rna-pdb-tools in concert with Jupyter Notebooks is easy to use for biologists, encouraging them to explore structural analyses on their own.

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370 Heteroformity: a measure of diversity for alternative splicing

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The advent of high-throughput sequencing of messenger RNA, coupled with the development of software (such as Salmon and Kallisto) that allows high-throughput processing of thousands of samples, makes possible a global description of alternative splicing at the level of a sample, a tissue or a species. We define heteroformity, a measure of transcript diversity as the fraction of transcript pairs drawn at random from a single gene that differ. This metric is inspired by the utility of mean heterozygosity in population genetics. The heteroformity of a gene thus varies between 0 (if there is only one isoform) and 1 (the limit, when so many isoforms are present that each transcript is different). The abundance-weighted gene heteroformity for a sample can be visualized as a cumulative distribution, allowing immediate inference of some phenomena.

Application to 11,688 human samples from 30 tissues in the Genotype-Tissue Expression (GTEx) project revealed some general patterns. In all samples, about 25% of transcripts are in genes with very low heteroformity. In contrast, the top quartile of transcripts are in genes with over 0.5 heteroformity. Tissues differ; greater levels of alternative splicing are consistently observed in reproductive and nervous tissue. Nevertheless, there is great individual variation, with specific heart samples varying over three-fold in total gene-level heteroformity. We note that sample heteroformity need not correlate with differences in alternative splicing between samples, and partitioning genes with respect to their overall heteroformity vs. their differential alternative splicing reveals patterns of developmental regulation.

We are currently applying heteroformity to diverse biological samples and exploring its properties as a robust and useful metric.

371 Systematic characterization of reverse transcriptases-related proteins and their diversities in prokaryotes

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Reverse transcriptases (RTs) are enzymes that polymerize DNA from RNA templates. RTs are usually thought to exist mostly in eukaryotes and viruses, but they are also present in bacteria. Bacterial RTs seem to be ancestors of eukaryotic RTs, and several types are identified i.e. group II introns, retrons, CRISPR/Cas-associated RTs, diversity-generating retroelements and Abi-like genes. Recently, several studies have reported that there are multiple uncharacterized and highly diverse RT-like sequences in bacteria. However, their evolutionary relationships and the processes of diversification are not well understood. In this study, we collected myriad of RT-like sequences from bacteria and archaea genomes and systematically characterized RT evolution. Using known RT-related sequences as queries, sequence homology search was performed against 85,613 bacteria and archaea genomes in the RefSeq database. As a result, we obtained 149,156 RT-related proteins, and they were found almost in all phyla registered in the database except for a few species in proteobacteria, bacteroidetes and tenericutes. These obtained sequences were clustered into 1,816 clusters based on sequence identity with especially small (less than 10 sequences) clusters eliminated. Although not all the sequences were annotated to have RT domain/activity, at least they had partial RT homologous regions. Possible evolutionary scenario of RT-related proteins will be discussed together with their possible sequence motifs.

372 Withdrawn

373 Withdrawn

374 The codon stabilization coefficient is a reliable parameter to indicate the most biologically accurate mRNA half-life measurements in *S. cerevisiae*.

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Messenger RNA half-life is a key aspect of gene expression regulation. Different methods of mRNA half-life measurements are available, but, unfortunately, datasets generated with *Saccharomyces cerevisiae* transcriptome show poor correlation between themselves, hampering our understanding of the mRNA turnover process. An ideal dataset would be in accordance with other cellular parameters, such as protein expression, translation efficiency and tRNA abundance. In order to clarify this subject, we analyzed mRNA half-life datasets from nine different groups in search of the most accurate method of measurement. Surprisingly, when we compared mRNA half-life with these different cellular parameters, we found little to no correlation on all datasets. Since codon optimality is one of the major determinants of mRNA stability, we used the codon stabilization coefficient (CSC), a correlation between the frequency of occurrence of each codon in mRNA and mRNA half-lives, as reference for mRNA half-life measurement accuracy. After CSC calculation for each dataset, we were able to find strong positive correlations between the CSC and the different cellular parameters in three of the analyses (Coller, Becskei and Cramer groups), indicating that these datasets would be more reliable. We then proceed to validate the CSC as a strong mRNA stability indicator/reference through a number of analyses, which confirms it as a precise mechanism. We conclude that the CSC is a strong parameter to indicate mRNA half-life measurement accuracy.

375 Computer-aided design of RNA structures and targeting

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In spite of the clear relation between structure and function in RNA, and the apparent ease with which base-pairing can be predicted, designing new sequences that fold the same way as known structures remains a challenge. This is especially true for structures that include pseudoknots. We devised software to achieve this by using the inverse folding approach (*Enzymer*), as well as to target RNAs with ribozymes (*Ribosoft*). In each case, we validated experimentally the software. With *Enzymer*, we were able to design active pseudoknotted hammerhead ribozymes, as well as an active, and glucosamine-6-phosphate inducible, glmS ribozyme. With *Ribosoft*, we were able to target several RNAs, leading to efficient gene targeting with "extended hammerhead ribozymes". Finally, we created a database (*RNAstem*) to catalog stems and small RNA motifs to help retrieve relevant data on any small RNA motifs that could be found in any of the RNA structures in the database of RNA families (*Rfam*). This database allowed some interesting findings relative to switching mechanisms of riboswitches in general. It also showed promise to find motifs that would be usable as drug targets in noncoding RNAs.

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376 RNAcentral: The unified database of ncRNA sequences with comprehensive genome mapping and improved quality controls

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The RNAcentral database (http://rnacentral.org) is a comprehensive collection of all types of non-coding RNA (ncRNA) sequences across a broad range of organisms. RNAcentral integrates over 25 expert resources, such as miRBase, LNCipedia, HGNC, and Ensembl, to provide a faceted text search and sequence search across all databases. RNAcentral currently contains over 11 million ncRNA sequences and keeps growing.

To unify and reconcile annotations from different sources, RNAcentral implemented a new quality control procedure that annotates all RNAcentral sequences with RNA families from the Rfam database. The quality controls warn users about partial sequences, potential contamination, and misannotations. This allows the users to identify and exclude problematic sequences from search results. In addition, RNAcentral uses Rfam annotations to select or generate informative descriptions for ncRNA sequences and to assign RNA types based on Sequence Ontology to all sequences.

RNAcentral also maintains a mapping of all ncRNA sequences from key species to reference genomes, making RNAcentral one of the largest sources of genome-level ncRNA annotations. Even ncRNA sequences without annotated genomic locations or coming from non-reference assemblies are placed in a unified genomic context. The data are available in an integrated genome browser, a set of track hubs, and in multiple downloadable formats.

RNAcentral has begun to import functional information for ncRNA. So far we have included information about modified nucleotides from MODOMICS and PDB and a high quality set of tRNA structures from GtRNAdb.The RNAcentral website has been continuously improved with an updated text search interface and a feature viewer displaying Rfam annotations and modified nucleotides. We welcome feedback about the resource and invite new member database to join RNAcentral.

377 Elucidating the genetic heterogeneity of acute myeloid leukemia through the widespread profiling of splicing variations.

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RNA splicing is a fundamental cellular mechanism in charge of encoding genomic complexity among higher eukaryotes. Throughout myeloid differentiation, stage-specific regulation of RNA splicing is suggested to be coordinated by the differential expression of multiple splicing factors. Notably, myeloid malignancies such as acute myeloid leukemia (AML) have been shown to harbor a high frequency of somatic mutations in splicing factors, suggesting the widespread deregulation of splicing as a potential disease driver. I hypothesize that splicing deregulation is a layer of unexplored heterogeneity in AML, thereby contributing to the discrimination and classification of AML patient differences. I have begun developing an RNA-Seq data mining pipeline to identify common and distinguishing patterns of RNA splicing and gene expression in AML patient samples. Both annotated and novel spliced isoforms are being identified and quantified by using MAJIQ, a new computational framework developed by the Barash Lab.

In my initial analysis of 10 patient samples I find that measures of gene abundance do not capture the full scope of transcriptomic variations across AML patients. Commonly mutated genes in AML such as the FLT3 kinase, ZRSR2 splice factor, and the EZH2 epigenetic factor, show underlying RNA splicing differences among our patient cohort. Local splicing variations for these genes include annotated differential exon usage and de novo intron retention events, which are predicted to interfere with proper protein translation by introducing premature stop codons. These preliminary findings motivate the genome-wide discovery of potentially interesting splicing events and the elucidation of RNA-splicing mechanisms with the intent of shedding light into AML patient genetic heterogeneity. Moreover, I anticipate that the development of an efficient pipeline to survey the diversity of complex splicing events will motivate and guide the screening of splicing patterns as biomarkers in AML and other diseases.

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378 Discovery and characterization of putative non-coding RNAs regulating methylases

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In bacteria, the majority of known RNA regulatory elements are found in the 5'-UnTranslated Regions (UTRs) of mRNAs. These include elements such as riboswitches (RNA "receptors" that bind a metabolite to control downstream gene expression), as well as RNA binding proteins, such as ribosomal protein leaders. Our interest in studying UTR regions is to discover new noncoding RNA (ncRNA) regulating elements. Numerous ncRNAs are crucial regulators involved in many physiological processes in response to different conditions. We focus on ncRNAs associated with a specific function, our study aims to discover *de novo* ncRNAs in 5'UTRs upstream of genes involved in RNA modification. We hypothesize that self-regulation of certain genes that code for RNA-modifying enzymes may occur through their 5'UTR. We are particularly interested in RNA methylation. In fact, certain conserved structures upstream of RNA methylase genes have been reported in the literature (23S-methyl motif and MraW), their mechanism remains however unknown.

To reach our objective, we use bioinformatics tools to find interesting candidates. Afterwards, we study experimentally the potential candidates to confirm their role and mechanism of action. Thus, by using Ribogap (a database which allows simplified access to intergenic regions in prokaryotes) and the GraphClust pipeline (which allows to find conserved RNA structures) we selected a list of interesting motifs for more investigations. Some of our interesting candidates are being tested experimentally by using gene reporters to verify if they impact the expression.

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379 R2C2: Increasing Accuracy of MinION Sequencing Reads

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Oxford Nanopore Technology's (ONT) long-read MinION sequencer can sequence hundreds of thousands of long DNA molecules. ONT's main limitation however, is raw read accuracy, which is about 90%. As a result, ONT has developed 2D/1D2 library preparation protocols which make it possible to read both template and complement of a DNA doublestrand, which increases the read accuracy to about 93%. To further increase read accuracy, we propose our new method, called rolling circle to concatemeric consensus (R2C2), which uses rolling circle amplification (RCA) of circularized DNA. RCA produces long molecules containing repeats of the original DNA molecule. To analyse these long molecules, we have also developed an analysis pipeline called concatemeric consensus caller with partial order alignments (C3POa) using Smith-Waterman alignments to detect repeats, and partial order alignments to combine these repeats into a consensus sequence. Depending on how many repeats are read, consensus reads can reach accuracy greater than 99%.

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380 The Junction Usage Model (JUM): A method for comprehensive annotation-free analysis of tissue-specific alternative pre-mRNA splicing patterns

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Alternative pre-mRNA splicing (AS) greatly diversifies metazoan transcriptomes and proteomes and is crucial for gene regulation. Current computational methods of AS analysis from Illumina RNA-seq data rely on pre-annotated libraries of known splicing events, which hinders AS analysis with poorly annotated genomes and can further mask unknown AS patterns in tissues with complex AS modes. To address this critical bioinformatics problem, we developed a method called the Junction Usage Model (JUM) that uses a bottom-up approach to identify, analyze, quantitate and categorize global AS profiles without any prior transcriptome or genome annotations. JUM accurately reports global AS changes in terms of the five conventional AS patterns and a previously uncharacterized "Composite" category composed of inseparable combinations of conventional patterns. JUM stringently and accurately classifies the difficult and disease-relevant pattern of intron retention (IR), reducing the false positive rate of IR detection commonly seen in other annotation-based methods to near negligible rates. When analyzing AS in RNA-samples derived from Drosophila heads, human tumors and human cell lines bearing cancer-associated splicing factor mutations, JUM consistently identified ~ twice the number of novel AS events that were missed by other methods. Computational simulations showed JUM exhibits a 1.2-4.8 times higher true positive rate at a fixed cut-off of 5% false discovery rate. In summary, JUM provides a new framework and improved method that removes the necessity for transcriptome annotations and enables the detection, analysis and quantification of previously known and novel AS patterns in complex metazoan transcriptomes with superior accuracy.

381 Prospects for recurrent neural network models to learn RNA biophysics from high-throughput data

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Computational models of RNA secondary structure are well-developed but often fall short in making quantitative predictions of the behavior of multi-RNA complexes. Recently, large datasets characterizing hundreds of thousands of individual RNA complexes have emerged as rich sources of information about RNA energetics. Meanwhile, advances in machine learning have enabled the training of complex neural networks from large datasets. Here, we assess whether a recurrent neural network model, Ribonet, can learn from high-throughput binding data, using simulation and experimental studies to test model accuracy but also determine if they learned meaningful information about the biophysics of RNA folding. We began by evaluating the model on energetic values predicted by the Turner model to assess whether the neural network could learn a representation that recovered known biophysical principles. First, we trained Ribonet to predict the simulated free energy of an RNA in complex with multiple input RNAs. Our model accurately predicts free energies of new sequences but also shows evidence of having learned base pairing information, as assessed by in silico double mutant analysis. Next, we extended this model to predict the simulated affinity between an arbitrary RNA sequence and a reporter RNA. While these more indirect measurements precluded the learning of basic principles of RNA biophysics, the resulting model achieved sub-kcal/mol accuracy and enabled design of simple RNA input responsive riboswitches with high activation ratios predicted by the Turner model from which the training data were generated. Finally, we compiled and trained on an experimental dataset comprising over 600,000 experimental affinity measurements published on the Eterna open laboratory. Though our tests revealed that the model likely did not learn a physically realistic representation of RNA interactions, it nevertheless achieved good performance on test sets with the application of transfer learning and novel sequence-specific data augmentation strategies. These results suggest that recurrent neural network architectures, despite being naïve to the physics of RNA folding, have the potential to capture complex biophysical information. However, more diverse datasets, ideally involving more direct free energy measurements, may be necessary to train de novo predictive models that are consistent with the fundamentals of RNA biophysics.

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382 Transcription contributes modestly to the regulation of genome-wide differential microRNA expression in mammals

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Transcription initiates the cascade of gene expression and is often assumed to play a predominant role in determining how much gene products are ultimately expressed. The relationship between mRNA levels and protein levels has been studied extensively to reveal the degrees of transcriptional and post-transcriptional regulation of protein expression. How transcription globally controls the differential expression of non-coding RNAs, however, has received little attention. microRNAs (miRNAs) are a class of small, non-coding RNAs whose biogenesis involves transcription followed by extensive processing. Here, using hundreds of datasets produced from the ENCODE (Encyclopedia of DNA Elements) project we calculated the correlations between transcriptional activity and mature miRNA expression in diverse human cells, human tissues, and mouse tissues. While correlations vary among samples, most correlation coefficients are small. Interestingly, excluding miRNAs that were discovered later or weighting miRNA expression improves the correlations. Our results suggest that transcription contributes only modestly to differential miRNA expression at the genome-wide scale in mammals.

383 Mining the miRBase: what are we talking about when we talk about miRBase

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MicroRNAs are small non-coding RNA molecules commonly involved in the regulation of gene expression. Nowadays, small RNA Next-Generation Sequencing is the most common platform for detecting the presence of miRNAs and differences in their levels amongst conditions. Additionally, the technique has identified many novel miRNAs, as well as variants in existing miRNAs.

For studies seeking to identify changes in miRNA expression levels a miRNA reference is required. miRBase is the most widely used and generally considered to be the standard reference, with each miRNA entry containing genome location and sequence. miRBase is updated periodically, with each release including newly discovered miRNAs, modified entries and "dead" entries corresponding to incorrectly annotated miRNAs.

However, due to the magnitude of the annotation task, there can be significant delays between releases (the most recent update was June 2014). Thus, newly identified miRNAs may be missing and incorrect entries may be present. This can impact the identification of differentially expressed (DE) features, something that is not taken into account by most researchers who use miRBase.

To investigate these effects and to characterize miRBase, we examined each release from v9.2 to examine how the annotation has evolved, including dead entries in each release and how degeneracy has crept into the annotation. We found miRBase contains: (1) entries with different names but identical sequences; (2) entries with multiple annotated locations; (3) pre-miRNAs with extremely low estimated minimum free energy; (4) entries possessing reverse complementary; (5) entries with a polyA 3' end (which may impact sequencing with certain library kits and trimming).

We have generated curated annotation sets for the most recent miRBase releases. We show how these different annotations can generate distinct results in DE studies. These results are relevant to anyone performing miRNA studies using miRBase as their miRNA reference for mapping or annotation.

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384 Evolution of biomolecular innovations at the intersection of two RNA functions

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The diversification of life forms is underpinned by molecular innovations – new macromolecular structures and functions. The mechanisms by which molecular innovations arise through the process of evolution remains poorly understood. A systems view of innovation requires an understanding of genotype networks, which are networks of genetic sequences (genotypes) connected by mutational steps that maintain the same structure or function (phenotype). Innovations can occur at the intersection of two genotype networks. Here, we analyze the sequence and function of numerous RNA molecules (ribozymes) that lie on two different genotype networks. We find that the intersection of these two genotype networks is characterized by extensive functional overlap, such that over half the genotypes can catalyze both functions to some extent. We demonstrate through evolutionary simulations that this extensive enzymatic promiscuity enables rapid optimization of a new function. We also find that different starting points in a genotype network have consistently different rates of adaptation toward the new function. The results support models that invoke innovations before gene duplications, suggest that innovations are highly probable in regions where two genotype networks are proximal, and that the time needed for a population to discover an innovation may be predictable.

385 Activity-dependent modulation of cytoplasmic polyadenylation element binding protein 3 ribozyme

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The formation of stable memories plays an essential role in guiding behavior. During memory formation and encoding, changes in synaptic plasticity that encode behavioral experiences are involved in this process. Synaptic plasticity is a reflection of changes in strength of neural communication between axons and dendrites at synapses. Alternations in synaptic functions and synaptic transmission are critical to the storage of information in the central nervous system (CNS), thereby contributing to memory formation and memory consolidation. This process is dependent on the regulation of gene expression and *de novo* local protein synthesis. Cytoplasmic polyadenylation element-binding protein 3 (CPEB3), a translation regulator, has been suggested to facilitate neuroplasticity by modulating polyadenylation-induced mRNA translation through poly(A) tails elongation. *In vitro* selection from a human genomic library revealed a highly conserved mammalian sequence maps to an intron of *CPEB3* gene. Furthermore, a single nucleotide polymorphism (SNP) in the intronic sequence of CPEB3 is associated with human episodic memory. However, the role of the CPEB3 ribozyme in the mRNA maturation and translation in neurons remains to be fully elucidated. Using primary neuronal cultures, we demonstrated that CPEB3 ribozyme activity is correlated with the mRNA expression upon neuronal stimulation, suggesting this activity-dependent manner might contribute to neuroplasticity. Moreover, CPEB3 ribozyme activity is inhibited in the presence of antisense oligonucleotides. Future work will focus on knocking down CPEB3 ribozyme in mouse hippocampus and uncovering the role of CPEB3 ribozyme in memory formation.

386 Tandem hammerhead ribozymes in metazoan genomes give rise to abundant circRNAs involved in new types of retrotransposons

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The simple hammerhead ribozyme (HHR) is a small self-cleaving RNA motif found widespread in DNA genomes[1]. We previously reported that dimeric type-III HHRs in flowering plants are part of a new family of non-autonomous LTR retrotransposons, the *retrozymes*, which accumulate as RNA circles of ~700 nt and use the machinery of LTR retrotransposons for their mobilization[2]. In this work, we have found that tandem copies of canonical type-I HHRs in metazoan genomes belong to a new family of non-LTR retrozymes. Molecular analyses in somatic and germinal tissues of diverse animals from cnidarians to vertebrates show that these retroelements abundantly express as small (~300 nt) circRNAs, which should parasitize non-LTR retroelements. Bioinformatic mining of type-III HHRs in metazoan genomes revealed the presence of a new and highly conserved ribozyme motif in the genomes of some bees, bumblebees and other hymenopters. These novel type-III HHRs show a large pseudoknot interaction but low self-cleaving activity, and follow a dimeric arrangement similar to the one described for plant retrozymes. Moreover, these putative retrozymes of hymenopters also contain the typical motifs required for mobilization by LTR retrotransposons, such as the PBS (tRNATrp) and the PPT, and might produce circRNAs of ~700 nt. These similarities between plant and hymenopter retrozymes allow us to suggest that circRNAs with type-III HHRs may have followed events of horizontal transfer from pollinizing insects to plants, in a similar way as described for other arthropod retrotransposons[3,4]. In conclusion, our results indicate that tandem HHRs in eukaryotes give rise to abundant circRNAs, which show intriguing capabilities of crossing interspecies barriers.

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387 Fitness landscapes and genotype networks for understanding RNA evolution

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RNA has long served as a model of the genotype to phenotype relationship. Each RNA molecule has both a genotype in its nucleotide sequence and a phenotype in its structure or function. RNA molecules are also robust to mutations, meaning that some changes to the nucleotide sequence result in the same structure or function. Genotype networks are the collection of mutationally accessible genotypes that produce the same phenotype. The properties of genotype networks such as their size, connectivity, clustering and proximity to other genotype networks are critical for understanding RNA evolution, yet remain poorly characterized experimentally. Research in the Hayden Lab uses high-throughput sequencing to explore how genotype networks influence the evolution of RNA functions. We have studied several ribozyme systems to explore fundamental ideas of the mapping of genotype to phenotype. We have found that ribozymes can hide cryptic genetic variation that is phenotypically revealed only upon environmental change, and that certain modular RNA structures may facilitate this process. We have found that the two different genotype networks of two distinct ribozymes show substantial overlap that can facilitate evolutionary innovation. We have also developed computational simulations that allow populations of RNA molecules to evolve on our experimental genotype networks. These simulations enable an investigation of how genotype networks constrain or facilitate evolution, and lead to predictions that can be further tested in the lab. Future work will search for more genotype network intersections, explore properties of genotype sequence space over large distances, and continue to explore the role of environmental change in evolution. It is our hope that this research program will lead to general conclusions about the nature of genotype networks of evolving systems that are applicable to many levels of biological organization, and contribute to more predictable, designable and evolvable genetic systems.

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388 Directed Evolution of a Novel L-Ribonuclease Ribozyme against HIV-1 D-TAR Element

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Besides carrying genetic information in its primary sequence, ribonucleic acid (RNA) also participates in complex physiological processes by virtue of its *structure.* RNA is structurally very dynamic and can fold onto itself to form stable three-dimensional motifs *via* both secondary and tertiary interactions. This facilitates interaction of RNA with nucleic acids, proteins, metabolites and other cellular components, thus making RNA indispensable for biochemical processes such as transcription, translation, splicing, cellular localization and turnover. This structure-function correlation makes structured RNA a very attractive therapeutic target. Standard strategies for targeting RNA such as antisense technology and RNA interference (RNAi), rely entirely on Watson-Crick (WC) base pairing for sequence recognition rather than targeting its unique structural "signature". This can result in off-target hybridization due to partial sequence complementarity. L-nucleic acids - the enantiomeric form of naturally occurring D-nucleic acids - preclude canonical WC based interactions with the native nucleic acids. Thus, use of cross-chiral nucleic acids (L-DNA/L-RNA) based strategies help circumvent toxicity arising from off-target effects. Here, we report a new class of L-ribonuclease ribozyme developed *via* directed evolution, that recognizes and cleaves the structured RNA element, the HIV-1 D-TAR hairpin, independent of hybridization. The TAR hairpin is crucial for the viral replication and thus serves as an ideal physiologically relevant structured RNA target. Though RNA strand scission reaction is one of the most commonly catalyzed reactions by both physiologically occurring ribozymes and artificially evolved deoxyribozymes, canonical WC base-pairing is still the mode of substrate recognition in these cases. Our L-ribozyme however, is incapable of forming contiguous WC base pairing interactions with the native RNA target and recognizes the target through tertiary interactions only. This implies higher sensitivity and specificity in ribozyme activity. Also, since L-nucleic acids are nuclease resistant, evolution of such a L-ribonuclease ribozyme has potential impact in therapeutics as alternative RNA silencing agents.

389 Specificity for adenine is established during the hydrolytic cycle of poly(A)-specific ribonuclease (PARN)

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Poly(A)-specific ribonuclease (PARN) is a processive poly(A) degrading exoribonuclease. It degrades poly(A) tails present on a subset of mRNAs and noncoding RNAs, including among others certain snoRNAs, miRNAs and precursor rRNAs. Human patients with genetic lesions in PARN suffer from a spectrum of syndromes called telomere biology disorders (TBD), which are associated with short telomeres. Here, we have investigated molecular mechanisms behind PARN's preference to degrade poly(A). We have used enzyme kinetics and divalent metal ions as mechanistic probes to show that PARN's poly(A) specificity is tightly linked to a translocation event during the hydrolytic cycle of PARN action. We postulate that a catalytically essential divalent metal ion participates in communicating the identity of the base to the hydrolytic activity at the scissile phosphodiester bond. Given that PARN acts processively the coordination site for this divalent metal ion will be reorganized for each round of hydrolysis. In conclusion, our study establishes a mechanistic link between PARN's processive mode of action, hydrolytic activity and preference for degrading poly(A).

390 Probing the Cpf1-crRNA Interaction with Ribochemical Modification

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Clustered regularly interspaced short palindromic repeat (CRISPR) is a bacterial and archaeal adaptive immune system. The core enzymes of CRISPR are RNA-guided endonucleases that sequence-specifically cleave foreign double-stranded DNA. Several classes of these enzymes exist and are being adapted for biotechnology, such as genome engineering. Cpf1 is a type V CRISPR-associated (cas) enzyme that naturally uses only one guide RNA, in contrast to the type II CRISPR-Cas9 enzymes. Thus, Cpf1 may represent a simpler and more practical tool for applications like gene editing or therapeutics. To better understand the functional requirements for Cpf1-crRNA interaction and develop modified crRNAs suitable for synthetic biology and therapeutic applications, we are performing mutagenesis and chemical modification of the crRNA. We have focused on the protein-interaction motif of the crRNA. We are incorporating base changes and chemical ribose modifications at the 2' position that alter hydrogen-bonding capacity, sugar pucker, and flexibility. Chemical modifications include 2'-deoxy, 2'-fluoro, 2'-*O*-methyl, and 2'-fluoro arabinonucleic acid. Crystal structures of Cpf1 are being used to place modifications and test requirements for RNA flexibility, 2'-hydroxyl polar contacts, A-form helical conformation, and pseudoknot stability. Biochemical endonuclease activity, gene editing efficiency, Cpf1 binding affinity, and ribonucleoprotein (RNP) stability will assess the tolerance and effects of modification. Characterizing structure-function requirements for Cpf1-crRNA interaction will facilitate better design and tuning of Cpf1 enzymes.

391 The m^{6}A RNA methyltransferase METTL16 targets the U6 snRNA, various non-coding RNAs and pre-mRNAs

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N^6-methyladenosine (m^{6}A) is a highly dynamic RNA modification that has emerged as a key regulator of gene expression as it can be recognised by specific m^{6}A binding proteins ("readers") and can be reversed by the action of the demethylases ("erasers") ALKBH5 and FTO. A methyltransferase complex containing METTL3 and METTL14 has been identified as a human m^{6}A "writer" that introduces modifications within a characteristic RRACH motif (R = A or G; H = A, C or U). However, transcriptome-wide mapping of m^{6}A sites revealed that many such modifications lie within different sequence contexts, suggesting that they are introduced independently of this complex and that additional m^{6}A methyltransferases remain to be identified in human cells. Based on its homology to the *E. coli* rRNA m^{6}A methyltransferase YbiN, METTL16 represents a putative human m^{6}A writer protein and using crosslinking and analysis of cDNA (CRAC), we identify METTL16 target RNAs in human cells. Our data reveal that METTL16 predominantly binds to the U6 snRNA, which forms the catalytic core of the spliceosome. The methylation target of METTL16 is A43, which lies within an evolutionarily conserved ACAGAGA box of U6. This sequence base-pairs with 5' splice-sites of pre-mRNAs, implying that METTL16-mediated modification of this site is important for splicing regulation. Identification of METTL16 interaction partners revealed RNA-dependent interactions with La, LARP7 and the capping enzyme MEPCE, demonstrating that METTL16-mediated modification occurs on an oligouridylated precursor form of U6 during early stages of assembly of the U6 snRNP. We further identify various (pre-)mRNAs, including *MAT2A* that encodes the S-adenosylmethionine synthetase, and (long) non-coding RNAs, such as MALAT1, XIST, 7SK and the vault RNAs as additional METTL16 targets. We show that, in contrast to the METTL3-METTL14 complex that typically installs modifications in proximity to stop codons, METTL16 predominantly associates with the introns of pre-mRNAs, suggesting that these two enzymes are responsible for installing distinct subsets of m^{6}A modifications that have different cellular functions. The identification of METTL16 as an active m^{6}A methyltransferase in human cells expands our understanding of the mechanisms by which the m^{6}A landscape is installed on cellular RNAs.

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392 Pseudouridine synthase 7-like protein PUS7L is required for infection by hepatitis C virus

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Pseudouridine is the most abundant RNA modification in human cells. The conversion of uridine to pseudouridine is carried out by pseudouridine synthase (PUS) proteins, which results in a complex, regulated pseudouridine landscape across a diverse range of cellular RNAs, including messenger RNAs (Carlile et al. Nature 2014). A recent screen identified the uncharacterized human PUS protein, PUS7L, as an important host factor in the life cycle of hepatitis C virus (HCV) (Marceau et al. Nature 2016), an unexplained requirement that we have validated in our laboratory. Pseudouridine has been shown to affect RNA structure, RNA-protein interactions, and cellular recognition of foreign RNA by innate immune sensors - all of which have the potential to significantly impact viral infection - but the RNAs targeted by PUS7L and the pseudouridine landscape of infected cells remain unexplored. Here, we investigate the roles of pseudouridine and PUS7L in the HCV life cycle. We have interrogated the HCV genome for the presence of pseudouridine and identified 43 novel sites including a conserved uridine in a functionally important domain of the IRES. Additionally, we have detected 601 pseudouridines occurring throughout the host transcriptome, including 240 novel sites in mRNA. Work is ongoing to comprehensively quantify changes to pseudouridylation patterns in the host transcriptome following HCV infection and assign each pseudouridine of interest to the activity of specific PUS proteins. This work provides the first evidence for pseudouridylation of the genome of a human pathogenic virus and establishes a role in the HCV life cycle for a previously uncharacterized PUS protein, PUS7L.

393 Relationship between Structure and Function in the Archaeal Box C/D Complex

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2'-O-methylation of RNA is a common modification that results in increased regional rigidity and thus plays a role in RNA folding and function. In eukaryotic and archaeal organisms, this methylation can be accomplished either by a single protein or by a Box C/D ribonucleoprotein (RNP) complex. The archaeal Box C/D complex is assembled from three proteins, Fibrillarin, Nop5, and L7ae, and a guide RNA, which work together to recruit the target RNA. S-adenosyl methionine is the methyl donor in the reaction. Study of the structure of these proteins and of the interactions among components of the complex, will help us better understand the operation of this complex.

Haloferax volcanii, a halophilic archaeal species, is used here to test, in-vivo, a variety of mutations in Fibrillarin and Nop5, to understand the role of structural components of these proteins in the complex. Mutant genes to produce alanine-substituted proteins were introduced into strains lacking the native proteins. The ability of these mutants to produce 2'-O-methylation at known rRNA or tRNA sites was determined by limited-dNTP-mediated reverse transcription reactions. Mutants were selected based primarily on homology modelling and literature review. Mutants that did not allow modification include those that targeted the Fibrillarin-Nop5 interaction, and those that inhibit Nop5's ability to bind and align the guide RNA.

394 Enzymatic properties of Vaccinia RNA capping enzyme

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Eukaryotic messenger RNA carries a unique structure at its 5' end referred as the 5' cap. As the mRNA is being transcribed, RNA capping enzyme, while bound to the transcribing RNA polymerase II, catalyzes a multi-step reaction to turn the 5' triphosphate group into a cap structure where a N7-methylated guanosine nucleoside is linked to the 5' triphosphate at its 5' position. The cap structure, m7Gppp group, is essential for the majority of ribosomal translation of mRNA and plays a major role in innate immunity against foreign RNA of the cells. Hence, the generation of capped RNA is essential for the production of biologically functional mRNA for research and therapeutic applications. Here we present our findings of the enzymatic properties of vaccinia RNA capping enzyme (VCE), the widely used enzyme to generated capped RNA in vitro. We found that VCE binds to ssRNA with high affinity, and to dsRNA with lower but sufficient affinity for RNA capping to take place. In addition, loading of ssRNA or dsRNA stimulates VCE's triphosphatase activity (the first enzyme activity of RNA capping.) We also found that VCE accepts 3' modified GTP as cap donor, therefore facilitating the modification of 5' triphosphate or diphosphate RNA by affinity tag such as desthiobiotin for enrichment or fluorescent group such as FAM for visualization.

395 Genome-wide analysis of RBP targets in plants

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Alternative splicing (AS) generates different transcripts from the same precursor mRNA (pre-mRNA) by selecting distinct splice sites. It largely increases complexity of transcriptome and provides additional layer of gene regulation. Splicing regulators, which are mainly RNA binding proteins (RBPs), are the major players recruited by regulatory cis elements on pre-mRNA either activate or suppress selection of splice site and assembly of spliceosome to control AS. Modulating the activities of these factors may change their selectivity for RNA targets that directly or indirectly alter global AS events and further affect biological processes in the cell. Therefore, identification of RNA targets and dynamic interactions between these splicing factors and RNAs allows us to explore potential functions of these factors in regulation of AS. We established a method called targets of RNA binding proteins identified by editing (TRIBE) that can detect RNA targets of RBPs globally. The method allows us to identify RNA targets of splicing factors by using small amount of cells without complex biochemical processes for plant systems. We further developed a data analysis pipeline that allow to identify dynamic interactions between RBPs and their RNA targets in response to environmental changes. The system will be the first quantitative method in plant research to globally identify RBP-RNA interactions differentially regulated in different cell types or by environmental factors. By taking the polypyrimidine tract binding protein (PTB) as an example, we showed this method can be adopted in plant systems for us to compare global changes of RBP-targeted transcripts during environmental changes. Detailed results will be discussed.

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396 An orphan 3'-5' polymerase implicated in noncoding RNA processing.

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Until the discovery of $tRNA^{His}$ guanylyltransferase (Thg1) from *Saccharomyces cerevisiae* (Sc), nucleotide polymerization was believed to exclusively occur in the 5'-3' direction. Thg1 shifts this paradigm by catalyzing the non-templated addition of a required guanosine to the 5' end of tRNAHis in a 3'-5' direction. Enzymes exhibiting similarity to ScThg1, called Thg1-like proteins (TLPs) catalyze a Watson-Crick dependent 3'-5' polymerization. TLPs have been found in all three domains of life, including eukaryotic organisms such as *Dictyostelium discoideum* (Ddi). However, the roles and mechanisms of TLPs compared to their relatively more well-studied Thg1 counterparts are less understood.

Previous work by our group has demonstrated the functions of two TLPs in the model eukaryote *D. discoideum*. These TLP enzymes, DdiTLP2, and DdiTLP3 catalyze a Watson-Crick dependent 3'-5' polymerization, and are responsible for mitochondrial tRNAHis maturation, and mitochondrial tRNA 5'-editing, respectively. However the biological function of a third TLP enzyme encoded in *D. discoideum*, DdiTLP4, remains unknown. In vitro studies suggest DdiTLP4 can act on several small, noncoding RNAs (ncRNA) in addition to tRNAs. Moreover, depletion of DdiTLP4 causes a severe growth defect in *D. discoideum*. Now we have the first evidence to suggest that the essential function of DdiTLP4 is due to its role in small RNA processing and its activity on specific ncRNA substrates. Depletion of DdiTLP4 followed by RNA-Seq was used to identify in vivo substrates of DdiTLP4 and enabling the identification of any type of RNA whose 5'-end sequence is altered in the absence of DdiTLP4 activity. This work comprises the first comprehensive insight into 3'-5' polymerase substrate specificity, including into non-tRNA related activities associated with these enzymes. Furthermore, investigation into the biological function of DdiTLP4 has provided greater understanding of the 5'-end maintenance machinery of eukaryotes and into diverse biological roles for 3'-5' polymerization.

397 Roles in rRNA methylation for the C-terminal domain of erythromycin resistance methyltransferases, members of the rRNA-Adenine Dimethylase family

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rRNA is extensively post-transcriptionally modified in all organisms. Ribosome maturation and efficient translation depend on rRNA methylation as does resistance to protein-synthesis targeting antibiotics in some bacteria. We have investigated the role of the non-catalytic C-terminal domain of erythromycin resistance methyltransferases in driving specific methylation of A2058 of 23S rRNA by the catalytic Rossmann-fold methyltransferase domain in these enzymes. We are investigating whether there are idiosyncratic or conserved mechanisms for specific methylation across erythromycin resistance methyltransferases. Our studies also provide insights broadly into the mechanisms of methylation by proteins in the rRNA-Adenine Dimethylase family, which are involved in ribosome maturation in all three domains of life.

398 TRMT1-catalyzed RNA modifications modulate translation to ensure proper proteostasis and neurodevelopment

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Here, we present our new findings that link key cellular and developmental processes to tRNA modifications catalyzed by the human tRNA methyltransferase 1 (TRMT1) enzyme. Intriguingly, mutations in TRMT1 have been identified as the cause of autosomal-recessive intellectual disability (ID) in certain human individuals but the molecular basis for this disorder remains enigmatic. Through gene editing to generate TRMT1-deficient human cells, we have recently shown that TRMT1 is responsible for catalyzing the dimethylguanosine (m2,2G) base modification in nuclear and mitochondrial-encoded tRNAs (Dewe et al. 2017). TRMT1-knockout cells are deficient in m2,2G modifications in tRNA, exhibit decreased global protein synthesis and display perturbations in redox homeostasis. Furthermore, we have performed transcriptomics and proteomics on the TRMT1-knockout cells to determine the cellular pathways that require TRMT1-catalyzed tRNA modifications. Through these global approaches, we have discovered an unanticipated role for m2,2G modifications in modulating proteostasis and neuronal differentiation. Our results provide a blueprint for unraveling the developmental networks that are dependent upon TRMT1-catalyzed tRNA modifications and their connections to cognitive disorders.

399 PseudoU writers of the Epitranscriptome: Identification and Characterization of Pseudouridine synthases

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Throughout all kingdoms of life, pseudouridine (Y) modifications of tRNAs, rRNAs and snRNAs are among the most abundant RNA modifications. In bacteria, archaea, and eukaryotes, this fairly simple conversion of uridine to Y is synthesized by unguided, probably stand-alone pseudouridine synthases (PUS). While the function of Y modifications in other RNA classes has been extensively studied, little is known about the role of Y in mRNA. However, latest findings that alterations in mRNA modifications are associated with multiple human diseases and cancers, together with the therapeutic use of Y and other mRNA modifications recently put the spotlight on the role of modifications in mRNA. In our lab, we concentrate on the identification and characterization PUS enzymes and their role in pseudouridinylation of mRNA. For this purpose, we designed, expressed, and purified multiple archaeal and eukaryotic PUS enzymes. We find that while some PUS variants show a high substrate specificity for tRNAs and other known natural targets, several of our PSU enzymes are able to efficiently introduce Y into various mRNA templates in vitro. We are in the process of determining the substrate features that define PUS binding sites on mRNA, as well as protein engineering to optimize our current PUS variants. Our research is aimed at understanding the role of Y mRNA modifications, the enzymes that are involved in writing the modification and believe that our work will provide enzymatic approaches to targeted mRNA modifications.

400 Withdrawn

401 The role of APOBEC3 proteins in renal cell carcinoma

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APOBEC3 proteins belong to the **AID/APOBEC** (activation-induced cytidine deaminase/apolipoprotein B mRNA editing enzyme, catalytic polypeptide) protein family, a class of Zn-dependent cytidine deaminases. In humans, there are seven members of the **APOBEC3 (A3)** protein family (A3A-D and A3F-H) which have evolved through a complex history of gene duplications (*Conticello et al. 2005*). **A3** proteins have the ability to bind single-stranded DNA/RNA. Consequently, **A3** proteins play an important role in human health and genomic stability by hypermutating and thereby restricting viral propagation or retrotransposition. Furthermore, **A3** proteins can act as mutator of genomic DNA if the expression is deregulated (*Knisbacher et al. 2016*). Indeed, **A3** proteins were implicated to act as mutators in multiple cancer types and have been linked to poor outcome (*Salter et al. 2016*). While the antiviral function of **A3** proteins is well studied, the contribution of **A3** to cancer needs to be determined.

By using publicly available datasets (TCGA), a significant upregulation of **A3C/A3G** has been detected in renal cell carcinoma (RCC). Kidney cancer is among the 10 most common cancers in western countries (*Ljungberg et al. 2011*). However, treatment especially of metastasizing RCC remains challenging, since these tumors are highly resistent to conventional antitumor therapies (chemotherapy and radiotherapy). RCC encompasses several histological subtypes with the highest incidence of clear cell RCC (ccRCC, *Motzer et al. 2009*). Analyzing primary tissue samples confirmed an upregulated expression of **A3C/A3G**, especially in ccRCC.

Since the physiological role of these proteins has not been analyzed in kidney cancer cells so far, we aim at determining the function of **A3** proteins in the ccRCC-derived cell lines 786-O and 769-P. Preliminary results suggest that **A3C/A3G** promote the expression of mRNAs that are usually expressed in the hematopoietic system (e.g. LPXN and RAC2). Therefore we hypothesized that **A3** proteins could contribute to kidney cancer cell properties by promoting the expression of immunologically relevant genes, which is a hallmark of kidney cancer. Accordingly, we also aim at characterizing the molecular features associated with **A3** genes (promoter, 3' UTR) to evaluate **A3** proteins as a novel target for treatment of metastasizing ccRCC.

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402 Maize Active RNA Editing Complexes Include PPR, DYW, OZ, RIP/MORF, and ORRM Proteins

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Most land plants modify the primary sequence of mRNAs in mitochondria and chloroplasts through cytidine to uridine (C-to-U) RNA editing. Editing of RNA bases has been linked to proteins with extensive N-terminal pentatricopeptide repeat (PPR) sequences and a C-terminal DYW deaminase domain. The DYW domain is thought to be the enzymatic component of the RNA editing complex, though it also has been associated with specific RNA cleavages in vivo. Additional proteins from the OZ, RRM, and RIP/MORF families also are thought to be components of the editosome. Unfortunately the set of proteins that form an active editosome is unknown, and recombinant proteins encoding the DYW deaminase have yet to be shown to possess deaminase activity. We have observed very high levels of editing activity in vitro using maize chloroplast extracts compared to other published reports for other species. We have fractionated active extracts using size exclusion chromatography and immunoprecipitation to investigate the nature of the active complex. We have discovered that proteins orthologous to Arabidopsis editing factors are present in fractions that correlate with activity, suggesting they are part of a large complex. We have used a reverse genetic approach to confirm the criticality of the putative RNA editing factor OZ1. We have also used the extracts to screen for inhibitors based on inferences about the RNA editing mechanism and the suspected transition state. A lack of inhibition by known transition state analogs of related enzymes hint at differences in substrate recognition for the DYW-deaminase compared to other nucleotide deaminases.

403 Structural and functional features of H/ACA ribonucleoprotein complexes from different species

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H/ACA RNPs catalyze pseudouridine formation in ribosomal and spliceosomal RNAs. Between different organisms, these complexes vary in their protein composition, the number of guide RNA hairpins, and the presence of specific protein domains. As current structural knowledge however only comprises the highly conserved core organization of the H/ACA RNP, we developed approaches to functionally and structurally characterize species-specific domains, and to compare conserved features between H/ACA RNPs from Pyrococcus furiosus and Saccharomyces cerevisiae.

We have developed and established pipelines to site-specifically label proteins and RNA to be reconstituted into catalytically active H/ACA complexes for single molecule FRET spectroscopy. We use a combination of biochemical tools and single molecule FRET spectroscopy to characterize the assembly of complexes. We investigate the differences between the requirement of L7Ae or Nhp2 for archaeal and yeast complexes, respectively. Furthermore, we assess the binding of substrate RNA to the complex and analyze differences in the structural response of the complex towards this binding event.

With respect to the species-specific number of hairpins present within the H/ACA RNA, we find that there is a correlation between this number and the presence of certain domains within the H/ACA complex. We characterize these domains structurally and functionally, and try to establish links between species-specific structural and functional features of H/ACA RNPs.

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404 Structual and functional study of tRNA 2'-phosphotransferase Tpt1p in *Saccharomyces cerevisiae*

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In yeast, tRNA splicing is catalyzed stepwise by specific enzymes. The endonuclease initiates intron excision of the pre-tRNA, yielding 5’ and 3’ half-RNAs containing 2′, 3′-cyclic phosphate and 5’-hydroxyl, respectively. Subsequently, the tRNA ligase joins the two-half RNA fragments, generating a ligated tRNA molecule with a 2′-phosphate at the splice junction. Finally, the 2’-phosphotransferase Tpt1p transfers the 2′-phosphate from tRNA to NAD, producing ADP ribose 1’-2’ cyclic phosphate and mature tRNA. Tpt1p is essential in yeast, since its deletion is lethal. Tpt1p belongs to a family of 2’-phosphotransferases that are found conserved from *E. coli* to human, despite their functions are unclear (such as: the 2’-phosphotransferase activity is not required in *E. coli*). To understand the structural and functional basis of Tpt1p in enzyme activity specificity, we have tried to crystallize Tpt1p protein and solved the crystal structure of the C-terminal domain of Tpt1p at 1.45 angstrom resolution. The structure of Tpt1p-C shows to be conservative to the 2’-phosphotransferase from *A. pernix*, which forms globular structure containing helix-turn-helix connected to two antiparallel β-sheets. In our structure of Tpt1p-C, NAD, the receptor of 2′-phosphate, localizes in the core of the β-sheets, clarifying this region as a functional domain for substrate recognition. Besides, by using biochemical assays, we have determined the binding of Tpt1p and 2′-phosphorylated tRNA substrates in vitro. Our result suggests that the hairpin structure of the tRNA is important for Tpt1p binding.

405 Roles of Fragile X proteins in RNA editing regulation

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Adenosine-to-inosine (A-to-I) editing is a highly prevalent post-transcriptional RNA modification mediated by ADAR enzymes. A-to-I editing not only diversifies gene expression but also helps to distinguish self from non-self RNA. Although recent studies made it possible to identify trans-acting factors of RNA editing, the extent to which other trans-acting factors are involved and how the editing is regulated are incompletely understood. Here we report new regulators of A-to-I editing, discovered by combining bioinformatics and biochemistry approaches. We found that Fragile X mental retardation protein (FMRP) biochemically interacts with ADAR1 and ADAR2 proteins, respectively. Intriguingly, we also observed protein interaction between ADAR1 and Fragile X-related protein FXR1P, but not between ADAR2 and FXR1P. In addition, we curated an extensive set of FMRP and FXR1P targets and observed that different editing sites are involved in distinct target-specific regulation by the two Fragile X proteins. FMRP appeared to regulate site- selective editing sites and FXR1P often acted on promiscuous sites in long double- stranded RNA (dsRNA) structures. These results link the Fragile X proteins with the RNA editing pathway and suggest that these proteins are novel regulators of ADAR activity.

406 Components of human mitochondrial RNase P act together to bind, methylate and process mitochondrial pre-tRNAs

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Human mitochondria contain an essential three component RNase P enzyme complex (mtRNase P) that cleaves the extra 5' end of precursor mitochondrial tRNAs ((mt) pre-tRNA). The catalytic unit of mtRNase P is Mitochondrial RNase P Protein 3 (MRPP3) that forms a functional complex with MRPP1 and 2. The MRPP1/2 sub-complex, a sub-component of mtRNase P, is responsible for the methylation of the 9th base of (mt)tRNAs. MRPP1 catalyzes the S-adenosyl-methionine (SAM) dependent methyl-transfer reaction, while MRPP2 serves as a scaffold protein. mtRNase P is associated with several human mitochondrial diseases and its key role in mitochondrial function is emerging. Despite its importance in human mitochondria, it is largely unknown how the different mtRNase P components work together to interact, process and modify its natural substrates, mitochondrial pre-tRNAs. Hence, we investigated how mtRNase P exerts its functions using six *in vitro* transcribed pre-tRNAs. We found that mtRNase P is able to bind, process and methylate its natural substrates. However, not all investigated pre-tRNAs were efficient substrates for mtRNase P. Since some were predicted to be *in vivo* substrates for mtRNase P, we speculated that they may require additional factors in the mitochondria so as mtRNase P can bind and process them efficiently. In agreement, we found that the presence of SAM enhances mtRNase P binding and processing for some substrates. Additionally, while we found that the methyltransferase and pre-tRNA processing functions of mtRNase P were mostly independent from each other, we also noted a modest enhancement of methylation of mitochondrial pre-tRNA$^{Leu(UUR)}$ in the presence of MRPP3 suggesting that the different functions of mtRNase P are not entirely de-coupled. Overall, this is the first comprehensive study on how the components of mtRNase P act together to cleave and methylate pre-tRNAs in human mitochondria.

407 Deciphering effects of *Adar* on *Drosophila* metamorphosis.

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One of the most prevalent type of RNA editing is the conversion of adenosine to inosine in double-stranded RNAs that is mediated by adenosine deaminases acting on RNA (ADAR) enzymes. A→I RNA editing can lead to a codon change as the nucleoside inosine (I) is interpreted as guanosine (G) by ribosomes, resulting in a diversification of protein function.

The ADAR family of proteins is present in all metazoans. In *Drosophila*, a single *Adar* is present on the distal X chromosome and is an orthologue of vertebrate *ADAR2*. In spite of major progress in the identification of editing sites, little is known about the regulatory mechanism of ADAR proteins in normal development and in disease.

In this present study, we performed a genetic screen that has uncovered a novel effect of *Adar* on ecdysone signaling which is a crucial regulator of *Drosophila* development. Ubiquitous expression of *Adar* with the *act*5c-Gal4 driver results in pupal lethality with defects in ecdysis and head eversion. The lethality caused by ubiquitous expression of *Adar* can be rescued by blocking ecdysone synthesis and signaling.

Tissue specific over-expression of *Adar* in the Prothoracic Gland (PG) with *phm*-Gal4 causes extended larval life with a long delay in pupation, with major reductions in prothoracic gland transcripts encoding the enzymes of ecdysone synthesis and signaling. These defects may be due to either aberrant RNA editing or RNA binding by ADAR protein. We hypothesize that *Adar* expression in *Drosophila* is a prerequisite to regulate ecdysone signaling during metamorphosis.

Currently, we are dissecting regulation of the ecdysone pathway by *Adar* and pursuing loss of functions studies with *Adar* RNAi lines to decipher the role of *Adar* in metamorphosis of *Drosophila.*

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408 Surveillance of hypomodified tRNAs promotes bacterial tRNA quality control

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Transfer RNA (tRNA) is heavily decorated with diverse post-transcriptional modifications (tRNA modification). tRNA modification fine-tunes its structure and functionality, but the physiological function of several modifications remains undefined. Here, we focused on 4-thiouridine (s^4U), a sulfurated uridine derivative found in the body region of bacterial and archaeal tRNAs. A genome-wide screen had revealed that *thiI*, which encodes the enzyme that synthesizes s^4U, is important for the fitness of *Vibrio cholerae* (the cholera pathogen) in an animal infection model; however, the mechanism by which s^4U promotes bacterial fitness is unclear. s^4U is reported to raise the thermodynamic stability of tRNA in solution, but there had been no experimental evidence that s^4U promotes intracellular stability or functionality of tRNA. Here, comprehensive quantification of *V. cholerae* tRNAs revealed that the abundance of a subset of tRNA species was decreased in a Δ*thiI* strain. Multiple mechanisms, including rapid degradation of a subset of hypomodified tRNAs, accounts for the reduced abundance of tRNAs in the absence of *thiI*. Through transposon insertion sequencing, we identified additional tRNA modifications that promote tRNA stability and bacterial growth. Whole genome sequencing of suppressor mutants lacking multiple tRNA modifications suggests that degradation of hypomodified tRNAs is mediated by the RNA degradosome. Together, these observations support the existence of a previously unrecognized bacterial tRNA quality control system in which hypomodification sensitizes tRNAs to RNA degradosome mediated decay. Finally, we also found that the Δ*thiI* strain entered a dormant state when competed with WT cells, suggesting that the absence of tRNA modification triggers dormant cell formation. The pathway by which tRNA hypomodification leads to dormant cell formation requires further elucidation, but likely reflects a general link between translation deficiency and dormant cell formation.

409 Expression and function of an oxidative stress induced RNA repair system in *Escherichia coli*

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Oxidation of their DNA and proteins may lead to cell death and associated repair systems play major roles in bacterial physiology and virulence. Far less well studied and understood is how cells manage oxidative damage to RNA. The Rtc RNA repair system of the model organism and putative pathogen Escherichia coli consists of the universally conserved RNA cyclase RtcA and RNA ligase RtcB, and their transcriptional regulator RtcR; the latter being modulated by a putative RNA binding, amino-terminal CRISPR-associated Rossmann fold (CARF) domain. We aim to investigate the transcriptional regulation and the function of the Rtc system under oxidative stress using an *E. coli* Hpx$^-$ (*katG katE ahpCF*) mutant, lacking enzymes to scavenge hydrogen peroxide. A reporter based assay revealed increased rtc promoter activity in the Hpx$^-$ mutant when compared to the wild-type, with maximal levels of induction observed at 24 hours. The growth of the Hpx$^-$ mutant is impaired under aerobic conditions and this effect is more prominent when the rtc genes are not expressed as demonstrated by RT-qPCR. RNA sequencing experiments are currently in progress to investigate the transcriptome of the Hpx$^-$ mutant under conditions where the Rtc system is and is not active, revealing an effect on non-protein coding RNA in the case of an inactive Rtc system. Additionally, through the use of RtcB dependent adapter ligation followed by RNA sequencing, potential RNA targets of the RtcB ligase were identified; the majority were classified as non-protein coding transcripts such as ribosomal RNAs and small regulatory RNAs, further supporting the role of the Rtc system in ribosome homeostasis. Finally, putative physical interactions among the three Rtc proteins were explored, in vitro using gel filtration and in vivo using a two hybrid system, indicating that the Rtc proteins may form a dynamic and discrete regulatory - RNA repair complex. Together, our data suggest that the Rtc RNA repair system is part of a previously unrecognized adaptive response to oxidative damage to RNA.

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410 tRNA processing in human mitochondria

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The majority of mitochondrial diseases are linked to mutations in mitochondrial tRNAs, however how these mutations contribute to disease is unclear. Interestingly, most of the disease-linked tRNA mutations are not in the anti-codon region of the tRNA suggesting that these mutations impact processes other than translation in the tRNA life-cycle, such as tRNA charging or maturation. Here, we investigate how patient mutations in precursor tRNAs (pre-tRNAs) alter tRNA structure and impair pre-tRNA recognition by the three-component mitochondrial RNase P complex (mtRNase P) responsible for tRNA 5' end maturation. While there is a growing appreciation for the importance of the mtRNase P in human health, the mechanism of how human mtRNase P processes pre-tRNAs has not been established.

We investigated the binding and processing by mtRNase P of 11 human pre-tRNAs containing disease-causing point mutations. We find that only a subset of mutations influence substrate recognition, however all mutations impacted the rate of tRNA processing by mtRNase P. Most of the investigated mutant tRNAs also showed altered UV melting profiles suggesting that mutations may influence the folding and structure of pre-tRNAs. Additionally, we unexpectedly identified a sub-set of native human pre-tRNAs, (e.g. pre-tRNA^{Val} and pre-tRNA$^{Ser(UCN)}$ that do not appear to be efficient substrates for mtRNase P *in vitro*, leading us to propose that some pre-tRNAs may require further processing/modification(s), or an additional as of yet unidentified protein partner in order to be processed by mtRNase P. Consistent with this idea, we found that pre-tRNA^{Val} is bound and processed more efficiently in the presence of S-adenosyl methione (SAM). Taken together, our results shed light on the role of the mtRNase P and implicate its importance in the context of human health and mitochondrial diseases.

411 RNA editing is important for worm development and regulates RNAi

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A-to-I RNA editing is a conserved and widespread phenomenon in which adenosine (A) is converted to inosine (I) by adenosine deaminases (ADARs) in double-stranded RNA molecules. Although human RNAs contain millions of A-to-I editing sites, most of these occur in noncoding regions and their function is unknown. Knockdown of ADAR enzymes in *C. elegans* causes defects in normal development but is not lethal as in human and mouse, making *C. elegans* an ideal organism for studying the regulatory effects of RNA editing on the transcriptome. Previous studies in *C. elegans* indicated competition between RNA interference (RNAi) and RNA editing mechanisms, and observed that lack of both mechanisms can suppress defects that are observed when RNA editing alone is absent. Previously, we showed that 3'UTR edited genes as well as many pseudogenes and other lncRNAs are extensively downregulated in the absence of ADARs, while this downregulation is not observed with additional knockout of RNAi. Here, we show that the main function of ADARs and RNA editing in *C. elegans* is to regulate RNA expression and not the protein content in a cell. We also found that changes in the level of editing cause more developmental defects than a complete lack of editing. In addition, we found severe developmental defects caused by lack of *adr-1*, which are probably independent of its function in the RNA editing process. Finally, our results suggest that the role of RNA editing in prevention of RNAi on self-produced transcripts can effect the normal development of the worm.

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412 The landscape of miRNA editing in animals and its impact on miRNA biogenesis and targeting

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Adenosine-to-inosine (A-to-I) RNA editing regulates miRNA biogenesis and function. To date, fewer than 160 miRNA editing sites have been identified. Here, we present a quantitative atlas of miRNA A-to-I editing through the profiling of 201 pri-miRNA samples and 4694 mature miRNA samples in human, mouse, and Drosophila. We identified 4162 sites present in ~80% of the pri-miRNAs and 574 sites in mature miRNAs. miRNA editing is prevalent in many tissue types in human. However, high-level editing is mostly found in neuronal tissues in mouse and Drosophila. Interestingly, the edited miRNAs in neuronal and non-neuronal tissues in human gain two distinct sets of new targets, which are significantly associated with cognitive and organ developmental functions, respectively. Furthermore, we reveal that miRNA editing profoundly affects asymmetric strand selection. Altogether, these data provide insight into the impact of RNA editing on miRNA biology and suggest that miRNA editing has recently gained non-neuronal functions in human.

413 Dynamic RNA Splicing and Gene-Specific Edited Isoforms identified in human PBMCs using high-throughput sequencing and alternate allele specific quantification methods.

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The RNA editing landscape, or editome, in human cells have been challenging to assess despite improvements in depth and variant detection via high-throughput sequencing technology methods. To date, the majority of non-canonical RNA editing types are A-to-G (I), with rarer cases being C-to-U. Here we describe A-to-G edit within gene sites in human peripheral blood monocytes (PMBC) using a very deeply sequenced data set (> 1 Billion reads), and an adapted RNA-seq pipeline to reduce sequencing mapping false categorization (as those associated to alu and pseudogenes). Innovative quantitative methods can be used to validate these sites as digital droplet PCR and pyrosequencing. Furthermore, we identify that several isomers (alternatively spliced transcripts have different levels of editing. Alternate sequencing technology as PacBio also corroborate targeted RNA editing isoforms.

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414 Deciphering the code of structure-function relationship in A-to-I RNA editing

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RNA editing is a mechanism that regulates gene expression and fine-tunes biological functions in higher eukaryotes. In animals, the predominant type of RNA editing is adenosine to inosine (A-to-I), catalyzed by adenosine deaminases acting on RNA (ADARs). ADARs recognize double-stranded RNA and are essential in neuronal development. Several devastating diseases including cancers, suicidal depression, and amyotrophic lateral sclerosis (ALS) have been linked with abnormal A-to-I editing and ADAR mutations. Recent discoveries show that ADAR1 is an important immune suppresser that distinguishes self from non-self RNAs. However, basic questions regarding efficiency and specificity in ADAR editing remain to be answered especially for ADAR1. This work aims to quantitatively and systematically address how and to what degree a particular adenosine is selected for ADAR1 editing. We used mutagenesis by CRISPR in cells and *in vitro* chemical probing of RNA structure coupled with deep sequencing to systematically dissect features in RNA substrate that dictate efficiency and specificity of ADAR1 editing. We mapped secondary structures and RNA editing levels for more than one thousand RNA variants. We are applying deep learning methods to analyze these data. We identified clusters of RNA structural features that confer distinctive A-to-I RNA editing efficiency. This work uncovers the mechanism behind A-to-I RNA editing for understanding the roles it plays in various human diseases and lays the foundation for exploring ADAR enzymes for transcriptome engineering.

415 Structure and mechanism of a bacterial t6A biosynthesis system

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The universal N(6)-threonylcarbamoyladenosine (t6A) modification at position 37 of ANN-decoding tRNAs is central to translational fidelity. In bacteria, t6A biosynthesis is catalyzed by the proteins TsaB, TsaC/TsaC2, TsaD and TsaE. Despite intense research, the molecular mechanisms underlying t6A biosynthesis are poorly understood. Here, we report biochemical and biophysical studies of the t6A biosynthesis system from Thermotoga maritima. Small angle X-ray scattering analysis reveals a symmetric 2:2 stoichiometric complex of TsaB and TsaD (TsaB2D2), as well as 2:2:2 complex (TsaB2D2E2), in which TsaB acts as a dimerization module, similar to the role of Pcc1 in the archaeal system. The TsaB2D2 complex is the minimal platform for the binding of one tRNA molecule, which can then accommodate a single TsaE subunit. Kinetic data demonstrate that TsaB2D2 alone, and a TsaB2D2E1 complex with TsaE mutants deficient in adenosine triphosphatase (ATPase) activity, can catalyze only a single cycle of t6A synthesis, while gel shift experiments provide evidence that the role of TsaE-catalyzed ATP hydrolysis occurs after the release of product tRNA. Based on these results, we propose a model for t6A biosynthesis in bacteria

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416 Engineering an RNA-targeting CRISPR/Cas9 system to edit transcripts in living cells

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CRISPR-Cas9 technologies have transformed biological research by providing an efficient means to perform precise, sequence-specific genomic editing. However, these methods are intrinsically permanent in nature, and are limited by the requirement of a protospacer adjacent motif (PAM) as well as a dependence upon cellular replication machinery. It has been reported that catalytically-dead Cas9 (dCas9) can be repurposed to target RNA in human cells in a programmable and PAM-independent manner, leaving open the potential for sequence manipulation using RNA-targeting Cas9 (RCas9) strategy. Here, by fusing a catalytically active ADAR2 deaminating domain (ADAR2DD) to dCas9, we demonstrate a means to alter genetic information at the transcript level. This newly designed Cas9-mediated RNA Editing (CREDIT) system can direct adenosine to inosine (A-to-I) conversion on a base-specific level. To accomplish this, we have engineered a single-guide RNA (sgRNA) that has been modified with a region of homology capable of near-perfect RNA-RNA base pairing over the desired site of editing, forcing an A-C mispairing and the generating a ‘pseudo-dsRNA' substrate. We demonstrate that we can perform targeted deamination on both a reporter and endogenously expressed cellular mRNAs using these components, thus illustrating a broad context for RNA editing. We next engineer a minimal-sgRNA that is still capable of editing targeted mRNAs in the absence of a canonical 20nt spacer sequence necessary for Cas9-dependent DNA binding, further simplifying the design of our system and demonstrating an alternate means of RNA-targeting. Lastly, we perform both targeted and global RNA-sequencing to demonstrate the overall specificity and efficacy of our platform when expressed in live cells. Overall, CREDIT serves as a powerful and flexible RNA editing tool for the targeted and reversible manipulation of genes, and proves very promising in the context of both biological research and disease therapeutics.

417 Mutagenesis screens identify editosome protein domains that differentially affect RNA editing between life cycle stages of *Trypanosoma brucei*

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Uridine insertion and deletion RNA editing generates functional mitochondrial mRNAs in *Trypanosoma brucei*. Transcripts are differentially edited in mammalian bloodstream form (BF) and insect vector, procyclic form (PF) cells, correlating with changes in mitochondrial function and metabolism between life cycle stages. The mechanisms controlling the developmental regulation of editing are currently unknown. Editing is catalyzed by three distinct ~20S editosome complexes that contain a common set of 12 proteins but differ in the mutually exclusive presence of the KREN1, N2 or N3 RNase III endonucleases and their respective KREPB8, B7, or B6 partner proteins, that contain degenerate RNase III domains, and have no known catalytic functions. Editosome subunit composition appears to be identical in BF and PF. Here, we describe the development of high-throughput mutational scanning and complementation assays for multiple editosome proteins, including the endonuclease partner proteins KREPB8, B7, and B6. These screens have revealed specific single amino acid substitutions in several proteins, both in predicted domains and in regions without detectable homology to known motifs, that differentially affect cell survival, editing, and editosome integrity in BF and PF. We identified residues that are essential for PF survival but not BF, and also mutations with a reciprocal effect. Furthermore, we show that substitution of residues required for PF function also block differentiation from BF to PF. Collectively our results indicate that there must be functional and/or architectural changes in editosomes upon the developmental transition from to BF and PF. Furthermore, they support a central role for editosomes and editosome subunits in the regulation of RNA editing during the *T. brucei* life-cycle.

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418 PRIMA-seq: novel method for enrichment and sequencing of the RNAs with primary aliphatic amines

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Aside from four canonical nucleotides cellular RNAs contain an impressive number of over 170 chemically distinct modifications. They are added post-transcriptionally by various RNA-modifying enzymes and can be found almost at any position of the nucleobase, 2'-OH group of the ribose or phosphate moiety. By altering RNA structure, stability, cellular localization, binding with RNA or protein partners, engagement of the translation machinery RNA modifications, therefore, contribute to post-transcriptional regulation of gene expression. In order to understand the biological importance of different RNA modifications the field of epitranscriptomics urgently requires robust detection methods compatible with next-generation sequencing.

Several modified nucleotides like acp^3U, lysidine, precursors of wyosine, wybutosine and queuosine include amino acid like residues with free aliphatic amines. To map these modified bases with single-nucleotide resolution, we developed primary aliphatic amines RNA sequencing (PRIMA-seq).

PRIMA-seq was applied to total and poly(A)-selected RNA from HEK293 cells. In total RNA samples the vast majority of RT-stops came from U1248 in 18S rRNA corresponding to hypermodified m^1acp^3Y base which served as internal positive control. The acp^3U-containing tRNA species, like $tRNA^{Asn}$ and $tRNA^{Tyr}$ were also strongly enriched, indicating specificity of the method. Several promising candidates were also identified among some other tRNA species, snoRNAs and even mRNAs. Currently we are validating several of our top candidates by biochemical methods.

419 Human Pus10 protein produces pseudouridine 54 in select tRNAs

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A typical tRNA contains a 17-base stem-loop commonly called "TΨC arm", because it contains T (ribothymidine or 5-methyluridine), Ψ (pseudouridine) and C, at positions 54, 55 and 56, respectively, in most bacterial and eukaryotic tRNAs. Archaeal tRNAs contain Ψ55 but rarely have T54, and instead mostly contain Ψ54. Archaea lack orthologs of TrmA and TruB that produce tRNA T54 and Ψ55 respectively in bacterial and eukaryotic tRNAs. Pus10, a Ψ synthase distinct from TruB family members is known to produce both Ψ54 and Ψ55 in archaeal tRNAs. Pus10 homologs have been observed in nearly all sequenced archaeal and most eukaryal genomes, but not in yeast and bacteria. This coincides with the observation of Ψ54 in most tRNAs of Archaea and some tRNAs of animals, and the absence of Ψ54 in tRNAs of yeast and bacteria. Certain isoacceptors of mammalian tRNAs for Gln, Trp, Pro, Thr and Arg, as well as chicken tRNAs for Pro and Trp contain Ψ, instead of T at position 54. Several Ψ54-containing animal tRNAs have been identified as primers for DNA synthesis of retroviruses. So far, no tRNA Ψ54 synthase has been identified in eukaryotes. Although human Pus10 is known to be involved in apoptosis, its crystal structure suggests it to be a bona fide Ψ synthase.

Here we show that Pus10 in the cytoplasmic extracts of human cell lines catalyzes tRNA Ψ54 production. Cell extracts and recombinant Pus10 can produce Ψ54 in certain isoacceptors of Trp, Gln, Pro, Cys and Lys3. The tRNA for Lys3 is the primer for HIV DNA synthesis. Ala, Phe and Asp tRNAs are not modified by this enzyme. This matches with the natural presence and absence of Ψ54 in most of these tRNAs. The presence of $Am^{1}AAU$ sequence at tRNA positions 57-60 is required for maximum Ψ54 activity. Crystal structure based modeling has previously suggested that Pus10 binds the tRNA in the TΨC and acceptor arms. We found that the destabilization of the acceptor stem of the tRNA by non-Watson-Crick pairs considerably reduces Ψ54 activity. Although human Pus10 has neofunctionalized to mediate apoptosis, it still retains its Ψ synthase activity.

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420 Structural features of archaeal box C/D guide sRNAs required for dual 2'-*O*-methylation function

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Majority of archaeal box C/D sRNAs function as dual guides i.e., they are known to guide 2'-*O*-methylation of specific nucleotides in two separate targets using both terminal box C/D and internal C'/D' RNP complexes. *In vitro* assembly reactions using *Haloferax volcanii* box C/D guides assembled with their cognate proteins from *Methanocaldococcus jannaschii* provide us with a system to study the structural determinants of such a dual sRNA guided process. We report two unique box C/D guides, sR-h41 and sR-tMet in this study that are able to modify only a single target as opposed to the typical dual archaeal sRNPs guides. We have converted them into dual box C/D guides for *in vitro* reactions. These double nucleotide methylation modifications require each of their D and D' guide region to be at least 12 bases long, which has been previously reported as the optimum size for an archaeal guide/spacer sequence. In case of sR-h41 we had to introduce, in addition a K loop/K turn at the internal C'/D' region for it to function as dual guide system. Furthermore, we find that the K-turn motif present at the terminal box C/D regions of both sR-h41 and sR-tMet are essential for functioning of each of their guide regions. L7Ae binds asymmetrically to only the terminal box C/D motif in wild type sR-h41 single guide RNA, which is typically a feature of single guide eukaryotic box C/D sRNPs. However, in the newly created dual guide versions it binds to both box C/D and C'/D' motifs. The internal box C'/D' region must contain at least one GA dinucleotide, either in C' or D' region, for both guide regions to function. Increasing the GC-pairs in guide-target interaction of these dual-guide RNAs increased methylation efficiency. This study shows that overall sRNP architecture dictates the single or dual guide function of box C/D RNAs in Archaea.

421 A-to-I RNA editing regulates skeletal muscle specific exonization of Alu element

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A-to-I RNA editing is a post-transcriptional modification in which adenosine (A) is converted to inosine (I) in double-stranded region of mRNAs mediated by adenosine deaminase acting on RNA (ADAR). It is known that more than 90% of editing sites reside in Alu repeat elements in human transcriptome. Antisense strand of Alu element has a cryptic splicing signal, and a part of it is sometimes incorporated as an Alu-drived exon during splicing. Thus, exonization of Alu element poses a threat to integrity of transcriptome. We previously reported that A-to-I RNA editing has a functional role in preventing aberrant exonization of Alu elements (Sakurai et al., Nat Chem Biol., 2010).

Selenoprotein N1 (SEPN) regulates calcium flux in endoplasmic reticula and plays an important role in skeletal muscle differentiation and maintenance. The Alu element in the second intron in SEPN1 mRNA is exonized in skeletal muscle more frequently than any other tissues. We found that the aberrant Alu exonization was promoted by ADAR1 knocking down, indicating that A-to-I RNA editing has a preventive role in this aberrant splicing and controls expression level of SEPN1. High frequency of the Alu exonization can be explained by low expression level of ADAR1 in skeletal muscle. We are now investigating physiological role and molecular mechanism of this event mediated by A-to-I RNA editing during myoblast differentiation.

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422 Structural basis for preferential deadenylation of U6 snRNA by Usb1

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Precursor mRNA splicing is a crucial step in human gene expression and is catalyzed by the spliceosome. U6 snRNA is a key component of the spliceosome active site, and during its biogenesis is post-transcriptionally processed by the 3′-5′ exoribonuclease, Usb1. Usb1 removes terminal nucleotides and installs a 3′ terminal phosphate modification on U6 snRNA (1). Loss of Usb1 function is lethal in yeast, and in humans is associated with the disease poikiloderma with neutropenia (PN) (2). Interestingly, patients with PN have U6 snRNAs with 3′ ends that are both aberrantly elongated and oligoadenylated (3).

Here we report that human Usb1 preferentially catalyzes the deadenylation of U6 snRNA, providing a mechanistic explanation as to why the 3′ ends of U6 RNA are aberrantly adenylated in patients with PN. To elucidate the structural basis of Usb1 recognition and catalysis, we determined three different X-ray crystal structures of Usb1 complexed with RNA to high resolution (1.17-1.20 Å). The structures unambiguously define the catalytic acid and base histidine residues, and show that bound adenosine makes additional hydrogen bonds in the catalytic pocket and adopts in an unusual *syn* configuration, whereas uridine is bound in the *anti* configuration. QM/MM MD simulations based on the co-crystal structures indicate the mechanism follows a minimum free energy path where the nucleophile is deprotonated quickly, followed by a rate-limiting step that involves concerted phosphoryl transfer and leaving group protonation. We measured kinetic isotope effects that support this mechanism. Finally, we provide evidence that the U6 snRNA secondary structure is important for regulating the extent of processing by Usb1, and therefore the end processing of U6 snRNA by Usb1 may be regulated by the myriad RNA-RNA and protein-RNA interactions that control the structure of U6 snRNA during its life cycle (4).

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423 Design and optimization of a guide RNA for site-directed A-to-I RNA editing

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A genetic engineering technology is being used not only for basic research but medicinal and therapeutic application. Recently, genome editing technologies that utilizing CRISPR-Cas9 system is now strongly expected as one of the versatile tool for regulating biological process, and is using in wide variety of research field and practical usage. As the same of genome editing, RNA editing that can alter genetic information at RNA level also has a potential to become a useful genetic modification method. To date, several methodologies for site-directed RNA editing has been developed based on the concept of adenosine-to-inosine (A-to-I) RNA editing. A-to-I RNA editing is one of naturally occurring RNA modification mechanism that is catalyzed by an adenosine deaminases acting on RNA (ADARs). Since inosine is read as guanosine (G) in a protein synthesis, A-to-I RNA editing can lead to codon change resulting to alter protein function. Therefore, A-to-I RNA editing has a same biological impact to A-to-G point mutations in genome.

In this study, we constructed the novel guide RNA (AD-gRNA) enabling to induce the RNA editing activity of endogenous ADAR into the target-site, in order to establish a versatile RNA mutagenesis method. AD-gRNA was designed on the basis of the secondary structure of the GluR2 pre-mRNA containing R/G editing site, which is well known as a substrate for ADAR2. Resulted AD-gRNAs have both "ADAR2-recruting region (ARR)" for interacting with the double-stranded RNA binding domain of ADAR2 and "antisense region (ASR)" for programing the target-site by the complementary sequence to the target RNA. These guide RNAs actually introduced A-to-I mutations into the target-site, which was determined by the reprogrammable antisense region. Additionally, site-directed RNA editing could also be observed by simply introducing the guide RNA in ADAR2-over expressing cells. In this poster presentation, we will introduce the sequence design of AD-gRNA and the results of functional evaluation *in vitro* and in cells. Additionally, sequence optimization toward practical application will be discussed based on the results of the mutation analysis for AD-gRNA.

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424 A new tool for sequence-specific manipulation of N^6-methyladenosine

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Epitranscriptomics is an emerging research field focusing on RNA modifications and their cellular functions. Until now, more than 150 reversible and irreversible RNA modifications are known, but their biological roles are mainly unknown. The most abundant internal mRNA modification is N^6-methyladenosine (m^6A). The methylation is realized by a methyltransferase complex and can be recognized by various reader proteins in the nucleus and cytoplasm. The "demethylases" FTO and AlkBH5 remove the methyl group, resulting in a dynamically regulated modification in RNA. Current research elucidates several cellular roles of m^6A on splicing, translation and mRNA decay with impacts on stem cell development and cancer. Until now, the specific role of each m^6A site remains unknown. We aim to investigate the effect of single m^6A positions on its mRNA fate using sequence-specific demethylation. To reach this specificity, we want to make use of RNA-binding proteins. This approach will be realized *in vitro* and in human cancer cells.

425 Metabolic and chemical regulation of tRNA modification associated with taurine deficiency and human disease

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Modified uridine containing taurine, 5-taurinomethyluridine (τm^5U), is found at the anticodon first position of mitochondrial (mt-)tRNAs. We previously reported that τm^5U is absent in mt-tRNAs with pathogenic mutations associated with mitochondrial diseases. However, biogenesis and physiological role of τm^5U remained elusive. Here, we elucidated τm^5U biogenesis by confirming that 5,10-methylene-tetrahydrofolate and taurine are metabolic substrates for τm^5U formation catalyzed by MTO1 and GTPBP3. *GTPBP3*-knockout cells exhibited respiratory impairment and reduced mitochondrial translation. Very little τm^5U was detected in patient's cells with the *GTPBP3* mutation, demonstrating that lack of τm^5U results in pathological consequences. Taurine starvation resulted in down-regulation of τm^5U frequency in cultured cells and animal tissues. Strikingly, 5-carboxymethylaminomethyluridine ($cmnm^5U$), in which the taurine moiety of τm^5U is replaced with glycine, was detected in mt-tRNAs from taurine-depleted cells. These observations suggest that tRNA modifications are dynamically regulated via sensing of intracellular metabolites under physiological condition.

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426 Stable lariats bearing a snoRNA (slb-snoRNA) in vertebrate cells: Are they functional?

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In vertebrates, most snoRNAs are encoded within introns. Therefore, the first step of snoRNA processing involves the linearization of intron lariats by the debranching enzyme. Generally, this process occurs rapidly and efficiently. However, we have shown that some lariats escape linearization; these stable intronic sequence RNAs (sisRNAs) in the form of a lariat accumulate in the nucleus and can be exported to the cytoplasm. In this study, we found a subset of nuclear *and* cytoplasmic sisRNAs that overlap with snoRNAs: namely, stable lariats bearing a snoRNA (slb-snoRNA).

Commonly, snoRNAs are classified into two classes: (1) box H/ACA snoRNAs that guide pseudouridylation when assembled in RNP particles with the pseudouridine synthase dyskerin, Nop10, Nhp2, and Gar1, and (2) box C/D snoRNAs that guide 2'-*O*-methylation when associated with the methyltransferase fibrillarin, 15.5KDa, Nop56, and Nop58. In these RNPs, dyskerin and 15.5KDa protein are RNA binding proteins. To test if slb-snoRNAs are associated with canonical snoRNP proteins, we transfected HeLa cells with expression constructs for HA-tagged dyskerin and 15.5KDa protein. After co-IP and genome-wide RNA analysis we found dozens of slb-snoRNAs associated with dyskerin or 15.5KDa protein. Furthermore, in *Xenopus*we found that both nuclear and cytoplasmic slb-snoRA75 co-precipitated with HA-tagged dyskerin. Thus, these data suggest that slb-snoRNAs associate with typical snoRNA-binding proteins in both the nucleus and cytoplasm. The next question is whether slb-snoRNAs are capable of guiding post-transcriptional modifications. We switched to an in vivo yeast *S. cerevisiae* cell system. We expressed *Xenopus* slb-snoRA28 and linear snoRA28 in wild type and *dbr1Δ* mutant yeast strains. In *dbr1Δ* cells, expression of mature linear snoRA28, even at very low levels, was sufficient to pseudouridylate position 808 in yeast 18S rRNA. This pseudouridine is normally absent from yeast rRNA, but conserved in higher eukaryotes and mediated by snoRA28. However, the accumulation of slb-snoRA28 could not induce this modification. Additionally, in *X. laevis*, the snoRA75 sequence accumulates only in the form of slb-snoRNA and the corresponding position is not modified in this species. We conclude that slb-snoRNAs do not function as guide RNAs, but could regulate snoRNA availability or could have an independent function.

427 Synthesis of Post-Transcriptional Modifications of RNA Phosphoramidites

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In addition to A,C, G and U, over 100 naturally occurring, post-transcriptionally modified nucleotides have been discovered in tRNA, mRNA, and rRNA. These modified nucleotides of diverse complexity give RNA access to a broad range of chemical and biochemical functionality. Despite the rapidly growing awareness of the biological significance of post-transcriptionally modified nucleotides, post-transcriptionally modified RNAs can be difficult to isolate and purify. To provide experimental access to two important modified tRNA nucleotides, we have chemically synthesized the phosphoramidite forms of $PreQ_0$ (2-amino-5-cyano-7-β-D-ribofuranosyl-7*H*-pyrrolo[2,3-d]pyrimidin-4-one) and wyosine (3,4-dihydro-4,6-dimethyl-3-β-D-ribofuranosyl-9*H*-imidazo[1,2-a]purin-9-one). While their nucleosides have been previously synthesized, neither phosphoramidite has been reported in the literature. The $PreQ_0$ phosphoramidite was synthesized over 14 steps through the Vorbrüggen glycosylation to generate a 7-iodo-7-deazaguanosine intermediate which could undergo nitrile substitution. The wyosine phosphoramidite was synthesized over 10 steps by extending the purine backbone of guanosine via *N*-alkylation and zinc mediated cyclization. Both phosphoramidites were synthesized using thionocarbamate chemistry to provide protecting groups compatible with solid-phase RNA synthesis. Access to site selective incorporation of such nucleobases makes it possible to study the functionality of these post-transcriptional modifications in carefully controlled conditions, while also expanding the chemical diversity of currently available nucleosides. This work was supported in part by the NIH-Oxford-Cambridge Scholars Program and the intramural program of the National Heart Lung and Blood Institute, NIH.

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428 Determining the Antibiotic Resistance Mechanism of a Ribosomal RNA-methylating Enzyme

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A mechanism frequently used by bacteria to evade antibiotics that target the ribosome involves the use of RNA-modifying enzymes to alter antibiotic binding sites. The enzyme Cfr is particularly concerning in this regard. Cfr modifies ribosomal RNA (rRNA) within the peptidyl transferase center (PTC). Specifically, Cfr adds a methyl group to C8 carbon atom of a highly-conserved adenosine A2503 in 23S rRNA (*E. coli* numbering). Acquisition of Cfr by pathogens and the resulting methylation causes resistance to 8 classes of PTC-targeting antibiotics, including the two critical clinical antibiotics clindamycin and linezolid. To better elucidate the mechanism of Cfr-mediated resistance, we used directed evolution to generate Cfr variants that confer high levels of resistance to PTC-targeting antibiotics. Interestingly, the evolved variants of Cfr harbor mutations that appear far (>12 Angstroms) from the enzyme active site. We isolated 23S rRNA fragments from *E.coli* expressing the Cfr variants and determined the methylation state of A2503 by MALDI-TOF mass spectrometry. Our findings indicate that the evolved Cfr variants confer enhanced antibiotic resistance by increasing the extent of A2503 C8 methylation. Ongoing efforts involve investigating kinetic parameters and stability of the evolved Cfr variants to determine how the identified mutations elicit enhanced rRNA methylation and antibiotic resistance.

429 Investigating the ADAR interactome

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Adenosine deaminases acting on dsRNA (ADARs) has been shown to be essential for a normal development, and to have a role in preventing innate immune response to endogenous dsRNA. ADARs deaminate adenosine to inosine by hydrolytic deamination and is known as A-to-I editing. Our group was the first to demonstrate that this editing event in endogenous dsRNA prevents the interferon (IFN) signalling cascades from RIG-I and MDA5, two cytosolic receptors for dsRNA (Mannion et al. 2014). In accordance, mice lacking *Adar1* have an immune phenotype with heighten levels of type-I IFN and IFN stimulated genes (ISGs). Our group rescued the embryonic lethality of *Adar1*$^{-/-}$ mice to birth by generating a double homozygous mutant between *Adar1* and *Mavs* (Mitochondrial antiviral-signalling protein), adaptor, which is downstream of RIG-I and MDA5.

In humans, mutations in *ADAR1* have been shown to cause the autoimmune disorder Aicardi Goutières syndrome-AGS (Rice et al. 2012), where patients display heightened levels of IFN and ISGs. Furthermore, most of the mutations were shown to reduce editing activity of the protein. An exception is *ADAR1 D1113H* mutation that is located in deaminase domain of the protein.

To investigate whether this mutation cause perturbations in protein-protein interactions, we first had to determine the protein interactome for ADARs. We prepared stable HEK293T cell lines, expressing both isoforms of ADAR1 and ADAR2. These proteins were tagged with BirA and Strep-tag at either the N or C terminal of the protein. In addition, cells were treated with HMW Poly I:C and co-immunoprecipitation of endogenous ADAR1 was also performed.

Now, we have a comprehensive data set of protein complexes in which ADAR1 and ADAR2 acts, these data are consistent for all three set. Our data is in agreement with all previously published interacting partners of ADARs. In addition, we found that tags present at different terminus can influence interactions and protein stability.

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430 Profile of m6A RNA modification identified age-associated regulation of AGO2 mRNA stability

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Gene expression is dynamically regulated in a variety of mammalian physiologies. During mammalian aging, there are changes that occur in protein expression that are highly controlled by the regulatory steps in transcription, post-transcription and post-translation. Although there are global profiles of human transcripts during the aging processes available, the mechanism(s) by which transcripts are differentially expressed between young and old cohorts remains unclear. Here we report on N6-methyladenosine (m6A) RNA modification profiles of human Peripheral Blood Mononuclear Cells (PBMCs) from young and old cohorts. An m6A RNA profile identified a decrease in overall RNA methylation during the aging process as well as the predominant modification on protein coding mRNAs. The m6A-modified transcripts tend to be more highly expressed than non-modified ones. Among many methylated mRNAs, those of DROSHA and AGO2 were heavily methylated in young PBMCs which coincided with a decreased steady-state level of AGO2 mRNA in the old PBMC cohort. Similarly, downregulation of AGO2 in proliferating human diploid fibroblasts (HDFs) also correlated with decrease of AGO2 mRNA modification and steady-state level. In addition, the overexpression of RNA methyltransferases stabilizes AGO2 mRNA but not DROSHA and DICER1 mRNA in HDFs. Moreover, the abundance of miRNAs also changed in the young and old PBMCs which is possibly due to a correlation with AGO2 expression as observed in AGO2-depleted HDFs. Taken together, we uncovered the role of mRNA methylation on the abundance of AGO2 mRNA resulting in the repression of miRNA expression during the process of human aging.

431 Thermodynamic characterization of RNA duplexes under molecular crowding conditions

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The most widely used algorithm to predict RNA secondary structure from sequence uses a model based on thermodynamic parameters derived from optical melting experiments collected in a standard salt buffer to predict the RNA secondary structure with the lowest free energy. Standard salt buffer fails to account for other molecules which may affect RNA stability. In order to better predict thermodynamics and secondary structures of RNAs in vivo, it is essential to study RNA under molecular crowding conditions. DNA duplexes have been studied in the presence of crowding agents of various sizes; however, RNA studies are lacking. Furthermore, no systematic study has been completed to determine the effects of a small crowding agent on the thermodynamics of DNA or RNA sequences of varying lengths and composition. Here, thermodynamic data was collected for RNA duplexes with varying length and composition in the presence of a 20% (poly)ethyleneglycol (PEG) 200 solution. These thermodynamic data show that RNA duplexes are destabilized by 0.97 kcal/mol in the presence of a small crowding agent and that there is no direct correlation between the degree of destabilization and sequence length or composition. The observed destabilization is in agreement with the findings of DNA studies where PEG 200 is introduced to DNA duplexes. This data sheds light on the stability of RNA in simulated cell-like conditions and provides insight into how the stability of oligonucleotides of differing lengths and composition are affected by crowding.

432 Analysis of HCV RNA genome structure

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The HCV genome is a 9.6 kb single-stranded, 3.1 MDa RNA molecule that directs its own translation and replication. These activities are mediated by regions in and near the 5' and 3' untranslated regions that fold into discrete structures which bind and manipulate host cell and viral proteins to act on the genomic RNA. Additional structural elements that are essential for viral replication have been identified throughout the HCV ORF by our lab and others. Currently, we are using new state-of-the art structural probes and analysis techniques to further probe the entire HCV genome structure with the goal of uncovering new functional domains and long-range interactions. Ultimately, we plan to explore the dynamics HCV RNA structure in cells throughout the HCV replication life cycle.

433 Structural and functional study of *Flaviviridae* and *Picornaviridae* untranslated region in the pre-initiation complex .

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To promote their propagation, viruses use specific mechanisms to produce their own proteins. One of the strategies to hijack the host cellular translation machinery is known as Internal Ribosome Entry. This bypass is for the majority of the well characterized viruses due to the presence of structural elements in the 5' untranslated region (UTR) that recruit, the translation machinery on the start codon. The ensemble of these structural elements is denominated the "Internal Ribosome Entry Site" (IRES). Human hepatitis C virus (HCV) is the archetype of type III IRES, which is also encountered in other *Flaviviridae* and *Picornaviridae*. The secondary structure of most of them is identical to HCV IRES structure; however some of them harbour a specific sub-domain called IIId2. Previous studies have shown that the deletion of this sub-domain completely inhibits the IRES translation activity, but does not affect its interaction with the small ribosomal subunit or with the eukaryotic initiation factor 3 (eIF3). Our work aims at deciphering the role of IIId2 sub-domain to better understand the molecular mechanisms of viral translation. Thanks to a new RNA secondary structure strategy developed in the lab, we successfully modelled the structure of some of these IRES and of the corresponding mutants devoid of the sub-domain IIId2. These results showed that the effect of the deletion is very local and does not affect the global structure, in addition our results suggests that IIId2 interacts with the small ribosomal subunit. We are currently investigating the structural reorganization of the ribosome upon docking of the IRES, and the protein composition of the pre-initiation complex (PIC) paused on the different IRES. It will allow us to understand how the deletion of IIId2 is able to inhibit the progression of the PIC. Our work aims at reconstituting this complex step by step from purified components, in order to shed light on the sequential events necessary to its formation.

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434 Convergent Use of Hepatacoordination for Cation Selectivity by RNA and Protein Metalloregulators

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Members of the large *yybp-ykoY* family of bacterial riboswitches regulate intracellular Mn^{2+} concentration. Previously, crystal structures showed that Mn^{2+} directly interacts with these riboswitches within a binuclear binding site. A hexacoordinate, hard (*i.e.*, of low polarizability) binding site with six nonbridging phosphate oxygen (NBPO) ligands preorganizes the adjacent softer (*i.e.*, more polarizable) hexacoordinate site comprised of five NBPOs and a N7 from a highly conserved adenine. The presence of this softer nitrogen ligand was proposed to be solely responsible for Mn^{2+} selectivity (Price *et al*. *Mol Cell* 57:1110, 2015). This mechanism, however, does not explain how these riboswitches discriminate against cellular metal ions that are similar in chemical hardness to Mn^{2+}. Because of the widespread occurrence of *yybp-ykoY* riboswitches and their genetic association with diverse transporters, we explored whether members of this family sense additional divalent transition metals. Using *in vitro* reporter assays, we discovered that *yybp-ykoY* riboswitches from the *alx*, *ykoy* and *mntP* loci activate gene expression in response to Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+} and Ni^{2+}, in addition to Mn^{2+}. By calorimetric analyses, we found that these riboswitches exhibit the highest affinity for Cd^{2+} and Mn^{2+}. To elucidate the molecular basis for this selectivity, we determined structures of three representative *yybp-ykoY* riboswitch aptamer domains bound to Cd^{2+}. Our structures reveal a conformationally plastic cation binding site. This plasticity implies that the atomic radii of the bound cations is unlikely to give rise to selectivity. Our structure of a riboswitch derived from the *mntP* locus, which has the highest affinity for Cd^{2+}, contains a hexacoordinate Mg^{2+} in the hard site, and an unexpected heptacoordinate Cd^{2+} in the softer site. Heptacoordination is rare among transition metal ions, but is a property shared between Mn^{2+} and Cd^{2+}. Thus, the riboswitch can achieve selectivity by exploiting this unique coordination geometry. Remarkably, heptacoordinate cation binding by *mntP* riboswitch is similar to the Mn^{2+} and Cd^{2+} selectivity mechanism of the MntR gene regulator. Our results suggest an expanded role for *yybp-ykoY* riboswitch in bacterial metal ion homeostasis. This work was supported by the intramural program of the National Heart, Lung and Blood Institute, NIH

435 Diverse structural ensembles of the MALAT1 triple helix reveal putative target sites for drug discovery.

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RNA remains an underexplored class of drug targets owing, in part, to the limited number of RNA-focused screening assays. Here, we develop a combined experimental and computational screening pipeline to target a critical triple helix in the oncogenic lncRNA MALAT1. This triplex masks the lncRNA 3′ end and protects the entire 7-kb transcript from 3′-5′ exonucleolytic degradation, supporting MALAT1-mediated metastasis and proliferation in a several cancer types. Using a FRET assay we uncover large conformational variations within the MALAT1 triple helix (M1TH). Biochemical analysis reveals that a subset of the conformations sufficiently destabilizes the RNA 3′ end, leading to exonucleolytic degradation by RNase R. We hypothesize that small molecule-induced disruption of the triplex represents a viable approach to inhibit M1TH and degrade the lncRNA. To evaluate putative binding pockets within M1TH, we perform molecular dynamics simulations for two representative conformations: the native crystal structure and a disrupted triple helix. Comparison of these two 500 ns simulations reveals differences in overall stability and conformation of the disrupted M1TH relative to the native triplex structure, giving rise to a distinct free energy landscape. Structural analysis of the disrupted triplex reveals several new putative target sites for small molecule intervention. Therefore, we are developing a computational methodology to explore docking of small molecules to an ensemble of RNA conformations represented by our simulated native and disrupted M1TH structures. Subsequently, we will employ our FRET and RNase R degradation assays to validate small molecule binding and inhibition of M1TH. Taken together, we aim to identify selective small molecule lead compounds that preferentially bind to an inhibitory triplex conformation and promote degradation.

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436 A new classification and nomenclature of RNA conformation connecting backbone conformers and mutual position of nucleobases

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The RNA structure conformations might be characterized and classified by various theoretical approaches. The classifications based on sugar-phosphate backbone torsions or pseudo-torsions characterize well the local shape of RNA backbone, however, provide rather poor information about the mutual positions of the nucleobases. Even small variations of the torsion or pseudo-torsion angles that are within the range of well defined backbone conformers lead to high uncertainty in prediction of the mutual position of the nucleobases that are not neighbors in the sequence. On the other hand, the other branches of the methods that are rather focused on mutual positions of nucleobases provide only limited information of the local backbone conformation.

Here we show the classification of the RNA conformations based on the clustering of the transformation matrices connecting the reference coordinate frames representing consecutive nucleotides in the sequence. The origin of these coordinate frames are placed to the C1' atoms that are an intersection between sugars and nucleobases and their coordinate systems follow the orientation of nucleobases, so this representation takes into account both local backbone conformation and the mutual positions of the nucleobases. These transformation matrices connecting consecutive nucleotides form clusters, which mostly follows clusters identified in backbone dihedral space, although, in some cases, similar set of torsion angles sharing the same backbone torsional conformer might be found in different clusters representing different mutual position of consecutive nucleotides, and similarly, the same mutual conformation of the nucleotides might be realized by more that one combination of backbone torsional conformers. Due to the simplicity of above mentioned description, the mutual position of any two nucleotides in the structure might be evaluated from the local backbone conformers just by multiplying the transformation matrices, and can be easily transformed, e.g., to isosteric groups or G-vectors of the eRMSD metric.

437 Seed length drives structural flexibility of miR-34a-SIRT1 mRNA complex

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MicroRNAs (miRNAs) are short, non-coding RNAs that regulates messenger RNA (mRNA) translation. At the core of miRNAs activity lays the base pairing between Argonaute associated miRNAs (miRISC) and their targets [1]. The dominant interaction in miRISC target recognition is the Watson-Crick base pairing of nucleotides (nts) 2-8, the seed sequence [1,2]. Beyond the seed, miRNA-mRNA complexes (nts 9-22) are predicted to contain non-canonical structural motifs [1]. Experimental observations of such motifs and their implications in target regulation have, however, remained elusive.

We employ biophysical methods, NMR spectroscopy relaxation dispersion ($R_{1\rho}$) [3,4] and molecular dynamics to dissect the structural flexibility of the miR-34a Sirtuin 1 (SIRT1) mRNA binding site at atomic resolution.

While the biophysical characterization allows us to estimate binding affinity (K_d) and thermal stability (T_m) of the miRNA-mRNA duplex, $R_{1\rho}$ NMR data of the central bulge, measured on a validated hairpin model, indicates that the seed is in equilibrium with a transient and low populated excited state that comprises an additional GU base pair at its 3'-end (seed +1). By stabilizing seed +1 by a two-point mutation we quench the flexibility of the central bulge, without impairing the K_d or the T_m of the duplex. 3D structures of wild type and seed +1 constructs are calculated from NMR-informed molecular dynamics and successfully docked into the crystal structure of human Argonaute 2 (hAgo2) [2]. The functional impact of such structural modulation is studied *in vitro* with purified hAgo2-miR-34a complexes and *in vivo* by luciferase reporter assay, preliminary results are reported.

It is becoming increasingly clear that the ability exploit RNA-guided machineries strongly relies on the understanding of their structure-function relationship. We argue that including structural flexibility in the miRISC picture will help to better understand general miRNAs biology and the development of better oligonucleotide-based drugs.

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438 Dissection of the Energetic and Conformational Properties of a Ubiquitous Tertiary Structural Motif through High-throughput and Single-molecule Studies

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RNA's ability to fold into intricate tertiary structures and undergo precise conformational changes is essential for complex biological processes such as translation and pre-mRNA splicing. Although decades of research have revealed important characteristics of the RNA folding process, such as multiple folding pathways and misfolded states, a predictive and quantitative model of RNA folding is lacking. Previous work has revealed modularity of RNA structural motifs, and recently we have provided evidence for a generalizable model of RNA folding based on reconstitution of the energetic and conformational properties of individual RNA secondary and tertiary structural motifs. Here, we used a novel high-throughput platform-based on in situ transcription of RNA on a next-generation sequencing chip-to dissect the energetic and conformational properties of thousands of sequence variants of the ubiquitous tetraloop/tetraloop-receptor (TL/TLR) tertiary motif, including variants abundant in Nature, in vitro-evolved variants not found in Nature, and hundreds of single and higher-order mutants. Each TL/TLR was inserted into up to fifty different scaffolds-derived from a ‘tectoRNA' dimer that assembles through formation of two TL/TLRs-and the binding dissociation constants (Kd) of thousands of TL/TLR-scaffold combinations were measured directly and in parallel. The set of Kd values for each TL/TLR across structural scaffolds provided a ‘thermodynamic fingerprint' that reported on the conformational preferences of the TL/TLR. Comparison of the thermodynamic fingerprints revealed a wide range of TL/TLR stabilities and distinct types of conformational behaviors, ranging from TL/TLRs with narrow conformational ensembles that can be modeled by a single specific conformation to variants with broad conformational ensembles. Differences in binding affinity and conformational preferences between TL/TLR variants mirrored their natural abundance, suggesting that Nature selects TL/TLRs based on overall stability and conformational constraints. Analysis of the thermodynamic effect of mutations, in conjunction with smFRET studies, revealed sequence features that dictate TL/TLR stability and conformational preferences. Overall, our results provide additional support for a reconstitution model in which RNA folding is quantitatively described from the properties of secondary and tertiary structural motifs and our quantitative dataset can be used to engineer RNAs with specified stabilities and defined conformational dynamics.

439 Impact of Modified Nucleobases on Base Pairing in RNA Experimental Structures

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Posttranscriptional modifications greatly enhance the chemical information of RNA molecules, contributing to explain the diversity of their structure and functions. A significant fraction of RNA experimental structures available to date present modified nucleobases, with half of them being involved in H-bonding interactions with other bases, i.e. ‘modified base pairs'. To this end, we did a systematic investigation of modified base pairs, in the context of experimental RNA structures. In addition, non-natural (synthetic) modifications have been introduced in nucleic acid structures for targeted applications. This prompted us to also analyze the impact of synthetically prepared non-natural modifications on the H-bonding propensity in the context of nucleic acid structures. For this, set of PDB structures solved by X-ray crystallography at a resolution of 3.5 Å or better and containing RNA molecules with posttranscriptional modifications were analysed, in order to identify the modified base pairs and classify their geometry. As a result of this analysis, we obtained 573 base pairs containing at least one modified base. Base pairs containing non-natural modifications were also modelled starting from available experimental structures in the PDB. The geometries of the base pairs were optimized with a Density Functional Theory (DFT) approach. Interaction energies, defined as the difference between the energy of the base pair and the energy of the isolated free bases, were evaluated on the DFT optimized geometries at the MP2 level of theory. Our structural analyses show that most of the modified base pairs are non Watson-Crick like and are involved in RNA tertiary structure motifs. Similar analyses were also performed on base pairs involving some non-natural modifications of particular biotechnological interest, in the context of RNA or DNA structure. The combined bioinformatics and quantum mechanics studies we performed help provide a rationale for the impact of the different modifications on the geometry and stability of the base pairs they participate in, and possibly predict the effect of newly designed modifications.(1-4)

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440 SHAPE-JuMP: Detecting RNA-RNA interactions via covalent linkage and reverse transcription

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For many RNAs, the higher-order structure of the molecule is critical to its function. However, discerning this structure, especially for large molecules, is a challenging and unresolved problem. We have developed SHAPE-JuMP (selective 2′-hydroxyl acylation analyzed by primer extension and juxtaposed merged pairs) to probe RNA tertiary structure at large scales. A bi-functional small molecule is used to covalently link two neighboring nucleotides that are close in three-dimensional space. The site of this linkage is then reported via an engineered reverse transcription enzyme that "jumps" across the crosslinked nucleotides, creating a cDNA with a deletion that identifies the site of the crosslinked nucleotides. Massively parallel sequencing and alignment analysis are then used to report tertiary contacts all across RNA molecules under a diverse array of biologically informative conditions. These tertiary contacts will improve our understanding of RNA structure and can be used to improve three-dimensional modeling of RNA structures.

441 Fold and protein binding of the U12-U6$_{atac}$ snRNA complex of the minor spliceosome

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The splicing of precursor messenger (pre-m)RNA, by which noncoding introns are excised and flanking coding exons are ligated, is catalyzed by the spliceosome, a dynamic ribonucleoprotein assembly comprising five small nuclear (sn)RNAs and >100 proteins. Proteins maintain critical roles in the assembly and regulation process, but the central core is defined by RNA components that are directly involved in catalysis. The spliceosome's catalytic core is a complex formed between U2 and U6 snRNAs for the major spliceosome (~99.7%) and U12 and U6$_{atac}$ snRNAs for the minor variant. The coexistence of this divergent and low-abundance minor spliceosome in many eukaryotic organisms ranging from plants to humans, provides a naturally occurring variant that performs identical chemistry as the major, despite limited (~60%) sequence conservation. To help understand how critical ion-binding sites are brought together to catalyze the first step of splicing, and to develop a general structural model of the pre-mRNA active site, we have studied structural features of the U12-U6$_{atac}$ snRNA pairing from human and plant minor spliceosomes to compare them with what is known about the U2-U6 snRNA pairing from the human major spliceosome. Using solution NMR, we show that the U12-U6$_{atac}$ snRNA complex of human and *Arabadopsis* (plant) spliceosomes adopt similar folds, and in spite of notable differences between the major and minor spliceosomal snRNA complexes, the relative positioning of catalytically essential elements is equivalent, creating an active site defined by similar architecture. We also show, by electrophoretic mobility shift assays, that the human spliceosomal protein RBM22, which has been suggested to facilitate folding the U2-U6 snRNA complex into a catalytically active form, binds the protein-free U2-U6 and U12-U6$_{atac}$ snRNA complexes with similar affinity (3.2+0.5mM and 8.4+0.7mM, respectively).

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442 Capturing The Light Triggered Release of Adenine Inside Adenine Riboswitch Crystals

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Limited structural dynamics about RNAs exists, thus severely impacting our understanding of how to RNA functions. To date, obtaining RNA structural information by X-ray crystallography has been limited by the need to obtain sizeable crystals of diffraction quality and the susceptibility of RNA to radiation damage. Serial femtosecond crystallography (SFX) has overcome both these roadblocks and has provided dynamic structural information to also be "captured". This technique uses X-ray free electron lasers to deliver femtosecond pulses to provide diffraction of nano- and microcrystals in their mother liquor at room temperature, nearly radiation damage free. Recently, SFX has been employed to surpass the limits of time-resolved data collection at synchtrons. Where time-resolved ligand mixing experiments could only be performed by mutation or freeze trapping, SFX allows crystals to be mixed with their substrate prior to diffraction because ligand diffusion into the microcrystals is faster than the reaction itself. Using this technique, we have collected time-resolved data of the adenine riboswitch when mixed with its ligand adenine. Surprisingly we found that a large conformational change must occur to accommodate ligand binding, which results in rearrangement of the crystal lattice. Because this reaction is triggered by diffusion, the reaction initiation is heterogeneous and thus the conformational change may occur in an unsynchronized fashion. From these mixing experiments, we have seen a loss of diffraction after mixing, indicating that many of these crystals do not survive the large conformational changes induced by ligand binding. To further study the rearrangement that occurs in the crystal lattice, we have explored using a photocaged ligand instead, where the photocaged ligand is soaked with the crystals. At a set of times prior to diffraction, the ligand is released upon UV light exposure. Preliminary experiments indicate that the photocaged molecule is released and induces a conformational change in crystallo as observed by microscopy and changes in the crystal unit cell dimensions.

443 Influence of conformationally restricted nucleosides on model RNAs structure.

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RNA performs important functions in the cell and more than 75% of DNA is transcribed into RNA. There is a relatively small number of three-dimensional RNA structures in the database but that piece of information is very useful in improving the prediction of RNA structure. Studying more RNA motifs can make the prediction process more accurate. Moreover, knowledge about those motifs can give us a better understanding of the interactions determining these motifs. In the presented studies we used oligonucleotides with restricted conformations. We synthetized oligoribonucleotides carrying AG/GA tandem mismatch containing several guanosine analogues. This mismatch carries great potential due to unpaired G residue, which is very often responsible for RNA interactions with other molecules. Selected analogues incorporated in oligonucleotides are conformationally restricted which means that their puckering or N-glyosidic bond is more or less fixed. By modifying 8 position of nucleobase we determined the *syn* conformation of the base and by modifying the sugar ring the puckering is affected. By chemically changing the sugar residue of the nucleotide we can increase the North and South puckering conformation in such a nucleotide. Using UV melting methods, NMR spectroscopy and PAGE we examined a series of RNA model oligonucleotides with self- and non-selfcomplementary sequences.

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444 Local-to-global signal transduction at the core of a manganese specific riboswitch

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The *yybP-ykoY* class of riboswitches regulates the expression of genes involved in Mn^{2+} homeostasis in bacteria through directly sensing the Mn^{2+} ion. The riboswitch adopts an H-shaped structure with two docked stem-loops connected by a coaxially stacked 4-way junction. Loops L1 and L3 form the docking interface between the stems and contain two divalent metal ion binding sites. While one site allows binding of either Mg^{2+} or Mn^{2+}, the other site strongly favors binding of Mn^{2+}. The ability of the RNA to selectively discriminate and respond to Mn^{2+} over Mg^{2+} in the cellular environment makes this riboswitch a particularly intriguing gene regulatory system. However, the mechanism of this exquisite metal ion sensing riboswitch is still poorly understood. Here, we have solved the structures of a Mn^{2+}-sensing riboswitch from *Xanthomonas oryzae* that revealed partially unfolded conformations in the ligand-sensing region. Molecular Dynamics (MD) simulations of the structures show a locally polymorphic environment of the two metal binding sites, suggesting that Mn^{2+} binding may impact the RNA through a local-to-global conformational cascade. MD simulations further indicate that the stacking in L3 and conformations of the ligand-binding sites are sensitive to the identity of the metal ions in those sites. Using single-molecule FRET (smFRET), we observe a previously unknown undocked conformation that samples transient docked states in the presence of Mg^{2+}. By contrast, we directly demonstrate that a stable docked conformation is formed only upon the addition of sub-millimolar Mn^{2+}. Our observations further reveal cooperative binding of Mn^{2+} facilitated by concomitant binding of Mg^{2+}. Overall, our work reveals that fast docking of the loops observed in the presence of Mg^{2+} pre-organizes the riboswitch into a compact global structure with a local binding site poised for Mn^{2+}, which once occupied results in the stably folded conformation. Such high specificity and cooperativity between Mn^{2+} and Mg^{2+} in folding of a riboswitch underscores the importance of the coupling of local with global structure of RNA in general, laying the foundation for multiple roles of RNA structural motifs in regulating transcription.

445 Suppression of alternate pseudoknot fold expands the human telomerase template repertoire.

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Telomerase is a specialized reverse transcriptase that uses an internal RNA template to processively add DNA repeats to telomeres. The human telomerase catalytic core consists of two components, the reverse transcriptase TERT, and the telomerase RNA hTR with an essential template/pseudoknot domain. To understand human telomerase specialization for synthesizing TTAGGG repeats, we carried out extensive template mutagenesis and screened the mutants for activity. We observed that template mutations can independently alter telomerase activity, repeat addition processivity, rate of repeat addition and the repeat permutation profile. Variation was not only dependent on the mutated template position, but also on the sequence change. Interestingly, we discovered that some template mutations trapped hTR in a proposed alternate conformation with a template-inaccessible pseudoknot fold. Using a compensatory mutation strategy that suppresses the alternate pseudoknot fold, we were able to recover activity for various altered template mutants. We propose a model where an alternate pseudoknot fold intermediate plays a role in active human telomerase assembly, which would establish a common principle of folding intermediates for telomerase RNAs from ciliates and vertebrates en route to telomerase ribonucleoprotein assembly and activation.

446 Simulating the miR-34a-mSIRT1 conformational rearrangement using Replica Exchange Molecular Dynamic simulations to assess miRNA activity

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Micro RNAs are small non-coding RNAs which regulate at least 30% of all protein translation. A single miRNA is complementary to partial sequence (seed region) of many mRNAs and beyond the seed region is extensively imperfect and follows different patterns. Understanding of the structure-function of miRNA and miRNA-mRNA recognition requires fundamental insight in the structural thermodynamics and mRNA binding stability. In this work, we will perform Replica Exchange Molecular Dynamic (REMD) simulations using NMR constraints provided by our experimental collaborators to model the dynamics and motion of miR-34a-mSIRT1 and characterize possible states adopted by miR-34a-mSIRT1. These transient states are in equilibrium with ground state which enable us to assess the mRNA stability and explain intrinsic preference of miRNA for each target. We will run REMD simulations over a broad range of temperature to capture these thermally accessible transient states by clustering tool of GROMACS using RMSD. The states we will report are in an agreement with secondary structures from NMR data. We conjecture the structural preferences of highly bulged region enable us to explain the microRNA preferences for target recognition.

447 Monitoring the 30S binding dynamics to a tbpA riboswitch in real time

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Riboswitches are regulatory elements located in the 5'UTR of mRNAs. They control gene expression by altering their structure in response to a small metabolite. In this study we focus on the thiamin pyrophosphate (TPP)-sensing riboswitches that are the most commonly found and have been identified in the three domains of life. In *Escherichia coli*, three riboswitches responding to TPP are involved in the control of *thiC*, *thiM* and *tbpA* operons. In contrast to *thiC* and *thiM* riboswitches that are regulating thiamine biosynthesis, the *tbpA* riboswitch is predicted to modulate the *thiBPQ* operon involved in ABC thiamin transport. The secondary structure of the *tbpA* riboswitch suggests that it operates at the translational level. In this mechanism, TPP binding to the riboswitch results in the sequestration of the Shine-Dalgarno sequence and in the inhibition of translation initiation. To directly visualize riboswitch translational control, a fluorescent 30S subunit was designed. Employing such an approach will allow to monitor 30S binding on *tbpA* riboswitches at the single-molecule level. Toeprint and RNase H assays have been used to confirm binding between the fluorescent 30S subunit and *tbpA* transcripts. Furthermore, by incorporating two fluorescent dyes on the *tbpA* riboswitch, we will also be able to monitor the FRET intensity. This will allow the monitoring of FRET and the 30S binding dynamics simultaneously on a single *tbpA* riboswitch. Overall, the comprehension of the 30S subunit binding to the t*bpA* riboswitch at the single-molecule level will provide insight into TPP riboswitch regulation mechanisms.

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448 Altering the rate of translation by ribosome-induced conformational switching of a structured 3'UTR.

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In addition to the well-known 5' cap and 3' poly(A) tail, it is becoming increasingly clear that signals based on structured RNA elements within a message's 3' untranslated region (3' UTR) can enhance translation in eukaryotes; these are poorly understood. To address this, we are using a powerful model system from the turnip yellow mosaic virus (TYMV). The TYMV viral RNAs are 5'-capped but instead of a poly(A) tail, they terminate in a highly structured 3' UTR that contains two structural domains: an upstream pseudoknot domain (UPD) and a tRNA-like structure (TLS). These domains work together to enhance translation, making this system an intriguing model for learning fundamental rules of how a complex structured 3' UTR affects protein synthesis. Using a combination of biochemistry, translation assays, structural biology, and biophysics, we have discovered that the two RNA domains form a conformationally dynamic higher-order architecture in which interactions between the domains affect translation enhancement. Furthermore, the RNA structure appears to act as a ribosome-induced structural switch through programmed global conformational changes. Using multi-dimensional chemical mapping, mutagenesis, and functional assays we have characterized this interaction and identified novel point mutants that affect the interaction and either increase or decrease function. Finally, we have solved the structure of the entire 3' UTR by x-ray crystallography, giving novel insight into the architecture and structural details of the RNA and illuminating how a structured, dynamic, multi-domain RNA at the 3' end can regulate translation.

449 RNA Duplex Destabilization Due to Cosolvent Effects

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RNA thermodynamic parameters are currently determined for a buffered 1 M NaCl solution, which is not characteristic of the native cellular environment containing large macromolecules and organelles as well as small-molecule crowding agents like metabolites, sugars, and amino acids. Cosolvents, such as alcohols and amides, share many of the same properties as common small-molecule crowders and can be used to mimic the effect of these molecules. By studying the properties, structures, and degree of destabilization due to each cosolvent, biologists and chemists can gain insight into how small molecules might affect RNA stability within the cell. To study these effects, eight self-complementary RNA duplexes of varying length, fraction of GC content, and sequence composition were chosen. Optical melts were performed on these sequences in a buffer consisting of standard salts, 10 mol% cosolvent, and 90 mol% water to obtain thermodynamic parameters and melting temperatures for each sequence and cosolvent. Comparison of ΔG°37 and Tm values shows that all RNA duplexes were destabilized in all cosolvent environments when compared to the standard aqueous buffer. On average across all studied sequences, cosolvents destabilized select RNA duplexes from 1.4-4.8 kcal/mol when compared to the predicted nearest-neighbor ΔG°37. It was also noted that shorter duplexes were less destabilized than longer ones and that sequence is important in determining degree of destabilization.

450 Structural insights into improved riboswitch regulation from natural variation

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Riboswitches are ubiquitous RNA motifs that regulate gene expression in response to specific metabolite binding. For example, ZTP riboswitches regulate genes essential to purine and folate biosynthesis by binding ZTP or closely related ZMP, which accumulates when cells are starved of folate. ZTP riboswitches bind ZMP and ZTP with a highly conserved two-domain core interspersed with variable regions of largely unknown function. We previously demonstrated that the variable linker connecting the two conserved domains modulates the affinity of the riboswitch for ZMP in a length-dependent manner (Jones and Ferré-D'Amaré, *NSMB* 2015). However, the role of secondary structure in these regions remains a mystery-why do some bacteria utilize ZTP riboswitches with additional structural regions while others do not? To answer this question, we solved the structure of the *Clostridium beijerinckii* ZTP riboswitch at 2.52 Å resolution via X-ray crystallography. Compared to our previous structure of the *Fusobacterium ulcerans* ZTP riboswitch, the *C. beijerinckii* RNA contains an additional helix connecting the conserved domains and stacking on a conserved helix of the first domain. In transcription assays mimicking the riboswitch's authentic activity in regulation transcription termination, we show that the *C. beijerinckii* riboswitch has a greater dynamic range than the *F. ulcerans* riboswitch. We hypothesize that the additional helix stabilizes the folding of the first domain, which is transcribed first, and thus reduces misfolding, leading the *C. beijerinckii* riboswitch to be a superior switch with a greater dynamic range. Taken together, this work demonstrates that natural variation in riboswitches can play a structurally stabilizing role to augment riboswitch output.

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451 Nucleobase-Water Stacking Interactions in RNA Molecules: Bioinformatics and Quantum Mechanics Analysis

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The folding of RNA molecules relies on a spectrum of stabilizing forces, with base-base stacking and base-base, base-backbone and backbone-backbone H-bonding interactions as the primary factors. Nevertheless, a survey of the RNA structures in the Protein Data Bank (PDB) revealed that, apart from the base-base stacking, numerous instances were identified where a nucleobase is stacked on a water molecule. This analysis evidenced that the nucleobase-water stacking can involve all the four nucleobases (A, U, G, C) and occurs in a wide variety of RNA molecules, including tRNAs, riboswitches, and in the ribosomes. This prompted us to perform an accurate statistical analysis of the nucleobase-water stacking interactions observed in crystallographic structures. Further, to have a clear evaluation of the energetic strength of these hitherto uncharacterized interactions, we performed state-of-the-art quantum chemical calculations, similar to those used to characterize stacking and H-bonding interactions between bases (1-5).

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452 NMR studies of an RNA thermosensor

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RNAs play myriad regulatory roles in biological systems. Riboswitches are noncoding RNAs that modulate gene expression as a function of ligand or metabolite concentration. RNA thermosensors (RNATs) are "riboswitch-like" regulatory elements located in the 5' untranslated region of a messenger RNA that control the translation of the downstream gene as a function of temperature. In *Listeria monocytogenes*, the expression of virulence genes is controlled by PrfA, a transcriptional activator whose own expression is temperature regulated. The secondary structure of the prfA RNAT has been predicted based on chemical probing and mutagenesis studies. Here, we present our progress towards the three-dimensional structure determination of the prfA RNAT. We employed a deuterium-edited nuclear magnetic resonance (NMR) spectroscopy approach to obtain high-quality spectroscopic data of both low temperature (translation inhibited) and high temperature (translation permissive) states of this large RNA. We show that the prfA RNAT undergoes a cooperative structural remodeling event over a very narrow temperature range. Additionally, we have employed a GFP reporter assay to characterize the effects that various structurally stabilizing and destabilizing mutations have of the temperature sensing mechanism.

453 Chemo-enzymatic synthesis of position-specifically modified RNA for biophysical studies including light control and NMR spectroscopy

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Interest in RNA structure determination has increased in recent times due to the discovery of many functional RNAs and the existence of a clear structure-function-relationship for RNA. Current approaches of RNA sample preparation for structure investigation with NMR spectroscopy, which has become a useful method for RNA structure-function analysis, are usually time-consuming. Here, we present a high throughput method for rapid and native preparation of RNA samples with purities comparable to PAGE purification. We show the applicability of the screening procedure by monitoring the change in ligand affinity with increasing RNA chain length for the 2'dG-sensing riboswitch from Mesoplasma florum.[1]

Further, a chemo-enzymatic method for synthesis of position-specifically modified RNA for structure-function investigation with NMR spectroscopy was established. Utilizing this three-step enzymatic method, we have synthesized RNA fragments containing photocaged nucleobases, azobenzene C-nucleosides or 13C,15N labelled nucleosides at a specific position. We applied this approach for the synthesis of a light-inducible 2'dG-sensing riboswitch and detected ligand-binding and structural reorganization upon irradiation by NMR spectroscopy. This method opens the possibility to incorporate a wide range of modifications including nucleotides carrying non-natural nucleobases at any desired position of RNAs of any lengths with standard laboratory equipment and beyond the limits of solid-phase synthesis.[2]

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454 Computer Folding of guanine quadruplexes

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Guanine quadruplexes represent key players in many cellular processes and are important as drug targets. However, despite of many of structural studies focusing on guanine quadruplexes, there is still limited knowledge on their folding. The crucial problem of study of folding of biomolecules is that there are no experimental methods to monitor folding at the atomistic level. Nevertheless, the computer folding can help to visualized structural details of folding of biomolecules and can allow to understand the process at the atomistic resolution. Today, the folding of biomolecules represents an important challenge of computational structural biology.

In this study, we focused on the folding of guanine quadruplexes using an extended set of standard and enhanced sampling MD simulations. The aim of this study was to examine folding landscape of RNA and DNA parallel G-hairpins with propeller loop and to identified folding and unfolding pathway of the parallel G-hairpins. Our results show that the general mechanism of formation of the parallel G-hairpins can be extended also for propeller loops with other sequences. Moreover, we demonstrated that the folding mechanism of DNA parallel guanine quadruplexes may differ from folding pathway of RNA parallel guanine quadruplexes due to presence of antiparallel and hybrid guanine quadruplex topologies on the folding landscape, which increases the complexity of the folding process.

455 The formation of intramolecular secondary structure brings mRNA ends in close proximity

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A number of protein factors regulate protein synthesis by bridging mRNA ends or untranslated regions (UTRs). mRNA can form extensive intramolecular secondary structure *in vitro* and *in vivo*. Prior computational predictions suggested that the formation of RNA secondary structure tends to bring the 5' and 3' ends of RNA into close proximity. Using experimental and computational approaches, we show that mRNAs from various organisms, including humans, have an intrinsic propensity to fold into structures in which the 5' end and 3' end are ≤ 7 nm apart irrespective of the mRNA length. The 3' poly(A) tail is not involved in intramolecular basepairing interactions, which bridge the ends of the 5' and 3' UTRs. In addition, single-molecule Förster resonance energy transfer (smFRET) measurements demonstrate that instead of adopting a single structure, each mRNA folds into a dynamic ensemble of structures with multiple end-to-end distances.

Computational estimates performed for ~22,000 human mRNAs indicate that the inherent closeness of the ends is a universal property of most, if not all, mRNA sequences. Only specific RNA sequences, which have low sequence complexity and are devoid of guanosines, are unstructured and exhibit end-to-end distances expected for the random coil conformation of RNA. Our results suggest that the intrinsic proximity of mRNA ends may facilitate binding of translation factors that bridge mRNA 5' and 3' UTRs. Furthermore, our studies provide the basis for measuring, computing and manipulating end-to-end distances and secondary structure in mRNAs in research and biotechnology.

456 Withdrawn

457 Co-crystal structure of a T-box riboswitch 3′ domain in complex with its cognate tRNA

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T-box riboswitches are bacterial gene-regulatory mRNA elements that sense and regulate amino acid availability through a multi-partite mRNA-tRNA interaction. T-box RNA is comprised of two conserved domains, a Stem I domain and a 3' antiterminator domain connected by a flexible linker. Stem I domain recognizes the overall shape and anticodon of a cognate tRNA with sequence and structural specificity. The antiterminator domain detects the molecular volume of the tRNA 3' end to sense aminoacylation. This readout determines the formation of either an intrinsic transcription terminator or antiterminator. Despite the recent progress in elucidating the T-box structure and mechanism, the architecture of a full-length T-box in complex with its cognate tRNA and the detailed interactions between the tRNA 3' region and T-box 3' domain remain poorly defined.

In this study, we develop a method to produce, assemble, and isolate stoichiometric complexes of the full-length T-box RNA bound by its cognate tRNA. We define a minimal region of the T-box that is both necessary and sufficient to bind an uncharged tRNA selectively, and determine the co-crystal structure of the complex at 2.7 Å. The crystal structure shows that 3' end of the cognate tRNA is buried inside the antiterminator. A conserved G•U wobble pair at the base of helix A2 is adjacent to the ribose 3'-hydroxyl group of the terminal adenosine of the uncharged tRNA. This juxtaposition creates steric clash between the universal amino group of the esterified amino acid and the uridine nucleobase, therefore providing a general mechanism to sterically exclude any aminoacyl tRNA.

We also report a cryo-EM structure of the full-length T-box riboswitch in complex with its cognate tRNA. The EM structure reveals that T-box Stem I domain and the newly defined 3' domain simultaneously bind two faces of the cognate tRNA, orchestrated and facilitated by a highly ordered inter-domain single-stranded linker. Taken together, the structures show that extensive intermolecular stacking allows the uncharged tRNA to be sandwiched in between the T-box Stem I and antiterminator domains to form a continuous helical stack, which stabilizes the antiterminator to transcribe downstream genes.

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458 Utilizing Chemical Tools to Explore the Role of Secondary and Tertiary Structural Motifs in MALAT1-driven Cancer Processes

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Long non-coding RNAs (LncRNAs) are classified as transcripts greater than 200 nucleotides (n.t.) in length that are not translated into proteins; despite this fact, lncRNA are implicated in various cellular processes such as the regulation of alternative splicing under normal expression levels as well as cancer metastasis when expression is dysregulated. These malignant cancer phenotypes may be enabled by lncRNA's formation of modular structures. For example, the human lncRNA Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) is found in nuclear speckles and is involved in pre-mRNA processing, but overexpression of MALAT1 is causative in colorectal cancer metastasis. It is well established that a triple helix at MALAT1's 3'-terminus promotes MALAT1 accumulation in HEK293T cells and destabilization or deletion of this triple helix-forming sequence promotes MALAT1 degradation. However, the existence and role of the triple helix in driving MALAT-1 related oncogenic functions is currently unknown. Recently, small molecules offering spatiotemporal control have shown selectivity towards particular RNA structure elements and have shifted their equilibrium. We hypothesize that such small molecules may be used to favor the triple helical as well as alternate structures at the 3'-terminus of MALAT1 and elucidate their impact on the oncogenic properties of MALAT1. So far, our secondary structure analyses support the formation of a triple helical structure previously observed by crystallography; however, there is additional evidence for the formation of tandem hairpin structures. Here, we will use a variety of techniques to identify the structures and structure populations of MALAT1. Small molecules will then be used to selectively shift the equilibrium to favor the lowly populated structures and measure their effect on MALAT1 stability via *in vitro* deadenylation assays and *in cellulo* transcript accumulation assays. This work will potentially provide the first small molecules that alter MALAT1 stability by shifting the structure population equilibrium and will thereby reveal the structure-function relationship between the modular 3'-terminus of MALAT1 and cancer metastasis.

459 Screening assays to identify small molecule inhibitors targeting the MALAT1 triple helix

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Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is a ~7-kb long noncoding RNA that promotes tumor metastasis and cancer proliferation. Its high abundance in cancer cells is due to a 3' triple helix that masks the 3' terminus of the transcript and prevents its degradation by the exosome. Disruption of the MALAT1 triple helix (M1TH) in vitro by mutation or altered buffer conditions promotes transcript degradation. We hypothesize that small molecule (SM)-induced disruption of the triple helix will similarly promote M1TH degradation and lead to the discovery of novel cancer therapeutics. We develop two orthogonal in vitro assays to evaluate SM-induced disruption of the triple helix. First, our combined FRET/differential scanning FRET (DS-FRET) assay simultaneously determines SM-induced conformational and stability changes of the triplex. Currently, we have used this assay to screen a 32-compound library of M1TH-binding compounds as a training set. While all compounds were previously shown to bind M1TH by a small molecule microarray screen, only a subset of these compounds induce changes to its conformation or triplex stability. We have identified multiple classes of hits based on responses to both signals, and confirm that some compounds preferentially interact with distinct conformations of the triple helix. Second, we are developing a biochemical degradation assay using RNase R to directly monitor SM-induced 3'-5' exonucleolytic triplex degradation. These assays can be used independently as primary screens, or successively as a primary screen/secondary follow-up assay. We anticipate that evaluating both conformational changes and functional output will increase the likelihood of identifying active hits rather than simply RNA binders. Following assay development using our 32-compound training set, we will implement screens utilizing these assays against a pilot library composed of compounds sharing physicochemical properties with known RNA-binding molecules.

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460 An RNA Pseudoknot Stimulates Human T-cell Lymphotropic Virus Type 1 *pro-pol* -1 Programmed Ribosomal Frameshifting

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An estimated ten million people are infected with human T-cell lymphotropic virus type 1 (HTLV-1). Approximately 5% of these infections are associated with adult T-cell leukemia/lymphoma, an aggressive form of leukemia/lymphoma. Establishment of new HTLV-1 infections is dependent upon the reverse transcription of the viral positive sense RNA genome and the integration of the resulting DNA into the host genome. Both processes require viral enzymes that are expressed by way of two independent one nucleotide (-1) programmed ribosomal frameshifts (PRF). These events occur at the *gag-pro* and *pro-pol* open reading frame junctions and are stimulated by *cis*-acting RNA elements within the viral transcript. Each frameshift site includes a heptanucleotide slippery sequence followed by a downstream structure. While -1 PRF and the slippery sequence of the *pro-pol* frameshift site has been established in HTLV-1, its structure has not been experimentally determined. Here, we report the HTLV-1 *pro-pol* frameshift site structure and demonstrate the functional relevance of this structure to -1 PRF. We combined nucleotide reactivities derived from chemical probing experiments with computational secondary structure prediction to determine the HTLV-1 *pro-pol* frameshift site secondary structure. Our results demonstrate that the frameshift site structure is a pseudoknot. This structure is consistent with the pseudoknot structure previously predicted using solely computational methods. Next, we evaluated the importance this structure to -1 PRF stimulation. We measured the *in vitro* frameshift efficiencies of the wild-type HTLV-1 *pro-pol* frameshift site and that of four frameshift site variants. The structures of these variants were also experimentally determined. We report a frameshift efficiency of 19.8(±0.9)% for the wild-type frameshift site. Significantly, the frameshift efficiencies produced by frameshift site variants that disrupt the pseudoknot are all reduced relative to the wild-type frameshift efficiency. These results support our hypothesis that a pseudoknot structure is critical to HTLV-1 *pro-pol* frameshift stimulation.

461 Use of SHAPE to study *in vivo* RNA folding in *Entamoeba histolytica*

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Selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) is a versatile sequence independent method to probe RNA structure *in vivo* and *in vitro*. It has so far been tried mainly with model organisms. We show that cells of *Entamoeba histolytica*, a protozoan parasite of humans is hyper-sensitive to the *in vivo* SHAPE reagent, NAI, and show rapid loss of viability and RNA integrity. We optimized treatment conditions with 5.8S rRNA and Eh_U3 snoRNA to obtain NAI-modification while retaining RNA integrity. We observed that the *in vivo* folding was different from *in vitro* and correlated well with known interactions of 5.8S rRNA with proteins *in vivo*. Interestingly, the Eh_U3 snoRNA also showed many differences between its *in vivo* and *in vitro* folding, which correlated well with conserved interactions with 18S rRNA and 5'-ETS. Further, Eh_U3 snoRNA obtained from serum-starved cells showed an open 3'-hinge structure, indicating disruption of 5'-ETS interaction. This could contribute to the observed slow processing of pre-rRNA in starved cells. Our work shows for the first time, the applicability of SHAPE to study *in vivo* RNA folding in a parasite and will encourage the use of this reagent for RNA structure analysis in other such organisms.

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462 Ligand-driven Changes in Spinach2 and Broccoli Aptamer Affinities.

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Although the folding of large RNAs can be complex and involve local folding minima that proteins can aid in overcoming, the folded structures of shorter RNAs such as aptamers are generally assumed to have reached a global energy minimum when bound with their ligands. Typically, ligand binding by aptamers is associated with a structural changes that occur in msec or shorter time frames, which can be exploited for detecting ligand binding by an aptamer, particularly of light-up aptamers. The kinetics of ligand binding to Spinach2 and Broccoli light-up aptamers similarly bind their DFHBI ligand in similar brief time spans. However, certain fluorinated ligands demonstrate a subsequent interaction with these aptamers that takes an extended time (half-life of 35 min) to achieve. The outcome of this slow transformation is a 3 to 10-fold increase in affinity for these ligands and the achievement of ultimate affinities that are 10-40-fold higher than for DFHBI. The change in affinity follows a first order reaction, which suggests a single rate-limiting event. We have identified a single adenosine in both aptamers for which the precise location of the amine group is essential to initiate the affinity change. SHAPE analysis of this aptamer is used to establish the effect of fluorinated ligand on aptamer shape. These results suggest that most ligand-occupied Spinach2 and Broccoli aptamer complexes are trapped in a local structural minimum that they can be shifted from by a hydrogen bond-driven event that is initiated on binding certain ligands. This work was funded by the National Institutes of Health.

463 Structure, dynamics and protein interactions of the 7SK RNP

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Transcription regulation in eukaryotic cells is a highly-regulated process, controlled in part by the availability of positive transcription factor P-TEFb, which is composed of CDK9 and CycT1. The non-coding 7SK RNA, in complex with several proteins, binds to P-TEFb and selectively sequesters this kinase into an RNP complex, preventing transcriptional activation. Tat-dependent transcription from the HIV-1 promoter appears to be much more sensitive to P-TEFb knockdown or inhibition by certain kinase inhibitors (incl. flavopiridol) than most other cellular genes and direct knockdown of the 7SK RNA reverses HIV latency in cell culture. Thus, disrupting the P-TEFb sequestration function of the 7SK RNA represents a plausible strategy for HIV latency reversal. Conformational changes in the 7SK RNA appear to contribute to release of P-TEFb and subsequent transcriptional activation. Exploratory studies from the current period of support show that it is possible to examine the structure of the 7SK RNP and to detect binding by most or all protein components of the P-TEFb-bound form in both Jurkat and primary donor cells. The Aimes of this project are to define the structure of the 7SK RNA and its network of protein interactions under conditions favoring P-TEFb bound (HIV transcriptionally inactive) and P-TEFb-free states and identify structures in the 7SK RNA that, when perturbed by small molecules, would favor release of P-TEFb from the 7SK RNA and subsequent activation of HIV transcription.

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464 Global structural remodeling as an evolutionary response to sequence duplication in RNA

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Multiple RNA and protein structures in modern biology feature repetitive elements that suggest evolution through duplication of smaller motifs; artificial duplications have also been employed to increase the efficacy of RNAs and proteins designed for biotechnology applications. Little experimental data is currently available concerning the structural and evolutionary consequences of duplicating biopolymer sequences in either of these contexts. To assess the effects of sequence duplication on the evolution of a functional RNA, we designed and mutagenized an RNA sequence containing two copies of an ATP aptamer, and subjected the resulting population of mutants to multiple *in vitro* evolution experiments under differing selection conditions. The populations were reselected for the ability to bind ATP, and were also subjected to altered selection pressures designed to favor mutants capable of binding to ATP and GTP simultaneously. While these altered selection pressures did not result in RNAs with clear dual-binding activity, they did favor mutants exhibiting large-scale changes in secondary structure that disrupt all of the initial base pairs, while preserving the original ATP binding motifs. These results show that extension of an RNA sequence via duplication enables a rearrangement of the secondary structure that retains the initial functionality of the RNA while conferring additional adaptive benefits.

465 Crystal structure determination of riboswitches and BsMiniIII endoribonuclease-assisted production of recombinant RNA

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RNA molecules play a variety of significant roles in cells, including regulation of gene expression, RNA processing, RNA splicing, RNA editing, RNA modification, protein synthesis, protein targeting, and telomere maintenance. Understanding of RNA three-dimensional structures has given extraordinary insight into the molecular basis for all of these processes. The major interest of our lab is devoted the molecular and structural biology of RNA and of RNA-modifying proteins. Within the first subject, we aim at providing structural information for representatives of selected RNA families using X-ray crystallography. As crystallization of RNAs remains a formidable experimental challenge, we further employ SAXS (small angle x-ray scattering) and structure probing methods like SHAPE (selective 2′ hydroxyl acylation analyzed by primer extension), as well as computational modeling of 3D structures of RNA molecules (SimRNA software). The resulting structural models are deposited in the RNArchitecture database – a classification system of RNA families, with a focus on structural information. Within the second subject, we study RNA-modifying proteins and apply the obtained knowledge to the engineering of these proteins. Previously, we have shown that Mini-III endoribonuclease from Bacillus subtilis specifically recognizes and cleaves ACC^U sequence in double stranded RNA substrates. Development of enzymes that would be able to cleave RNA in a sequence specific manner, similar to restriction enzymes that cut DNA, could greatly facilitate studies on the structure and function of RNA molecules. Currently, we are exploring potential applications of BsMiniIIIs in a wide range of RNA manipulation techniques. Particularly we aim at developing tools and protocols for the use of BsMiniIII in the production of homogeneous samples of RNA molecules with defined sequences and structures which can be further used in RNA crystallography and nanotechnology. Here we present our workflow and preliminary results.

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466 Unwinding the double pseudoknot of the HDV-like ribozyme family

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The Hepatitis delta virus (HDV) ribozyme is one of the best studied ribozyme.s The native fold of the ribozyme is complicated, having two pseudoknots involving five helical regions (P1.1, P1, P2, P3, and P4) [1]. The HDV ribozyme has a remarkably stable structure, demonstrating self-scission activity in up to 18 M formamide condition [2]. Until 2006 the HDV ribozyme was considered to be the only representative of a small ribozyme with such a complex fold. However a genome-wide search identified the human cytoplasmic polyadenylation element-binding protein 3 (hCPEB3) ribozyme as the first of the HDV-like ribozymes. Since this day, the HDV-like ribozyme family has largely expanded. Indeed, the HDV-like ribozymes are found in nearly all branches of life [4]. These ribozymes display the secondary structure in a nested double-pseudoknot with only six conserved nucleotides fulfilling functional or structural roles including the protonated form of cysteine C 75 (according to the HDV ribozyme nomenclature) essential for the catalytic activity of the ribozyme. To date, only the HDV ribozyme structure is available. In order to better understand the complex folding that leads to cleavage activity of the HDV- like ribozymes, we decided to use X-ray crystallography to solve the three dimensional structure of the HDV-like ribozymes.

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467 Relating thermodynamics to structure: A comparative NMR analysis of an RNA duplex before and after adenosine deamination

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An enzyme family known as adenosine deaminases that act on RNA (ADARs) catalyze the conversion of adenosine (A) to inosine (I), which often creates an I·U base pair. Previous thermodynamic characterization of RNA duplexes containing I·U pairs showed that, on average, an RNA duplex with an internal I·U pair is 2.3 kcal/mol less stable than the same duplex with a corresponding A-U pair. To better understand the conformational effects of adenosine deamination on RNA structure, scientists have begun to study the structure of inosine-containing RNA duplexes; however, no direct structural comparison has been made between an RNA duplex containing an I·U pair with one containing the corresponding A-U pair. In order to shed structural light onto the thermodynamic differences in stability, NMR data of an RNA duplex containing an internal A-U pair is compared to the same duplex containing an I·U pair. Solution structures of both duplexes will be compared.

468 Probing RNA secondary structure in the activated yeast spliceosome

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During eukaryotic splicing, noncoding sequences (introns) within precursor-messenger RNAs (pre-mRNAs) are removed and the remaining coding sequences (exons) are ligated together to form mature mRNA. Pre-mRNA splicing is achieved by a multi-megadalton RNA-protein complex called spliceosome. Unlike most RNA or protein enzymes, the spliceosome is a dynamic machinery that involves five main small nuclear ribonucleoprotein (snRNP) complexes that associate, rearrange and dissociate during at least seven distinct steps of splicing, including two chemical catalysis steps. In the splicing cycle, the role of pre-mRNA is often viewed as that of a passive substrate. However, recent reports have indicated that pre-mRNAs can actively influence regulation and catalytic efficiency of splicing. Our goal is to elucidate the impact of intron secondary structure on splicing activity.

To this end, we use Saccharomyces cerevisiae as our model system. Using a temperature-sensitive strain carrying a TAP-tagged Cef1 protein (Prp2-1, Cef1-TAP), we isolated yeast Bact complexes (the activated complexes right before the first catalytic step) with their native substrates. Bioinformatic analysis of RNA sequencing data found significant enrichment of >90% of the known pre-mRNA targets, as well as revealed several novel splicing mediated decay substrates. With this high-confidence list of spliceosomal substrates in hand, we attempted to probe the RNAs in Bact using selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP), achieving only low sequencing depth for the pre-mRNAs. We are now instead using the recently described DMS-MaP technique, wherein yeast cell extract (for in vivo RNA probing) or purified Bact complexes (for in vitro RNA probing) is treated with dimethyl sulfate (DMS). DMS is highly cell-permeable and reacts rapidly with unpaired adenines and cytosines at their Watson-Crick edges. The modifications introduced by DMS will be read as mutations during reverse transcription of the modified RNA. This procedure is particularly suitable for genome-wide profiling of low-abundance RNA. We anticipate that a transcriptome-wide dissection of RNA conformations along the splicing cycle will complement recent cryo-EM structures and help dissect the roles of pre-mRNA secondary structure changes in splicing regulation.

469 Benchmaring RNA Force Fields using Hairpin Loop Folding Free Energy Change

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Estimating experimentally-observable quantities from molecular dynamics simulation can be used to benchmark simulation performance. For RNA, UV optical melting experiments can be used to quantify the structural stability for the folding of small oligonucleotides. We are using all-atom simulations to estimate the folding stability of RNA to benchmark force fields and also to identify RNA sequences with unexpected stability to study experimentally. We use umbrella sampling molecular simulations of three dodecamer hairpins, for which there are experimentally determined free energies of unfolding, to estimate the free energy change of stretching along the end-to-end (5' OH to 3' hydroxyl oxygen) distance. This mimics the reaction coordinate of single molecule pulling experiments. We estimate the free energy change of the transition from the native conformation to a fully extended conformation with no hydrogen bonds between non-neighboring bases. The fully extended conformation begins at 45 Å for each dodecamer. Each simulation was performed four times using the standard AMBER ff10 force field and each window, spaced at 1 Å intervals, was sampled for one microsecond, resulting in 552 μs of total sampling. We analyzed error along the estimated free energy curves (FEC) with the variance in the derivative of the FECs, a novel approach that indicates which windows vary in free energy. We compared differences in the simulated free energy estimates to analogous differences in experimental values using thermodynamic cycles. This avoids the problem of sampling the random coil, which provides little sequence specific information but is difficult to sample thoroughly. The residuals between differences in free energy changes estimated by simulation and those measured experimentally are 1.5 ± 0.6, 0.5 ± 0.6, and 1.0 ± 0.6 kcal/mol. This suggests that further improvements are necessary for the AMBER force field, but that the force field may be accurate enough to guide the choice of experiments.

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470 Time-resolved crystallographic studies of the adenine riboswitch aptamer domain

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Developments in serial crystallography using X-ray free electron lasers (XFELs) have begun a revolution in structural biology to the study structures of biochemical reactions in real time. Such experiments rely on the use of nano/microcrystals, whose large surface-to-volume ratios allow for increased crystal elasticity and rapid diffusion of ligands. We are interested in using time-resolved crystallography to understand the structural basis for gene regulation by riboswitches by determining the detailed conformational changes that occur upon ligand binding. Using this method for studying the adenine riboswitch aptamer domain (rA71), we determined the structure of a ligand-bound intermediate, and demonstrated that the rA71 molecules convert to the stable ligand-bound state *in crystallo*, resulting in a change to the crystal space group. Our results posed two intriguing questions: 1) How can a macromolecular crystal undergo such a phase transition, which involves substantial intra- and inter-molecular rearrangements, while maintaining detectable Bragg diffraction? 2) For those crystals that maintain diffraction throughout the transition, albeit at lower quality, can one capture discrete transitional structures during the ligand-triggered reaction and phase transition? We then performed a time-resolved series of mixing experiments for rA71 with delay times of 10s, 25s, 75s, 100s, 125s, and 175s after mixing with adenine. Preliminary data analysis through guided indexing and three-dimensional merging of diffraction patterns reveals crystallographic changes occurring within 10s, and that by 75s, the majority of crystals convert to one of two ligand-bound lattices. One of these corresponds to the known ligand-bound orthorhombic space group. The other is a pseudo-merohedrally twinned monoclinic lattice that is related to yet distinct from the bound orthorhombic lattice. In both cases, the bound conformation of rA71 is the same; however, the two lattices are non-superimposable. With additional data and further analysis, we aim to create a "molecular movie" of the conformational switching and lattice transition.

471 Structural determinants of exoribonuclease-resistant RNA elements

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Folded RNA elements (xrRNAs) that block processive 5'->3' cellular exoribonucleases to produce biologically active viral non-coding RNAs were discovered in mosquito-borne flaviviruses (e.g. DENV, WNV, ZIKV), where they play an important role for viral pathogenicity. During infection with these viruses, the host exoribonuclease Xrn1 is first recruited to the viral genome and then selectively stopped by xrRNAs in the viral 3'UTR. This selective protection from exoribonucleolytic degradation might represent a new mode of RNA maturation. However, it was unknown if this RNA structure-dependent mechanism exists elsewhere in biology, and if so, whether a conserved fold is required to block the 5'->3' exoribonucleolytic degradation machinery.

We demonstrate the existence of authentic xrRNAs not only in divergent members of the *Flaviviridae* family (Tamana bat virus (TABV) and members of the *Pegivirus* genus), but also in Dianthoviruses, plant-infecting viruses of the *Tombusviridae* family. These results demonstrate that xrRNAs are widespread among *Flaviviridae*, and that convergent evolution has created RNA structure-dependent exoribonuclease resistance in different contexts. This establishes exoribonuclease-resistance as a general RNA maturation mechanism and defines xrRNAs as an authentic functional class of RNAs.

Interestingly, xrRNAs from different virus families use very different folding mechanisms to confound exoribonucleases, but all rely on a shared unique topological feature: a pseudoknot that creates a protective ring around the 5' end of the RNA structure. Here we present the biochemical, virological and structural characterization of xrRNAs from divergent *Flaviviridae* and Dianthoviruses, and describe similarities and differences of their RNA-based exoribonuclease-resistance. Together, these data provide the framework for understanding the essential features of exoribonuclease-inhibition and thus lay the groundwork for identifying these elements elsewhere in biology.

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472 Versatile Tools Towards Real-time Single-molecule Biology

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Biological processes performed by proteins interacting with and processing DNA and RNA are key to cell metabolism and life. Detailed insights into these processes provide essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go awry.

The next scientific breakthrough consists in the actual, direct, real-time observations and measurements of the individual mechanisms involved, in order to validate and complete the current biological models.

Single-molecule technologies offer an exciting opportunity to meet these challenges and to study protein function and activity in real-time and at the single-molecule level.

Here, we present our efforts for further enabling discoveries in the field of biology and biophysics using both the combination of optical tweezers with single-molecule fluorescence microscopy (C-Trap).

We show the latest applications of these technologies that can enhance our understanding not only in the field of DNA/RNA-protein interactions but also in the fields of RNA mechanics and genome structure and organization.

These experiments show that the technological advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new venues in many research areas.

473 Is there a riboSNitch in the 3' UTR of Lipoprotein Lipase?

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Lipoprotein lipase deficiency (LPLD) is a genetic disorder that changes metabolism of fats resulting from a reduction in the amount of functional lipoprotein lipase. More than two hundred different causative genetic mutations have already been identified; however, a non-coding single nucleotide mutation in the 3' untranslated region (UTR) has also been associated with disease through genome wide association studies. Therefore we hypothesized that this single nucleotide change could be a riboSNitch that changes the structural ensemble of the RNA with possible functional consequences. First we used the algorithm SNPfold to predict whether or not this mutation would significantly change RNA folding and examined structural predictions. Based on these predictions, we designed small RNA constructs to evaluate whether or not the non-coding mutation changes the structural ensemble using both RNA structure mapping and mobility on native gels. The findings can contribute to our understanding of the mechanism of disease in LPLD.

474 An mRNA structure that affects aberrant expression of a proto-oncogene by eIF3 recognition

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Regulation of translation initiation is a critical step in protein synthesis and its deregulation is observed in human cancer. Translation rates for specific mRNAs are modulated by 5'-UTR cis-acting RNA structures required for recruitment of the 13-subunit, 800-kilodalton eukaryotic initiation factor 3 (eIF3) to activate translation initiation. However, little is known at the structural level regarding how eIF3 selectively recognizes 5'-UTR mRNA structure. Here, we use NMR to determine the structure of a 56-mer RNA stem-loop located in the 5'-UTR of the proto-oncogenic c-JUN mRNAs that is recognized by eIF3 and essential for efficient translation. C-JUN is aberrantly transcribed in glioblastoma cells, suggesting this interaction might mechanistically link eIF3 upregulation and tumor formation. The structure resembles eIF3-binding moieties from viral internal ribosomal entry sites, suggesting similar modes of eIF3 recognition. Structural and mechanistic insight derived from this study will contribute to a long-term effort for understanding the role of eIF3 in translational control in disease states and ultimately developing therapeutics targeting mRNA-eIF3 interactions.

475 Blind prediction of noncanonical RNA structure at atomic accuracy

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Prediction of RNA structure from nucleotide sequence remains an unsolved grand challenge of biochemistry and requires distinct concepts from protein structure prediction. Despite extensive algorithmic development in recent years, modeling of noncanonical base pairs of new RNA structural motifs has not been achieved in blind challneges. We report herein a stepwise Monte Carlo (SWM) method with a unique add-and-delete move set that enables predictions of noncanonical base pairs of complex RNA structures. A benchmark of 82 diverse motifs establishes the method's general ability to recover noncanonical base pairs *ab initio*, including multistrand motifs that have been refractory to prior approaches. In a blind challenge, SWM models predicted nucleotide-resolution chemical mapping and compensatory mutagenesis experiments for three *in vitro* selected tetraloop/tetraloop receptors with previously unsolved structures (C7.2, C7.10, and R1). As a final test, SWM blindly and correctly predicted all noncanonical pairs of a Zika virus double pseudoknot during a recent community-wide RNA-puzzle. Stepwise structure formation, as encoded in the SWM method, enables modeling of noncanonical RNA structure in a variety of previously intractable problems.

476 Effects of *in vivo-like* Conditions on G-quadruplex Formation

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Recent studies suggest that in vivo, RNA is less structured than originally predicted. Despite the extensive in vitro work completed on G-quadruplex structures, they remain relatively elusive in vivo due to sequestration of both the Watson-Crick and Hoogsteen faces of the guanines. To more closely mimic intracellular conditions, literature values for various ion concentrations were found for prokaryotic and eukaryotic cells. G-quadruplex sequences were exposed to both prokaryotic- and eukaryotic-like conditions, and folding was measured using a variety of biophysical techniques. Results indicate that spermine, a polyamine prominent in eukaryotes with a +4 charge, disfavors the folded state of certain G-quadruplex sequences. This indicates that some G-quadruplexes may be globally unfolded in eukaryotic cells with the mechanism that spermine interacts with the Hoogsteen face of the guanines, shifting the equilibrium towards unfolding. G-quadruplex unfolding could lead to interesting scenarios of gene regulation caused by spermine or potassium fluctuations.

477 Dissecting binding modes of near-cognate ligand analogs of the GTP class II RNA aptamer

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The GTP class II aptamer is one of 11 different classes of GTP-binding RNA aptamers that were in vitro selected by the SELEX procedure[1]. Despite its small size of 34 nucleotides it binds its cognate ligand GTP with a K_D in the nanomolar range. We solved the structure of the

RNA-GTP complex by solution NMR with a low overall rmsd (0.5 Å) in order to gain insight in the structural diversity of GTP recognition by different aptamers[2]. The RNA-GTP complex folds into a compact structure comprising inter- and intramolecular base triplets and even a base quartet with a stably protonated adenosine. The intricate interplay of hydrogen bonding interactions amongst the individual residues of the RNA and the recognition mode for GTP gave rise to extended studies of further RNA-ligand complexes of near-cognate GTP analogs.

For a detailed understanding of the role of individual functional groups of the ligand GTP to binding affinity and specificity we initiated structural and thermodynamic studies of the aptamer in presence of GTP analogs. Investigations using GMP and guanosine in the presence of different concentrations of Mg^{2+} show the influence of the negative charge of the phosphate group to ligand binding. Substituted analogs or analogs with perturbed hydrogen bond donors or acceptors as e.g. 6-Thio-GTP, 2`-dGTP or 2`-F-dGTP reveal the contribution of individual functional groups to ligand binding. Interestingly, the bacterial second messenger molecules 5',3- cdiGMP and 5',3-cGAMP are also recognized by the aptamer but lead to an altered structure of the binding pocket whereas the eukaryotic second messenger 2',3'-cGAMP is rejected by the aptamer. This suggests that the aptamer could be further developed into specific artificial sensors for bacterial cyclic dinucleotide second messenger molecules.

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478 An automated protocol to identify RNA sequence families that adopt similar tertiary structures: GNRA and UNCG tetraloops as a test case

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One of the significant challenges in RNA 3D structure prediction from sequence is the limited understanding of how noncanonical regions, such as those found in secondary structure motifs, fold into 3D structures. The RNA Classification of Secondary Structure Motifs (CoSSMos) database allows researchers to search for secondary structure motifs among 3D structures deposited in the Protein Data Bank. We have developed an automated protocol for searching the CoSSMos database for tertiary structure motifs among secondary structures that does not require a priori knowledge of structural motifs. In this method, RNA segments of the same secondary structure are grouped based on structural similarity. Sequence analysis and structural characterization are performed on each group. As a proof-of-concept, from the >4400 tetraloop structures deposited in the PDB, this protocol reveals groups corresponding to the well-documented GNRA and UNCG tetraloop motifs. Further application of this protocol may reveal undocumented tertiary structure motifs among secondary structures.

479 RNA-based regulation of gene expression to improve the biotechnologically relevant bacteria *Methylobacterium extorquens*

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Genetic tools based on RNA have emerged due to the ease with which they can be adapted to different gene targets according to predictable sequence base pairing; due to their efficiency in rapidly altering genome sequences; and due to gene expression in systems that have been traditionally challenging to work with. The clustered, regularly interspaced, short palindromic repeat (CRISPR) system is a good example of how RNA can be used to guide the Cas9 protein to target specific endogenous genes for genome editing. By using a mutant Cas9 with no endonuclease activity, the same guide RNA can instead be used to downregulate gene expression without producing a knockout. Another system of gene regulation is based on small RNAs (sRNAs) acting jointly with the Hfq protein. Synthetic sRNAs can be customized to repress the expression of a specific mRNA by including a sequence complementary to the target. This study focuses on *Methylobacterium extorquens*, a model organism to study the metabolism of one carbon compounds. This proteobacteria can metabolize methanol, a cheap raw material that can be produced from renewable sources and can be used as a biotechnological tool for production of value-added products. The engineered sRNA system and CRISPR system have already been proven useful in other proteobacteria, such as *Escherichia coli*, but they have not been used in *M. extorquens*. The Green Fluorescent Protein (GFP) was used as a reporter gene to evaluate repression by the CRISPR-Cas9 system and sRNAs. Once these systems are shown to be efficient in targeting the reporter gene, they can be used to target any other endogenous genes. We have already adapted *M. extorquens* for the production of succinic acid from methanol. The development of these genetic tools to perform gene silencing or overexpression within *M. extorquens* could further help improve our strain and encourage the use of *M. extorquens* for a methanol based-bioeconomy in the hope to become more independent of regularly increasing oil price.

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480 Expanding the RNA Mango Toolbox Through Multi-stem and Split Designs

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RNA Mango is a recently developed fluorogenic aptamer with a fluorescent signal comparable to GFP. This genetically encodable RNA label opens up numerous potential avenues for fluorescent quantitation and localization of RNA. The crystal structure of the RNA Mango confirmed a quadruplex structure, with a closing base paired stem that interrupts one face of the quadruplex. This has lead to a few successful designs that use the RNA mango to replace existing stem loops in structured RNA molecules. However, many loops in non-coding RNA molecules are functionally important, and are involved in either tertiary structure or protein binding interactions. The utility of Mango could be enhanced by alternative orientations of the quadruplex within or between paired regions of RNA. Here, we show data on the interruption of the RNA Mango at two faces of the G-quadruplex simultaneously and the resulting fluorescence of these constructs. We further demonstrate that these constructs enable a split RNA mango where two separate molecules come together to create the aptamer binding site. This split design opens the door for the detection of RNA-RNA interactions. We demonstrate the utility of this split design by detecting the interactions between the naturally occurring crRNA and tracrRNA of the CRISPR bacterial immune system. Importantly, some of our two stem and split designs are as bright as the original RNA Mango, making them viable alternatives for incorporating Mango into RNA structures of interest. Our results expand the RNA Mango toolbox and open up new labeling strategies.

481 A Study of Transcriptional Activation by the Transcription Factor Gal4 in Saccharomyces cerevisiae by 3D Orbital Tracking and in vivo RNA labelling

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Understanding what is going on at the molecular level within individual cells is challenging. But deciphering stochastic biomolecular processes is crucial for our understanding of gene transcription and the intricacies of cellular metabolism. With 3D orbital tracking, we are able to visualize and monitor pre-mRNA and transcription factors in real-time using fluorescent tagging within yeast cells at a high sampling rate. Our study demonstrates that we can track these molecules of interest, fluorescent-labeled GFP (pre-mRNA) and JF 646 dye (transcription factors), during the process of transcribing a gene that codes for metabolizing galactose. This method allows us to directly observe the movement of a single molecule and determine the time lag between the GAL4 transcription factor binding to DNA and the activation of the mRNA synthesis. The data we collected improves our knowledge of the details of transcriptional kinetics and how single celled eukaryotic organisms regulate transcription. This will expand our research on the transcription processes in similar genes and in multicellular organisms like humans. 3D orbital tracking opens up a new window for exploring fundamental biochemical processes through a dynamic view of single fluorescent molecules in living systems at high speed.

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482 Chemical modifications on synthetic guide RNA for improved RNA stability and cellular viability in CRISPR-Cas9 genome editing

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The CRISPR-Cas9 system has enabled easy modification of the genome in mammalian cells and has revolutionized the genome engineering field. Synthetic single guide RNA (sgRNA) or CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) offer an advantage over IVT sgRNA by providing little-to-no effect on the cellular immune response and allow for incorporation of modifications. However, unmodified single and dual-guide RNA for CRISPR are susceptible to exonucleases that can limit efficacy and excessively modified gRNA can reduce cell viability. We have systematically studied chemically modified guide RNA (gRNA) with one to three 2'-O-methyl nucleotides and backbone phosphorothioate linkages (MS) on the 5' and/or 3' ends. Our results show that incorporating 2 MS modifications on the gRNA are optimal for improved stability and cellular viability and may provide modest improvement in gene editing efficiency.

483 Applying riboregulator to knock down chromosomal gene *cyabrB2* in *Synechocystis* sp.PCC 6803 for higher glycogen production

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Cyanobacteria are one of the attractive hosts for biofuel production but genetic approach to knock down the certain chromosomal gene in cyanobacteria is still limited. To develop a new knock down method in cyanobacteria, we focused on a post-transcriptional gene regulation system, riboregulator [1].

Riboregulator consists of a *cis*-repressed mRNA (crRNA) and a *trans*-activating RNA (taRNA). The crRNA contains an artificially designed *cr*-sequence which hybridizes to the ribosomal binding site (RBS) and forms an internal stem-loop structure, resulting in repression of target gene expression. On the other hand, the taRNA has a sequence complementary to the internal loop region of the crRNA, so that the taRNA can activate the translation of the target gene by hybridizing to the crRNA and exposing the RBS. In recent years, riboregulators have been improved so that it can induce the target gene strongly. However, we thought that one of the remarkable features of riboregulator is the specificity of the target gene repression by crRNA. In this study, we focused on this feature which can be applied to chromosomal gene knock down.

As the target gene, we focused on the *cyabrB2* gene, encoding global transcription regulator in cyanobacteria. It was reported that accumulation of glycogen granules in the cells was observed when the *cyabrB2* gene was disrupted [2].

In this study, we chromosomally inserted riboregulator in the upstream of the *cyabrB2* gene and evaluated the transcription and expression level of the target gene. Also, we evaluated the effect of the expression level of cyAbrB2 on the glycogen accumulation and the transcription levels of other genes regulated by the cyAbrB2 were determined.

In the integrated mutant, the *cyabrB2* gene was knocked down successfully. Also, the high amount of glycogen accumulation and the decrease of transcription levels of other genes, *ftsQ, ftsZ, sigE* were observed in the mutant when *cyabrB2* was knocked down by the riboregulator. Altogether, it was shown that riboregulator could be applied to a new knockdown tool.

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484 Non-canonical translation initiation signals for synthetic biology

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Modulating the frequency of translation initiation is a universal strategy for controlling gene expression. Translation initiation in bacteria is controlled by fine-tuning interactions between the translation initiation region (TIR) of an mRNA and the ribosome, typically by varying Shine-Dalgarno (SD)-anti-SD complementarity and levels of inhibitory RNA structure within the TIR. Natural translation initiation control elements have been repurposed to tune synthetic genetic circuits, however limitations exist regarding their predictability, portability, and dynamic range. Interestingly, new studies have revealed alternate strategies for mRNA-ribosome recruitment; exploring their mechanisms will afford new approaches for controlling translation initiation. Here we characterize a non-canonical translation initiation element: the TIR of the *E. coli rpsA* gene. Although lacking a canonical SD, the *rpsA* TIR is one of the most efficient drivers of translation initiation in *E. coli*. Additionally, the typically inhibitory secondary structure within the TIR is somehow necessary for its function. We have built a quantitative, dual-reporter *in vivo* screening tool, and have mapped the sequence-function relationships of the *rpsA* TIR using large-scale mutagenesis and next-generation sequencing. Our resulting pipeline has generated a library of synthetic structure-based ribosome recruitment devices, which can be used to fine-tune translation initiation over several orders of magnitude. Candidate variants were analyzed *in vitro* using non-equilibrium filter binding, which revealed little correlation between initiation strength and ribosome binding affinity. However, small angle x-ray scattering (SAXS) experiments to examine the structure of these variants suggest the specific RNA-fold is responsible for modulating translation initiation efficiency. Three-dimensional *ab initio* models of *rpsA* TIR variants were generated using SAXS data, which provided constraints for *in silico* modeling of RNA tertiary structure. Our integrated *in vivo*, *in vitro*, and *in silico* approach gives new insight into this non-canonical mechanism of translation initiation, and provides a new class of synthetic regulatory RNA devices for engineering biology.

485 Computational Design of Asymmetric Three-dimensional RNA Structures and Machines.

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The emerging field of RNA nanotechnology seeks to create nanoscale 3D machines by repurposing natural RNA modules, but successes have been limited to symmetric assemblies of single repeating motifs. We present RNAMake, a suite that automates design of RNA molecules with complex 3D folds. We first challenged RNAMake with the paradigmatic problem of aligning a tetraloop and sequence-distal receptor, previously only solved via symmetry. Single-nucleotide-resolution chemical mapping, native gel electrophoresis, and solution x-ray scattering confirmed that 11 of the 16 'miniTTR' designs successfully achieved clothespin-like folds. A 2.55 Å diffraction-resolution crystal structure of one design verified formation of the target asymmetric nanostructure, with large sections achieving near-atomic accuracy (< 2.0 Å). Finally, RNAMake designed asymmetric segments to tether the 16S and 23S rRNAs together into a synthetic single-stranded ribosome that remains uncleaved by ribonucleases and supports life in *Escherichia coli*, a challenge previously requiring several rounds of trial-and-error.

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486 Naturally intronless mRNAs are targeted to nuclear speckles for assemblingexport-competent mRNPs

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Nuclear speckles (NSs) are known to be splicing factor storage sites. Here, we unexpectedly found that many endogenous naturally intronless mRNAs that do not undergo splicing apparently associate with NSs. These associations do not require transcription, polyadenylation or the polyA tail. Rather, cis-acting exonic splicing enhancers (ESEs) present in intronless mRNAs and their trans-factor SR proteins function in targeting intronless mRNAs to NSs. Significantly, speckle targeting of mRNAs promotes TREX recruitment and TREX-dependent mRNA export. Furthermore, TREX that are concentrated in NSs, but not NXF1 mainly localizing at nuclear pores, is required for releasing intronless mRNAs from NSs. Together, these data indicate that ESEs target intronless mRNAs to nuclear speckles via SR proteins, resulting in efficient TREX recruitment and nuclear export. We propose that assembly of many export-competent mRNPs mainly occurs in nuclear speckles, and entering nuclear speckles could serve as a quality control step in mRNA export.

487 Regulation of mRNA export *in vivo* by the DEAD-box ATPase Dbp5

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mRNA transport from the nucleus to the cytoplasm via nuclear pore complexes (NPCs) is a fundamental step in eukaryotic gene expression. Although factors involved in mRNA transport have been characterized, the lack of information on molecular mechanisms of directional mRNA transport has hampered a comprehensive understanding of this essential process and its regulation. mRNA export depends on several NPC-associated proteins, including the DEAD-box ATPase Dbp5, which has been suggested to drive directional transport *in vivo*. Extensive work from several groups has proposed models for Dbp5 function *in vitro*, but whether this can be transferred to its role *in vivo* is unclear. Here, we use single molecule imaging to characterize the function of Dbp5 in nuclear mRNA export. We demonstrate that acute Dbp5 depletion in budding yeast using an inducible degron system causes rapid accumulation of labeled endogenous mRNA molecules at the nuclear rim in live cells, supporting a role of Dbp5 in late steps of mRNA export *in vivo*. Furthermore, we show that while steady-state localization of many factors involved in mRNA export is unaffected in the absence of Dbp5, nuclear dynamicity changes dramatically. Our work allows us to understand how the ATPase cycle of Dbp5 is coupled to mRNA export *in vivo,* and we propose a mechanistic model of how mRNAs are directionally exported through the nuclear pore complex.

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488 Delivery and detection of nuclear-encoded RNAs in mammalian mitochondria

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Mitochondria rely on the import of certain nuclear-encoded macromolecules to carry out biochemical processes like replication, translation, and membrane-mediated metabolic activities. Protein import in mitochondria has been well-documented and its mechanisms have been well-characterized. Nuclear-encoded RNAs have also been found to have important functions in the mitochondria. Observations that nuclear-encoded RNAs have a distinct presence in mitochondria suggest that efficient RNA import pathways have evolved for mitochondria. However, many of the key mechanisms involved with RNA import into mitochondria have yet to be defined.

We sought to understand RNA import pathways in mitochondria by identifying both RNA sequence motifs as well as RNA-binding proteins that would enable RNAs to traffic from the nucleus to the mitochondrial matrix. The RNA components of the mitochondrial RNA processing endoribonuclease (MRP) and ribonuclease P (RNase P) complexes are two endogenous, nuclear-encoded RNAs known to have functions in mitochondria. We have identified sequences in MRP and RNase P present in human mitochondria in an initial characterization of RNA sequences that may be important for trafficking to mitochondria. Using MRP and RNase P as known RNA targets, we optimized a mitochondrial fractionation- and quantitative PCR-based assay to determine the enrichment of nuclear-encoded RNA sequences in mammalian mitochondria. RNA immunoprecipitation was also used to detect non-mitochondrial encoded RNAs in mitochondria. These strategies will be used in future studies to screen more RNA sequence-structure motifs and assess their functions in mitochondrial RNA import. In addition to understanding endogenous systems of transport, we sought to develop artificial systems for transporting RNAs to mitochondria by re-engineering the localization patterns of RNA-binding proteins (RBPs). RBPs relocated to mitochondria were examined for their ability to bring specifically-bound RNAs to mitochondria. These artificial systems would enable the delivery of RNAs to mitochondria for gene therapy applications. RNA-based therapeutics are promising avenues in the future of gene therapy. In developing RNA-based therapeutics to correct genetic defects in mitochondria, it will be paramount to understand the endogenous cellular mechanisms for transporting nuclear-encoded RNAs to mitochondria and to have strategies to manipulate them.

489 Withdrawn

490 UPF2 promotes the secretion of CD81+ exosomes

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Exosomes are extracellular vesicles with a diameter of 40-150 nm, secreted from most cell types. They are believed to represent a mechanism of exchange of molecules (DNA, RNA, protein) from one donor cell to a recipient cell, and may also constitute a means of eliminating cellular materials. Exosomes are found in body fluids including blood, breast milk, urine, and saliva. The composition of exosomal material varies in persons with certain diseases, such as neurodegeneration and cancer; thus, there is great interest in employing exosomes in diagnosis, prognosis, and therapy. However, the processes of exosome biogenesis, secretion, and uptake are poorly understood, hampering progress towards employing exosomes in the clinic. In this project, we employed a screening approach to identify the factors involved in exosome biogenesis. We began by engineering human cervical carcinoma HeLa cells expressing the exosome marker CD81 as a fusion fluorescent protein, CD81-GFP. After confirming the expression of CD81-GFP in exosomes by Western blot analysis, we used the cells to identify factors affecting exosome biogenesis by screening whole-genome RNAi libraries. Among the top candidate genes, we discovered that UPF2 and SAE1 suppressed exosome biogenesis, while MED14, ATOX1, POLE, and DDX39B promoted exosome biogenesis. To confirm these findings, we first focused on UPF2, one of major protein mediators of nonsense-mediated mRNA decay (NMD). Interestingly, UPF2 downregulation using short interfering (si)RNA in HeLa cells, followed by CD81-GFP analysis by Western blot analysis, flow cytometry, and fluorescent microscopy, revealed that CD81-GFP expression rose intracellularly in cells after UPF2 silencing, while CD81-GFP secretion declined. Exosome number, however, was unchanged. Taken together, our results indicate that UPF2 silencing leads to a rise in intracellular CD81-GFP in cells and reduces the secretion of CD81-GFP-containing exosomes without affecting the total number of exosomes produced. We propose that exosomes are heterogeneous and that CD81-GFP-expressing exosomes are particularly dependent on the presence of UPF2. Studies are underway to investigate the protein composition of exosomes after UPF2 silencing by RNA sequencing and mass spectrometry. Given that UPF2 is implicated in NMD, we are particularly interested in testing if RNA substrates of NMD are differentially represented in exosomes.

491 Cis-element based export machinery recruitment at the absence of splicing

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The export of spliced mRNAs depends on the recruitment of TREX complex during splicing. While for naturally intronless mRNAs, how they are exported at the absence of splicing has long been an enigma. Our previous studies showed that cytoplasmic accumulation region (CAR) is critical for the export (Lei H, et al. PNAS, 2011; Nucleic Acids Res, 2013), supporting a model of cis-element based export machinery recruitment. However, multiple deletions or mutations in the predicted consensus element CAR-E in intronless HSPB3 reporter failed to show export defect, which raised the concern whether such element is prevalent in naturally intronless mRNAs.

To search for similar elements that may mediates the export of naturally intronless mRNAs, we performed bioinformatic analysis on the coding region of 679 naturally intronless mRNAs using MEME algorithm followed by experimental verification, which revealed consensus elements highly similar to CAR-E we previously defined. Using the coding region of intronless gene KRN1 as a reporter, which contains a cluster of 6 motifs within 100 nt at the 3' region, we showed that deletion of this region or introduction of multiple points mutations led to severe export defect. Insertion of this region to the 5' of b-globin cDNA promoted the export of the transcript. In vitro RNP pulldown followed by mass spec revealed candidate proteins bound on the motifs. Screen using siRNA knockdown identified protein X as a vital trans factor in the export of KRN1. RIP experiment suggested that the wild type transcripts but not the transcripts containing multiple point mutations could be pulled down by antibody against protein X. To further explore the mechanism of protein X in intronless mRNA export, we performed protein IP using HA antibody and nuclear extract of stable cell line expressing HA-tagged protein X. We are currently analyzing the proteins interacting with protein X.

Our results provide direct evidence that naturally intronless transcripts contain elements vital for the export and further support cis-element based recruitment of mRNA export machinery at the absence of splicing.

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492 Assaying RNA localization *in situ* with spatial restricted oxidation

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The past few years have witnessed the surging discoveries of indispensible roles that RNA plays in almost every aspect of cell functions. As more and more studies reveal unprecedented insights about RNA's impact on cell development and survival, current methods limit our capability to decipher RNA's biological function with high spatial and time resolution. Our novel approach relies on in situ biomolecule oxidation by singlet oxygen generated from spatially confined fluorophores. Singlet oxygen has limited diffusion range due to their extremely high reactivity inside cells. As a result, only the adjacent guanosines as well as electron rich amino acids (histidine, tryptophan, tyrosine, etc.) are oxidized by singlet oxygen. The oxidized molecules are susceptible to nucleophilic attack by propargyl amine (PA). We have successfully appended alkyne groups onto biomolecules such as RNAs and proteins. These chemical handles significantly facilitate the downstream analysis, including RT-qPCR and RNA-sequencing. By crosslinking RNAs to their binding proteins, we dramatically enhance our ability to assay cellular RNA localization within specific cellular compartments. We anticipate that this platform will provide the community with a much-needed methodology for tracking RNA localization within living cells, and set the stage for systematic large scale analysis of RNA localization in living systems.

493 Loss of Elongation-Like Factor 1 Spontaneously Induces Diverse, RNase H-related Suppressor Mutations in *Schizosaccharomyces pombe*

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An individual with healthy fitness may carry a detrimental genetic trait that has been masked by another genetic mutation. Suppressive genetic interactions, in which a mutant allele either partially or completely restores the fitness defect of another mutant allele, tend to occur between genes that have a confined functional connection. This fitness recovery through the acquisition of suppressor mutations is accelerated in cells bearing mutations that result in genome instability. Emerging views reveal that nuclear RNAs can also be mutagenic, suggesting that the defects in RNA processing and maturation may cause genome instability. Here, we report that loss of Elf1, an AAA+ family ATPase in fission yeast involved in RNA export, promotes genome instability. Although *elf1Δ* strains have severe growth defects initially, cells can quickly recover growth rates near to those of wild-type strains by acquiring mutations that suppress the *elf1Δ* growth defect. Cells without Elf1 are sensitive to DNA damage, have unstable chromosomes, and accumulate RNAs within the nucleus, suggesting that nuclear RNA retention promotes the formation of DNA-RNA hybrids associated with genome instability. Notably, the rate of suppression was further enhanced in *elf1Δ* cells when RNase H activities were abolished. Using whole genome sequencing, we mapped a few consistent suppressors of *elf1Δ*, suggesting previously unknown functional connections between Elf1 and these proteins. Our findings describe a mechanism by which *elf1*Δ indirectly suppresses its own immediate effects on growth rate, which restores the fitness of the population to survive.

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494 Stress granules are heterogeneous assemblies composed of distinct core substructures that contain unique RNA and protein compositions

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Stress granules are mRNA-protein assemblies formed from non-translating mRNPs. Stress granules are important in the stress response, related to neuronal mRNP granules, and aberrant stress granules contribute to some degenerative diseases. A poorly understood issue is the composition of mRNAs within stress granules and their spatial arrangements. Stress granules contain stable sub-structures referred to as stress granule cores, that can be of different protein compositions. Sequencing of G3BP positive stress granule cores revealed that long poorly translated transcripts enrich in G3BP cores. To determine the mRNAs in stress granules cores that lack G3BP (identified in Jain et al., 2016, Cell), we sequenced PABPC1 stress granule cores. We find that transcripts that localize to PABPC1 cores show significant overlap with transcripts that localize to G3BP stress granules and have a similar length and translation efficiency bias. However, there are some differences between the G3BP and PABPC1 stress granule core transcriptomes. Transcripts enriched in RNA-Seq of PABPC1 cores, but not G3BP cores, show a higher enrichment in stress granules *in vivo* than our previous estimates predicted and seem to localize to PABPC1 dense regions, suggesting that there are transcripts that localize to PABPC1 positive cores independent of G3BP. Indeed, the presence or absence of G3BP does not affect the localization of transcripts to PABPC1 cores during sorbitol induced stress. Consistent with this finding, during sorbitol induced stress we observe regions of PABPC1 staining that do not co-localize with G3BP. Interestingly, other stress granule RBPs such as FAM120A, TIA1, and PUM2 occupy these PABP positive G3BP-negative regions. Thus, our analysis argues that stress granules are the composed of a heterogeneous mixture of cores that have a unique RNA and protein composition and further expands our understanding of the way in which RNA and protein localize to stress granules.

495 Nopp140 is required for targeting Cajal body-specific (sca)RNPs to Cajal bodies and for proper modification of spliceosomal snRNAs

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Cajal bodies (CBs) are micron-sized nuclear organelles identified by the marker protein coilin and other components. They play a role in the inherited diseases dyskeratosis congenita and spinal muscular atrophy. Two kinds of RNPs are concentrated in CBs: spliceosomal small nuclear (sn)RNPs and modification-guide small CB-specific (sca)RNPs. The colocalization of snRNPs and scaRNPs, as well as exogenous expression studies, suggest that CBs are the sites of snRNA modification, but this has not been documented for endogenous particles. CB localization of scaRNPs depends on the protein Wdr79/TCAB1. However, the related intronic AluACA RNPs also associate with Wdr79/TCAB1, but are situated in the nucleoplasm, indicating that additional factors are involved in CB localization of scaRNPs. Here we show that one such factor is Nopp140, a phosphoprotein found in both nucleoli and CBs.

Specifically, we characterize several Nopp140 knock-down (kd) cell lines generated by CRISPR/Cas9 technology, using two small guide RNAs. Although there is no gross phenotype, combined indirect immunofluorescence and RNA fluorescent in situ hybridization (FISH) experiments demonstrate that CBs in Nopp140kd cells specifically lose Wdr79/TCAB1 and scaRNPs (core proteins and RNAs of both box C/D and H/ACA RNPs, including telomerase RNA, hTR). In contrast, coilin, SMN, and snRNP localization in CBs are not affected. Moreover, snoRNPs in nucleoli are unchanged, as are overall protein, RNA, and mRNA levels. The loss of hTR mimics the effect of dyskeratosis congenita mutations in Wdr79/TCAB1. Additionally, we found that certain modifications in spliceosomal snRNAs are reduced in the Nopp140kd cells in proportion to the reduction of Nopp140 itself. These effects are specific for phosphorylated Nopp140 because they are rescued by re-expression of Nopp140, but not in the presence of a casein kinase 2 inhibitor. Finally, Nopp140 seems to act upstream of Wdr79/TCAB1 because CB localization of Wdr79/TCAB1 is reduced in Nopp140kd cells and because Wdr79/TCAB1 overexpression rescues CB localization of scaRNPs. We postulate that Nopp140 distinguishes between the two-hairpin H/ACA scaRNPs and the single-hairpin intronic AluACA RNPs. In summary, we identify a Nopp140-mediated pathway for targeting scaRNPs to CBs, where they function to modify snRNAs.

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496 mRNA proximity Biotinylation- A new tool to identify interactome of a localized mRNA

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Localized mRNA translation is found in diverse organisms throughout phylogeny. Identifying the proteome of a localized mRNA gives us an opportunity to identify trans-regulatory factors and molecular mechanisms of localized translation. Using mouse embryonic fibroblasts ß-actin as model mRNA, we introduce a new tool to identify the proteome of localized mRNAs (mRNA proximity ligation) which allows specific biotinylation of proteins associated with or in the close neighborhood of the 3'UTR of the target mRNA. We demonstrate that this method allows identification of proteins stably or transiently interacting with the ß-actin zip code including the motor proteins responsible for mRNA localization like Myh10 (myosin II motor MYH10), we have identified additional ß-actin mRNA interacting candidates. Our tool potentially allows the identification of the interactome of any mRNA.

497 Subcellular transcriptome-wide analysis reveals that Fmr1 promotes neuronal RNA localization and translational repression through distinct target recognition mechanisms

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Fragile X mental retardation protein (FMRP) is an RNA-binding protein that is involved in both translational repression and RNA localization. Mutations in the Fmr1 gene are associated with abnormal cognitive development in both mice and humans. However, the RNA targets, mechanisms, and relative contributions to observed phenotypes of the translational repression and RNA localization activities of FMRP are largely unclear. To investigate these processes, we created an FMR1 null mouse neuronal cell line. We used subcellular fractionation followed by RNAseq to determine transcriptome-wide neurite-directed RNA localization patterns. By comparing RNA localization patterns in wildtype and FMRP null cells, we identified hundreds of transcripts whose efficient localization to neurites depends on FMRP. These RNA localization targets of FMRP display large enrichments of computationally predicted and experimentally verified G quadruplex-forming sequences, especially in their 3' UTRs, suggesting that FMRP recognizes G quadruplex- containing transcripts and promotes their localization to neurites. Intriguingly, many RNA localization targets of FMRP were components of transport machinery, including several kinesins. Human orthologs of mouse FMRP targets were also enriched in G quadruplex-forming sequences, suggesting that this mode of regulation may also be present in humans. The mislocalized transcripts were also highly enriched in published FMRP CLIP-seq datasets, indicating that they are likely direct targets of FMRP. To identify translational repression targets of FMRP, we performed ribosome footprinting in wildtype and FMRP null cells. We find that the translational repression targets of FMRP are largely distinct from its RNA localization targets and that G quadruplex sequences are not enriched in translational regulatory targets of Fmr1. Taken together, these observations suggest different modes of target definition for the RNA localization and translational repression activities of FMRP and raise the possibility of activity-specific therapeutic approaches.

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498 Splicing factor proline-glutamine rich (SFPQ) in motor axon development and neurodegeneration

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Cell structure and function are largely defined by the complement of proteins being expressed. Neurons are especially complex cells, expressing around 10,000 different protein types at any one time (Hanus & Schuman, 2013). They also have sophisticated morphologies, with functionally different cell compartments residing huge distances apart and from the nucleus. One remote region, the synapse, is highly metabolically active, experiencing rapid protein turnover during development. This presents a unique challenge for neurons: ensuring efficient protein dynamics far from the nucleus. Recent research has shed light onto the wealth of RNA processing events facilitating neuronal differentiation and homeostasis, such as axonal RNA transport and local translation. A better understanding of RNA processing has huge potential benefits in tackling neurodegenerative disease.

SFPQ is a multifunctional RNA-binding protein expressed ubiquitously in nuclei, implicated primarily in pre-mRNA splicing. Recently, extranuclear expression of SFPQ, intron-retaining transcripts and other spliceosomal proteins has been observed in motor neuron axons. The extranuclear roles of SFPQ remain enigmatic, despite a relatively good understanding of its nuclear functions. SFPQ null zebrafish embryos display a striking phenotype including no motility accompanied by failed motor axon extension, rescued upon expression of an exclusively cytoplasmic SFPQ variant (Thomas-Jinu et al., 2017). In mammalian sensory neurons extranuclear SFPQ is required for axonal localisation of mRNAs essential for axonal survival (Cosker et al., 2016). These findings strongly suggest that extranuclear SFPQ is vital for healthy motor axon extension and maintenance.

Using cell transplantation, we now show that SFPQ null motor neurons extend axons in a wild-type background. These axons appear a day later than those from normal motor neurons, fail to branch or form numerous synapses with muscle. Some show classical signs of degeneration. These observations reveal that axons, although morphologically abnormal, are capable of extension in absence of SFPQ providing the environmental conditions are permissive. We are now working to compare null and sibling axonal transcriptomes by RNAseq, and perform CLIP and mass spectrometry on axons to enable the identification of important axonal RNA and protein interactors supporting axon development and maintenance.

499 Mechanism of mRNA localization to a phase separated condensate

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Germ granules are a hallmark of all germ cells. Drosophila germ granules are phase separated condensates that instruct the formation of primordial germ cells (PGCs), the first cell lineage that forms in the early embryo. Filled with ribosomes, translation factors and decay regulators, germ granules are the hubs for post-transcriptional gene regulation needed to specify and maintain the germ cell fate. An estimated 200 maternally-deposited mRNAs are enriched in germ granules. Using single-molecule FISH and super-resolution microscopy, we have previously shown that localized mRNAs are distributed asymmetrically within the granule whereas core germ plasm proteins are distributed evenly throughout the granule. Multiple mRNAs organize into homotypic clusters that occupy defined positions within the center or periphery of the granule. Our new data demonstrate that mRNA itself drives the formation of homotypic clusters, provides the specificity for clustering, determines their position within granules and possibly even localizes itself to the granules through mRNA self-entrapment thereby forming homotypic clusters. Our work reveals a new regulatory mechanism for localization and for spatial organization of mRNAs within germ granules that may be applicable to other mRNA-bound membraneless organelles. This work was supported by the HHMI fellowship of the Jane Coffin Childs Memorial Fund and by the NICHD K99HD088675 grant awarded to TT. RL is an HHMI investigator.

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500 Mitochondrial concentration and targeting signal strength determine condition-specific mRNA localization to the mitochondria in budding yeast

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Cellular homeostasis controls organelle morphology. This, in turn, facilitates cell survival under fluctuating growth conditions and environmental stresses. Precise control of the localization of biological molecules to specific intracellular organelles is necessary for their proper function. Proximity-specific ribosome profiling has shown that many mRNAs are translated at the surface of the mitochondria. However, a comprehensive understanding of the relationship between mRNA localization, co-translational protein import, and the morphology of the mitochondria itself has not been addressed. To assess the potential influence of mitochondrial morphology on mRNA localization and translation, we developed methods to measure the distance between individual mRNAs and surface of the mitochondria as well as the velocity of mRNA movement along the mitochondrial surface. We compared these measurements to mitochondrial architecture under different growth conditions. First we performed rapid, 3D live-cell imaging of single molecule mRNA foci using the MS2-MCP system while simultaneously imaging mitochondria by spinning disk confocal microscopy in cells grown in microfluidic plates. We reconstructed and statistically analyzed the spatial relationship between various mRNAs and mitochondria using custom ImageJ routines and MitoGraph V2.0 software, which we previously developed to reconstruct 3D mitochondria. We found that, as mitochondrial concentration (mitochondrial volume vs. cytoplasmic volume) increased, the average distance between mitochondria and mRNA decreased, suggesting that mRNA localization is dependent on geometrical constraints. Furthermore, we found that the strength of this dependency was correspondingly increasing according to the strength of a mitochondrial-targeting signal of the translating complex, leading to increased number of mitochondrial localized mRNA. For example, *ATP3* mRNA does not associate with mitochondria during vegetative growth. However, upon shift to glycerol conditions, which cause respiration and an increase in mitochondrial concentration, the targeting signal of Atp3p can drive its translating complex localization to the mitochondrial surface. These observations suggest a key factor that determines mRNA localization is mitochondrial concentration itself and suggest that condition-specific mRNA localization can be further regulated by the propensity for a mitochondrial-targeting signal to drive mRNA localization to the mitochondrial surface. This is the first study to suggest the potential importance that organelle concentration has for translation in a spatial context.

501 RNA self-assembly contributes to stress granule formation and defining the stress granule transcriptome

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Stress granules are higher order assemblies of non-translating mRNAs and proteins that form when translation initiation is inhibited. Stress granules are thought to form by protein-protein interactions of RNA-binding proteins. We demonstrate RNA homopolymers or purified cellular RNA form assemblies *in vitro* analogous to stress granules. Remarkably, under conditions representative of an intracellular stress response, the mRNAs enriched in assemblies from total yeast RNA largely recapitulate the stress granule transcriptome. We suggest stress granules are formed by a summation of protein-protein and RNA-RNA interactions, with RNA self-assembly likely to contribute to other RNP assemblies wherever there is a high local concentration of RNA. RNA assembly *in vitro* is also increased by GR and PR dipeptide repeats, which are known to increase stress granule formation in cells. Since GR and PR dipeptides are involved in neurodegenerative diseases, this suggests that perturbations increasing RNA-RNA assembly in cells could lead to disease. Additional experiments illustrating the roles of RNA-RNA interactions in vitro and in vivo will also be presented.

502 Coordinated regulation of *hunchback* mRNA by Pumilio, Nanos and Brain Tumor Protein in *Drosophila*

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Pumilio (Pum), Nanos (Nos) and Brain Tumor (Brat) are three important RNA-binding proteins involved in post-transcriptional regulation of *hunchback* mRNA in *Drosophila*, which is rich in the anterior of the oocytes and plays an important role in regulating the development of head and thorax. It is known that the polar distribution of *hb* mRNA depends on the translation inhibition of Pum-Brat-Nos protein complex in the posterior pole. Pum and Brat recognize and bind to NRE(Nanos Response elements) of *hb* mRNA 3'-UTR. The Nanos protein is responsible for locating the non-translational protein-RNA complex in the posterior pole, and the abnormal translation activation of the *hb* mRNA in the posterior pole may affect the development of the abdominal ganglia of *Drosophila*. In order to study the structural and molecular mechanism of combinatorial control by Pum, Nos and Brat, we have assembled the quaternary complex of Pum-Brat-Nos-*hb* NRE2 and ternary complex of Pum-Brat-*hb* NRE2 in vitro, and screened crystals of them. Through biochemical assays, we found the cooperative binding of these three proteins to *hb* mRNA depends on the interaction of Pum and *hb* mRNA NRE2. The zinc-finger domain and C-terminal extension of Nos were determined to be essential and critical for the cooperative interaction.

503 Association between splicing efficiency and sub-cellular localization of coding and long non-coding RNAs in human cells

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Most mammalian mRNAs are spliced, and splicing is considered important for subsequent efficient nuclear export, but whether and how splicing efficiency regulates export of different gene subsets remains unclear. We set out to assess the importance of the splicing process in determining sub-cellular localization of transcripts at global scale, and to explore possible mechanisms of regulation of subcellular localization by splicing. To address these questions, we first analyzed RNA-seq data from subcellular fractions of 10 ENCODE cell lines, which allowed quantification of splicing and cytosol/nucleus expression levels. We found a strong correlation between splicing efficiency and cytoplasmic localization on a genome-wide scale in all examined cell-lines. In addition, when comparing pairs of cell types, increased splicing efficiency was correlated with increased RNA levels in the cytoplasm, suggesting that splicing underlies efficiency of nuclear export. Differences in splicing efficiency also explain some of the difference in nuclear export efficiency between the mRNAs, that are typically well spliced, and long noncoding RNAs (lncRNAs), whose splicing is mostly inefficient.

The mechanism of coupling of splicing and nuclear export is poorly understood. To characterize the export regulation of inefficiently spliced transcripts, we used RNAi to deplete the major RNA export factor NXF1 and the nuclear pore-associated protein TPR, and characterized whole cell and nuclear/cytoplasmic fractions using RNA-seq. NXF1 or TPR depletion allowed leakage of inefficiently spliced transcripts to the cytoplasm, suggesting that these factors play a role in nuclear restriction of intron-containing transcripts. Surprisingly, we also found that export of lncRNAs and genes with no or few exons, or long exons, is substantially more sensitive to NXF1 depletion, which reveals selectivity in the general NXF1-based export pathway, and suggests that genes with multiple introns are efficiently exported independently of NXF1. We are currently using additional perturbations to reconstruct the network of factors that determine the subcellular fate of poorly spliced lncRNAs and mRNAs.

Overall, our results provide the first transcriptome-wide view of the connection between RNA processing and nuclear retention, which is crucial for both the function of many lncRNAs and for regulation of protein production from mRNAs.

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504 Global nuclear mRNA retention by the FUBP family of RNA binding proteins as a novel mechanism of posttranscriptional gene regulation

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The Far Upstream Binding Protein family of RNA binding proteins has been proposed to act on both DNA and RNA, however, their molecular and biological function remains ill-defined. FUBP1 and FUBP3 were recently described as oncogenes, whereas FUBP2 (aka KHSRP) is thought to regulate inflammatory genes and differentiation processes. Other suggested roles for FUBPs are MYC transcription modulation, regulation of RNA stability, miRNA maturation, and splicing regulation, altogether generating a confusing picture of FUBPs function. Here we combined a systems-wide approach with traditional techniques to generate a comprehensive model of FUBPs function. We quantitated in human cell lines FUBPs levels of 1-2 million molecules per cell and confirmed that FUBP1/2 were restricted to the nucleus, while FUBP3 showed mainly cytoplasmic localization. We next identified the target mRNAs of each FUBP protein using PAR-CLIP and showed that the FUBP1/2 bind to pre-mRNA transcripts of ~20% of the protein coding genes. We then used CRISPR technology to generate single knock-out cell lines: Loss of FUBP2 resulted in upregulation of FUBP1 and 3, providing strong evidence for autoregulation within the FUBP family that was also evident in PAR-CLIP results showing FUBPs interaction with mRNA encoding their paralogs. Generation of double knockout cell lines failed since the FUBP family is essential, as was also evident by the lethality of combining knockdown of FUBP1 and 2. Dense cultures RNA-seq analysis combined with biochemical fractionation showed that FUBP KO results in an increase abundance of its target mRNAs in the cytoplasm with a compensatory decrease in nucleus. However, an opposite effect was observed when proliferating cells were analyzed: Although FUBP levels remain steady in these conditions, FUBPs target mRNAs remained predominantly nuclear upon FUBP KO. Nuclear retention is accompanied with lower overall abundance whereas increase export is accompanied with increase in overall abundance, implying that nuclear retention of target messengers leads to their degradation. This is also consisted with the fact that proteomic analyses failed to detect FUBPs protein co-factors that could lead directly to the degradation machinery. Our data highlight the importance of mRNA export in posttranscriptional gene regulation.

505 The NEXT complex controls the proper levels of miRNA precursors in plants

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The Arabidopsis thaliana SERRATE protein (SE), which is a homologue of the human ARS2 protein, is involved in two important pathways of plant RNA metabolism: miRNA biogenesis and pre-mRNA splicing. Originally, SE (a zinc-finger protein) was characterized as a protein involved in miRNA biogenesis, where together with DCL1 (a ribonuclease) and HYL1 (a double-stranded RNA binding protein) forms a core of the plant microprocessor. In this complex SE influences the efficiency and accuracy of pri-miRNA cleavages catalyzed by DCL1. SE together with another factor involved in miRNA biogenesis, the nuclear cap-binding complex (CBC), have been also ascribed to pre-mRNA splicing. We have shown that SE interacts with CBC and both influence alternative splicing of pre-mRNAs. In order to understand this dual role of SE in different pathways of RNA metabolism, we analyzed the proteins interacting with SE. To this end, we carried out co-immunoprecipitation (co-IP) of the FLAG:SERRATE fusion protein. The SE-bound proteins were identified by mass spectrometry. In our list of the proteins co-immunoprecipitated with SE we found all subunits of the Nuclear Exosome Targeting (NEXT) complex as well as subunits of the THO/TREX complex. The interactions between SE and NEXT complex subunits were confirmed by the yeast two hybrid system, pull-down experiments and FLIM-FRET analyses. Our results have clearly shown that SE forms a complex with NEXT, interacting directly with both ZCCHC8 and RBM7 subunits. Therefore, we performed also co-IP analyses using tagged ZCCHC8a and RBM7 subunits of NEXT. Among ZCCHC8a and RBM7 protein partners we detected SE but none of the subunits of THO/TREX. We studied also a function of the NEXT/SE interaction in miRNA biogenesis. The results have revealed that NEXT is necessary for degradation of 5' pri-miRNA fragments after excision of miRNAs from miRNA precursors by DCL1. Interestingly, loss of the NEXT function causes the accumulation of pri-miRNAs, suggesting the involvement of NEXT in regulation of the proper pri-miRNA level by degradation of incorrect/unprocessed miRNA precursors. Our results suggest that the interaction between SERRATE and the NEXT complex is important for the control of quality and levels of miRNA precursors in the plant cell nucleus.

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506 Defects in tRNA intron turnover create novel small RNAs: possible consequences to cell growth

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Transfer ribonucleic acids (tRNAs) are abundant molecules, comprising ~15% of cellular RNAs. Although the major biological role for tRNAs is to bring amino acids to the ribosome during protein synthesis, they also play many secondary roles. Defects in pre-tRNA biogenesis and processing cause numerous disorders, from neurodegenerative diseases to cancer. In eukaryotes, a subset of tRNA-encoding genes contains non-coding introns that must be removed in post-transcriptional tRNA processing; in *S. cerevisiae*, these account for 20% of pre-tRNAs. Through an unbiased screen of the yeast genome, we identified two proteins required for tRNA intron turnover of $tRNA^{Ile}_{UAU}$, the tRNA employed in our screen (Wu and Hopper 2014). We showed that the free $tRNA^{Ile}_{UAU}$ intron is first phosphorylated on the 5' end by the tRNA ligase/kinase Rlg1, then degraded by the 5' to 3' exonuclease Xrn1. Rlg1 also ligates the mature tRNA halves. By expanding our analysis to the other intron-containing pre-tRNAs, we identified four additional possible mechanisms for intron degradation, as well as the circularization of the $tRNA^{Trp}$ intron in vivo. Together, these findings indicate the specificity of tRNA intron degradation pathways. The evolution of multiple tRNA intron turnover mechanisms strongly suggests that elevated intron levels are harmful to cells. To test this, we devised a genetic approach to determine the biological consequences of aberrant accumulation of pre-tRNA introns. We introduced a multi-copy plasmid encoding a bacterial ligase, RtcB, into yeast, replacing Rlg1 in tRNA half ligation, but not phosphorylation of the intron 5' termini. As a result, the cells generate large quantities of introns that cannot be degraded, causing growth defects and cellular stress. One possibility for the growth defect is interference by free tRNA introns with particular mRNA functions.

507 Genome-Wide Analysis of mRNA Degradation Kinetics

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Alternative splicing allows for the production of multiple mRNA isoforms from a single gene. These isoforms encode for the diverse proteome needed for the cell to function properly. Extensive research has been carried out to identify mRNA isoforms and their steady-state levels. While insightful, these studies do not allow insights into the dynamics of how changes in steady state mRNA expression are established. In general, the expression level of mRNAs can be influenced by changes in transcriptional activity, alterations of mRNA stability or a combination thereof. To understand the dynamic behavior of mRNAs we used a genome-wide metabolic labeling approach to monitor the degradation kinetics of genes and exons. Hela cells were pulsed for 4 hours with 4sU media and subsequently chased for 24 hours with media lacking the label. During the chase period we extracted RNA at various time points. Total RNA was subsequently enriched for 4sU labeled mRNA and sequenced. After aligning sequencing reads we determined degradation rates by fitting time course profiles to kinetic equations describing first order decay behaviors. Our approach permitted the derivation of degradation rates for 4,250 genes and approximately 48,000 exons. As expected from previous studies fast decaying mRNAs are enriched for genes related to pol II transcription and RNA synthesis. Interestingly, our analysis pipeline identified over 1,000 kinetic outlier exons, which are characterized by displaying significantly different degradation profiles when compared to other exons within the same gene. In general, these outlier exons are alternatively spliced exons, suggesting that mRNA isoforms generated from a single gene can display strikingly different degradation rates. The mechanisms leading to different mRNA isoform degradation kinetics can be attributed to known and unknown mRNA degradation mechanisms.

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508 Phosphorylation-dependent regulation of deadenylation by the Tristetraprolin CNOT1-interacting motif

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The regulation of cytokines is of vital importance for achieving homeostasis following the induction of an immune response; an inability to do so promotes the development of chronic conditions such as auto-immunity and cancer. Tristetraprolin (TTP) regulates the translation and stability of pro-inflammatory cytokine mRNAs containing AU-rich elements (AREs) through the recruitment of components involved in translation repression, deadenylation, decapping, and exonucleotic degradation. Recently, the C-terminus of TTP has been shown to be a docking site for the CCR4-NOT deadenylase complex through its association with the scaffold protein CNOT1 (1, 2).This CNOT1-interacting motif (CIM) of TTP has been shown to be a site of phosphorylation (1, 3); however, the relevance of this phosphorylation event in TTP regulation is currently unknown. We have developed a phospho-specific antibody against the TTP CIM. Using this antibody we demonstrate that the CIM domain of TTP is phosphorylated during the early induction phase of the inflammatory response in fibroblast and macrophage cells. Through pulse-chase mRNA decay assays we find that the TTP CIM activates deadenylation without degradation of a tethered transcript. This process is inhibited when cells are treated to allow accumulation of phosphorylation of the CIM. Furthermore, we demonstrate that protein kinase C-alpha, or its downstream effector(s), targets exogenously expressed TTP-S316 in a p38 MAPK independent manner. Collectively, our findings suggest that the CIM acts in conjunction with other TTP domains to carry out translation repression and degradation, and that kinase pathway(s) play a central role in inhibiting TTP target mRNA deadenylation by targeting the TTP CIM, furthering the idea that cytokine mRNAs are regulated during the inflammatory response through fine-tuned signaling events acting on TTP.

509 Substrate specificity of the TRAMP and exosome complexes *in vivo*

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The exosome complex plays major roles in RNA 3'->5' processing and surveillance activities. The TRAMP complexes, which include the RNA helicase Mtr4 together with a RNA binding protein (Air1 or Air2) and a poly(A) polymerase (Trf4 or Trf5), are major cofactors for the nuclear exosome. Mtr4 is an essential cofactor for all characterized nuclear RNA degradation and processing activities of the exosome, whereas only surveillance activities are known to require the other TRAMP components. It remains unclear how these cofactors cooperate together on different substrate classes. Structural analyses of Mtr4 previously identified an "Arch" domain, which acts independently of the helicase activity and stimulates exosome activity.

In vivo UV-crosslinking (CRAC) was used to compare RNA targets of the different TRAMP and exosome subunits, Mtr4, Mtr4-Arch lacking the Arch domain construct, Air1, Air2, Trf4 and Trf5, as well as the exonucleases Rrp44 and Rrp6. Data analysis showed that Trf5/Air1/Mtr4 and Trf4/Air2/Mtr4 exhibits different substrate specificities. Consistent with the CRAC results, mass spectrometry indicated that Air1 interacts with both Trf4 and Trf5 while Air2 interacts almost exclusively with Trf4.

Mtr4, Trf4 and Air2 bind strongly at mRNA 5' ends, close to transcription start site (TSS), together with both Rrp44 and Rrp6. This suggests roles in degradation of promoter-proximal RNAs generated by early termination of transcription. Notably, Mtr4 exhibited lower association with TSS proximal region when Trf4 is deleted but was not affected by Air2 deletion suggesting that substrate specificity is provided by Trf4 and Trf5 respectively while Air1 and Air2 appear to be interchangeable. This was unexpected, since we had previously envisaged that RNA binding specificity would largely be determined by Air1 and Air2, which are Zn-knuckle RNA BPs.

To identify regions of Trf4 and Trf5 that confer target specificity, we are testing chimeric forms of Trf5 lacking the low-organized N-terminal and C-terminal domain or fused with the corresponding Trf4 regions.

We conclude that distinct TRAMP complexes assemble on different classes of transcripts, with Air1, Air2, Trf4 and Trf5 apparently performing distinct roles in specific surveillance and regulatory pathways.

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510 Mutant mRNA decay confers genetic robustness to mutations through triggering a transcriptional adaptation response.

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Robustness to mutations promotes organisms' well-being and fitness. The increasing number of mutants in various model organisms showing no obvious phenotype has renewed interest into how organisms adapt to gene loss. In the presence of deleterious mutations, genetic compensation by transcriptional upregulation of related gene(s) (also known as transcriptional adaptation) has been reported in numerous systems (reviewed in [1]); however, the molecular mechanisms underlying this response remain unclear. To investigate this phenomenon, we developed and analyzed several models of transcriptional adaptation in zebrafish and mouse. We first observed that transcriptional adaptation is not caused by loss of protein function, indicating that the trigger lies upstream. We find that the increase in transcript levels is due to increased transcription, and that chromatin becomes more accessible at the upregulated genes' regulatory regions. As mutations often lead to transcripts that are degraded by the mRNA surveillance machinery, we investigated this process and found a correlation between the degree of mutant mRNA decay and the transcriptional upregulation of the related gene(s). In order to assess the role of the mutant mRNA in triggering transcriptional adaptation, we generated alleles that fail to transcribe the mutated gene and found that they do not exhibit this response. In addition, they displayed more severe phenotypes than those observed in alleles exhibiting mutant mRNA decay. Moreover, genetic inactivation of the nonsense mediated decay factor Upf1 can also lead to the loss of transcriptional adaptation. Coupling of mRNA decay and transcription has been reported previously [2]. RNA-seq analysis of wild-type and mutant cells revealed upregulation of many genes and notably an enrichment for genes sharing DNA sequence similarity with the mutated gene's coding sequence, suggesting a model whereby RNA decay intermediates induce the transcriptional adaptation response. These results identify a new role for the mRNA surveillance machinery in buffering against mutations by triggering the transcriptional upregulation of related genes. In addition, these results will help design mutant alleles with minimal transcriptional adaptation-derived compensation.

[1] El-Brolosy and Stainier, PLOS genetics (2017).
[2] Haimovich et al., Cell (2013).

511 Withdrawn

512 Role of nonsense-mediated mRNA decay in the exit from pluripotency

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Embryonic stem cells (ESCs) are able to proliferate indefinitely in culture and to differentiate into cells of all the three germ layers. The mechanisms underlying their differentiation are poorly understood. Recent evidence from our and other labs suggests a role for RNA binding proteins in the exit from pluripotency. In particular, components of the nonsense-mediated mRNA decay (NMD) pathway have been identified in a haploid genetic screen for factors promoting the exit from pluripotency. NMD is a quality control mechanism, which promotes the degradation of target mRNAs, e.g. containing a premature termination codon (PTC).

The mechanism through which NMD is involved in the exit from pluripotency is still unknown. However, ESCs deficient for key factors of NMD, such as Smg5, Smg6 or Smg7, are no longer able to differentiate properly. In addition to a functional deficiency in commitment, these cells show also an upregulation of self-renewal factors compared to wild type cells. In order to dissect the molecular mechanism through which NMD controls the exit from pluripotency I am employing genetic disruption combined with detailed biochemical and functional validation of potential downstream mechanisms. Moreover, have assessed the NMD dependent regulation of transcriptome-wide mRNA half-life. Taken together all these data will help us understand how NMD regulates ESC fate decisions.

513 Proximal 3'UTR introns elicit EJC-dependent NMD during zebrafish embryonic development

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Post-transcriptional control of gene expression is essential for proper development, and is achieved largely by RNA-binding proteins. One such protein complex, Exon Junction Complex (EJC), is deposited 24 nts upstream of exon-exon junctions during pre-mRNA splicing. The EJC influences many aspects of post-transcriptional regulation, including Nonsense Mediated mRNA Decay (NMD). NMD is a surveillance system to degrade aberrant mRNAs and also degrades non-aberrant mRNAs containing 'NMD-inducing features' such as 3' UTR introns (3UI). Post-splicing, a 3UI leads to an EJC bound downstream of the stop codon - if the distance between the two is ≥ 50 nts, the mRNA is targeted for NMD by the key NMD-regulator Upf1. To study EJC function during development, we generated zebrafish mutants in EJC core protein genes *rbm8a* and *magoh*. Homozygous *rbm8a* and *magoh* mutants are paralyzed and have muscle and neural defects. As expected, RNA profiling reveals that annotated aberrant and natural NMD targets are significantly upregulated in EJC mutants. Surprisingly, some upregulated natural transcripts contain a conserved proximal 3UI (< 50 nts downstream of the stop codon). These 'proximal 3UI+ NMD targets' are similarly up-regulated in Upf1-deficient and NMD inhibitor-treated embryos, suggesting that this subset of rbm8a- and magoh-regulated transcripts is regulated via NMD. The same trend is observed in Upf1-deficient mammalian cells. One proximal 3UI+ NMD target transcript encodes Foxo3b, which has been shown to play a role in autophagy, skeletal muscle atrophy and negative regulation of Wnt signaling. We hypothesize that *rbm8a* and *magoh* mutant defects are due, at least in part, to increased Foxo3b activity. Preliminary experiments using CRISPR-mediated somatic knockout of *foxo3b* in EJC mutants shows mosaic phenotypic rescue and generation of *rbm8a*/*foxo3b* and *magoh*/*foxo3b* double mutants is underway. Our findings show that proximal 3'UTR introns (< 50 nts downstream of the stop codon) are a new, atypical NMD-inducing feature that may be critical for regulating gene expression during embryogenesis.

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514 Characterization of deNADding Enzymes in Eukaryotes

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The N^7- methylguanosine (m^7G) cap is a ubiquitous element on the 5′ end of eukaryotic mRNAs involved in pre-mRNA processing, stability, nuclear export and protein translation. Recent findings in diverse organisms have demonstrated that RNAs can additionally possess a non-canonical cap structure consisting of a nicotinamide adenosine dinucleotide (NAD^+) at their 5′ end in place of the m^7G. In contrast to the m^7G cap, the NAD^+ cap does not support mRNA protein translation but instead promotes mRNA decay in mammalian cells. The mammalian and fungal noncanonical DXO/Rai1 decapping enzymes efficiently remove NAD^+ caps and appear to function as deNADding enzymes in cells [1]. We now report mammalian cells contain multiple deNADding enzymes. Of the two Nudix hydrolases that can cleave free NAD^+, Nudt12 and Nudt13, we can now show that Nudt12 also possesses deNADding activity distinct from that of DXO. While the DXO family proteins remove the intact NAD^+ from the 5′ end of an RNA, Nudt12 cleaves within the NAD^+ cap diphosphate linkage to release nicotinamide mononucleotide and RNA ($N_{ic}p$ + pA-RNA). HEK293T cells harboring a disrupted Nudt12 or DXO genes ((Nudt12-KO and DXO-KO respectively), resulted in increased NAD^+-capped RNAs in the cells indicated that both Nudt12 and DXO are deNADding proteins in mammalian cells. Double knock-out previously in vitro identified DXO yeast deNADding homologues Dxo1 and Rai1 in yeast cells leaded to the accumulation of NAD^+-capped RNAs, indicating that both enzymes function to clear NAD^+ from the 5′ end of RNAs in yeast. Interestingly, exposure of cells to environment stress conditions altered cellular NAD^+ levels and in turn impacted levels of NAD^+-capped RNA, indicating NAD^+ capping is a modulated process that may be linked to the metabolic state of the cell.

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515 Proteasomal regulation of nonsense-mediated RNA decay in human muscle disease

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When the protein-coding capacity of an mRNA is compromised through the introduction of a premature termination codon, nonsense-mediated RNA decay (NMD) ensures that the aberrant RNA is degraded to prevent the accumulation of truncated and potentially toxic proteins. We recently discovered that this essential quality control process is inhibited in a human muscle disease called facioscapulohumeral muscular dystrophy (FSHD). We also showed that the expression of FSHD-causing transcription factor, DUX4, causes proteolytic degradation of UPF1, a core NMD factor. To understand how DUX4 modulates the post-transcriptional regulation of UPF1 level as well as to identify the extent of DUX4's post-transcriptional functions in an unbiased manner, we used paired RNA-seq and quantitative SILAC-based mass spectrometry to measure DUX4-induced changes to the cellular transcriptome and proteome. At the transcript level, DUX4 induced significant changes to various RNA binding proteins and splicing factors, which emerged as the most enriched category of genes affected by DUX4. Proteomics showed that DUX4 caused widespread uncoupling of RNA and protein levels of not just UPF1, but also several other NMD factors, including the endonuclease SMG6 and the 5'-3' exonuclease XRN1. Moreover, DUX4 expression alters the levels of various regulators of the proteasome function including several E1, E2, and E3 ubiquitin ligases. Based on these data, we hypothesize that DUX4 modulates the activity of the ubiquitin proteasome system to induce degradation of NMD factors, ultimately inhibiting NMD. Studying this novel regulatory mechanism further will allow us to uncover hitherto unknown regulators of NMD function that cause inter-tissue and inter-individual differences in RNA surveillance.

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516 A mechanism to protect normal long 3'UTRs from NMD drives failure of mRNA quality control in B cell lymphoma

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The human nonsense-mediated mRNA decay (NMD) pathway performs quality control and regulatory functions within complex post-transcriptional regulatory networks. Efficient and accurate detection of NMD substrates involves both degradation-promoting factors and proteins that safeguard normal mRNAs with potential NMD-inducing features such as long 3'UTRs. In this work, we identify hnRNP L as a protein responsible for preventing decay of mRNAs that would otherwise be NMD targets. Using transcriptome-wide approaches, we find that 3'UTRs with a high density of hnRNP L recognition motifs are less likely to bind UPF1 and be decayed. Further, we provide evidence that the regulation of a given transcript to NMD can be tuned based on its 3'UTR length and ability to recruit hnRNP L. Integrating these findings with the previously defined role of polypyrimidine tract binding protein 1 in NMD evasion enables greatly enhanced prediction of transcript susceptibility to NMD.

Unexpectedly, this system causes a failure of mRNA quality control in many B cell lymphomas, permitting overexpression of aberrant oncogenic BCL2 transcripts. Chromosomal translocations that append the *IGH* locus directly to the *BCL2* locus are frequent initiating events in development of follicular and diffuse large B cell lymphomas. These translocations cause transcriptional induction of BCL2:IGH fusion mRNAs which have 3'UTRs containing ~2.5 kb of the native BCL2 3'UTR but also several IGH-derived exons. These transcripts are predicted to be ideal NMD targets due to their multiple 3'UTR introns but are nevertheless able to produce high levels of BCL2 protein. We find that an extensive stop codon-proximal tract of hnRNP L binding sites responsible for shielding the normal ~5 kb BCL2 3'UTR from NMD is retained in the BCL2:IGH fusion mRNAs. Our data indicate that continued recognition of the fusion transcripts by hnRNP L allows these aberrant mRNAs to evade NMD, thereby promoting BCL2 overexpression and neoplasia.

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517 Transcriptome-wide analysis reveals target selection patterns during glucocorticoid receptor- mediated mRNA decay

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Glucocorticoid receptor-mediated mRNA decay (GMD) has been shown to elicit rapid degradation of target mRNAs in a translation-independent manner. Previous studies show that the sequential recruitment of glucocorticoid receptor (GR), Y-box-binding protein 1 (YBX1) and heat-responsive protein 12 (HRSP12) is required for efficient GMD. However, the molecular details of GMD induced by glucocorticoids (GC) remain unclear. In this study, we show that an intact GR site allows GMD to occur in both the nucleus and in the cytoplasm, as to act on pre-mRNAs and mRNAs. Also, through comparative analysis using as cross-linking and immunoprecipitation coupled to high-throughput sequencing (CLIP-seq) and mRNA sequencing, we show that cellular transcripts harboring a common binding site for GMD factors (GR, YBX1, and HRSP12) are preferential targets for GMD. Furthermore, our transcriptome-wide analysis suggests potential function of GMD in diverse biological and physiological events.

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518 Pat1 directly recruits cofactors to mRNA to promote decapping

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All mRNA contain a 5' methyl-7 guanine cap (m7G) which regulates processing, translation, and degradation. Bulk mRNA degradation begins with the trimming of the 3' poly(A) tail followed by removal of the 5' m7G cap by the Dcp1/2 holoenzyme. Decapping irreversibly commits an mRNA to exonucleolytic digestion and is thus highly regulated by multiple cofactors. The Lsm1-7:Pat1 complex binds the 3' end of deadenylated mRNAs and promotes decapping through an unknown mechanism. We show that the C-terminal domain of Pat1 (PatC) can directly interact with conserved short linear motifs in the C-terminus of Dcp2 but is unable to activate decapping. The presence of both the middle and C-terminal domains of Pat1, however, activates Dcp1/2 by increasing its affinity for substrate, which requires cooperation between residues on both domains of Pat1. We next demonstrate that the middle domain of Pat1 is necessary and sufficient to interact with Lsm1-7. The C-terminal domain, however, is required to enhance the RNA binding ability of Lsm1-7. Furthermore, we identify distinct regions of the middle domain that interact with Lsm1-7 and promote RNA binding, suggesting that the middle and C-terminal domains cooperate to enhance RNA binding of the Lsm1-7 complex. Using this reconstituted system, we establish the mechanistic basis for how Pat1 uses distinct regions to coordinate mRNA decay factors and promote decapping. Future work with additional mRNA decay factors will provide a quantitative view of how multiple proteins cooperate to promote mRNA decapping and degradation.

519 Sequence-specific PPR RNA binding proteins coordinate internal mRNA editing and 3' modifications in mitochondria of trypanosome

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Most mitochondrial mRNAs in *Trypanosoma brucei* undergo U-insertion/deletion editing to reconstitute open reading frames. Addition of short 3′ A-tail by mitochondrial KPAP1 poly(A) polymerase prior to editing protects mRNA from 3′-5′ degradation during the editing process. Conversely, completion of editing is manifested by A-tail extension into long A/U-heteropolymer. This unconventional mRNA modification by KPAP1 and RET1 TUTase stimulates translation by increasing mRNA affinity to the ribosome. The distinct roles and editing-dependent temporal separation of A-tailing and A/U-tailing events imply existence of sequence-specific factors that sense the mRNA's editing status and regulate 3′ additions. Here, we identify two novel pentatricopeptide-repeat containing (PPR) RNA binding proteins that fulfil these mRNA quality control and translational activation functions and determine their mechanism of action. We show that Kinetoplast Polyadenylation Factor 3 (KPAF3) specifically recognizes 3′ end of pre-edited transcripts thereby stabilizing mRNAs. KPAF3 also recruits KPAP1 poly(A) polymerase to stimulate short A-tail addition. Initiation of editing displaces KPAF3 leaving mRNA reliant on short A-tail as *cis*-stability determinant. We further demonstrate that Kinetoplast Polyadenylation Factor 4 (KPAF4) recognizes a contiguous stretch of 5-6 adenosines acting as short A-tail binding protein in vivo and in vitro. In this capacity, KPAF4 blocks 3′-5′ mRNA degradation of adenylated mRNAs by the mitochondrial processome and limits their uridylation by RET1 TUTase. The latter attribute prevents A/U-tailing, and therefore translational activation, of partially edited mRNAs. Collectively, our findings reveal previously unappreciated roles of PPR proteins as sequence-specific polyadenylation factor (KPAF3) and poly(A) binding protein (KPAF4). This study also reveals molecular mechanisms that couple mitochondrial mRNA editing, 3′ modification and translation.

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520 Translation-dependent RNA degradation through the Ski complex.

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The Ski complex is a conserved cofactor of the cytoplasmic exosome that assists with RNA 3'-to-5' degradation. Within the complex, the DExH-box RNA helicase Ski2 provides catalytic activity, and Ski3 and Ski8 are scaffold proteins. The Ski complex feeds RNA substrates into the exosome through a channel connecting their helicase and ribonuclease activities [1]. Increasing evidence suggests that cytoplasmic RNA surveillance and turnover are influenced by translation, and in the yeast S. cerevisiae the Ski complex binds the ribosome [2]. Whether the Ski complex interacts with the ribosome in higher eukaryotes and how this might influence Ski complex activity and RNA decay is unclear. To address these questions we combined transcriptome-wide UV Crosslinking and Analysis of cDNAs (CRAC), RNA-seq and mass spectrometry with genome engineering in mouse embryonic stem cells. Interestingly, the CRAC data revealed that mammalian Ski2 contacts 40S rRNA in a manner closely resembling the yeast cryo-EM structure, and binds mRNAs mostly in the 5' UTR and coding sequence but not the 3' UTR. Histone mRNAs were abundantly bound by Ski2 and upregulated upon Ski2 knock-out, suggesting that they are a major direct target. We also identified potential Ski complex adaptor proteins in the mass spectrometry data, and are testing whether these recruit the Ski complex to specific target RNAs. Furthermore, experiments with translation inhibitors revealed an expanded set of Ski2 substrates and offer insights into the dynamic interplay between translation and RNA decay. Overall, our data provide the first transcriptome-wide picture of the substrates, recruitment and regulation of the Ski complex in higher eukaryotes.

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521 α-proteobacterial RNA degradosomes assemble liquid-liquid phase separated RNP bodies

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RNA granules play an important role in organizing eukaryotic mRNA metabolism via liquid-liquid phase separation of mRNA decay factors into membrane-less "droplet" organelles in the cytoplasm. Here its shown that the bacterium *Caulobacter crescentus* Ribonuclease E assembles ribonucleoprotein (RNP) liquid-liquid phase separated droplets that we term bacterial RNP bodies (BR-bodies) that have similar cellular properties to eukaryotic P-bodies and stress granules. Ribonuclease E requires RNA to assemble a BR-body, and disassembly requires RNA cleavage, suggesting BR-bodies provide localized sites of RNA degradation. The unstructured C-terminal domain of Ribonuclease E is both necessary and sufficient to assemble the core of the BR-body, is functionally conserved in related α-proteobacteria, and influences mRNA degradation. Like eukaryotic P-bodies or stress granules, BR-bodies are rapidly induced under cellular stresses and provide enhanced cell growth under stress. To our knowledge, *C. crescentus* RNase E is the first bacterial protein identified that forms liquid-liquid phase separated RNP granules. Liquid-liquid phase separation provides an effective strategy for the subcellular organization of mRNA decay in these organisms as bacterial cells generally lack membrane bound compartments.

522 Assessing the role of UPF1 during premature translation termination

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Premature termination of translation at nonsense codons has potentially deleterious functions to the cell by producing C-terminally truncated polypeptides. Nonsense-mediated mRNA decay (NMD) represents a conserved quality control pathway in eukaryotes limiting accumulation of such aberrant protein products by recognizing nonsense-containing mRNA and targeting it to accelerated degradation. How the NMD machinery is able to monitor ribosomes to determine when termination is premature, and then communicate this information to the mRNA decay machinery remain key, yet undetermined questions.

UPF1, an essential component of the NMD machinery, is an RNA-dependent ATPase. We have previously shown that in yeast cells harboring ATPase-deficient UPF1, ribosomes stall on NMD substrates at or near the premature termination codon, demonstrating a functional role for UPF1 ATPase activity in facilitating translation termination and/or ribosome recycling. Moreover, the observed ribosome stalling required the RNA-binding and ATP-binding activity of UPF1, and NMD co-factors UPF2 and UPF3. We present further insights into the impact of UPF1 ATPase activity on premature translation termination. Ongoing efforts are directed at assessing the requirements for establishing a functional interaction between UPF1 and the terminating ribosome.

523 Structural and kinetic insights into stimulation of RppH-dependent RNA degradation by the metabolic enzyme DapF

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Critical to the survival of living organisms is the ability to precisely regulate the production of proteins needed to respond to an ever-changing environment. Because the rate of protein biosynthesis depends on the availability of mRNA for translation, the modulation of mRNA lifetimes represents a commonly used strategy to influence protein production. In *Escherichia coli*, the decay of many mRNAs proceeds through a 5'-end-dependent pathway triggered by conversion of the triphosphate initially present at the mRNA 5' end to a monophosphate. This conversion occurs in two steps that involve the removal of the terminal phosphate by an unidentified enzyme to generate a diphosphorylated intermediate followed by the removal of an additional phosphate by the RNA pyrophosphohydrolase RppH to produce a monophosphorylated RNA product vulnerable to attack by the endoribonuclease RNase E[1]. RppH is also capable of converting a 5' triphosphate to a monophosphate in a single step without generating a diphosphorylated RNA intermediate, albeit more slowly than it acts on diphosphorylated substrates.

In *E. coli*, the activity of RppH is stimulated by binding to DapF, a diaminopimelate epimerase involved in lysine and cell wall biosynthesis. However, our high-resolution crystal structures of the RppH-DapF complexes have not revealed detectable DapF-induced allosteric changes in the structure of RppH[2]. To understand the mechanism by which DapF potentiates RppH activity, we have examined the effect of DapF binding on the reactivity of various diphosphorylated and triphosphorylated RNAs with RppH. These studies have revealed that the stimulatory effect of DapF depends on the length of the RNA substrate and is greatest for substrates that appear long enough to reach from the active site to DapF in the ternary complex. Furthermore, for RNAs too short to reach DapF, DapF binding increases the reactivity of those that are triphosphorylated (presumably by a structurally subtle allosteric mechanism) but not those that are diphosphorylated. These unexpected observations demonstrate the versatility of RppH by showing that it employs multiple activation strategies in response to the binding of its protein modulator.

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524 Cell cycle arrest-induced changes in CELF1 function and target mRNA decays

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Posttranslational modifications of RNA-binding proteins are often dysregulated in cancer, resulting in abnormal mRNA expression and turnover rates. For example, aberrant phosphorylation of RNA-binding proteins appears to alter their functions and causes abnormalities in binding to GU-rich sequences in 3' Untranslated Regions (3'UTR) of mRNAs, which results in mRNA degradation. Phosphorylation decreases the binding by CELF1 to mRNA 3'UTR, and it allows the networks of target mRNAs to rapidly accumulate in the cell and be translated into proteins. Members of these mRNA networks, coding for regulators of cell proliferation, are overexpressed in cancer. We hypothesize that the phosphorylation and functional inactivation of CELF1 contributes to an oncogenic phenotype by preventing the degradation of mRNA transcripts that promote cell growth and cell proliferation.

We conducted series of cell cycle arrest experiments in malignant T cell lines, with various chemotherapeutic drugs and radiation exposure.

When malignant cells were arrested at G1/S or G2/M phases of the cell cycle, we detected significant dephosphorylation of CELF1, similar to the level in normal, non-dividing T cells. Dephosphorylation of CELF1 led to restored binding to GU-rich mRNAs. It was also significantly correlated with increased decay of transcripts that promote cell growth and prevent apoptosis.

These results suggest that blocking phosphorylation of CELF1 could be used as an approach to inhibit malignant proliferation, by controlling the expression of hundreds of GU-rich mRNAs simultaneously. Specific kinase signaling pathways will be discussed.

525 Novel factors of *Arabidopsis thaliana* NMD mRNA surveillance pathway

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Nonsense-mediated mRNA decay (NMD) is a conserved mRNA surveillance mechanism to prevent the production of potentially harmful proteins by eliminating aberrant mRNAs carrying premature translation termination codons (PTC). The key NMD effectors, ATP-dependent RNA helicase UPF1 together with UPF2 and UPF3, form a core of the NMD complex in all eukaryotes. In addition, in vertebrates this process involves SMG1, SMG5-9, the ribosome, the exon-exon junction complex (EJC) and eukaryotic release factors ERF1 and ERF3A. Recent studies revealed a whole set of new additional and auxiliary NMD components, including RNA helicases, subunits of the eukaryotic initiation factor, transcription-export (TREX) complex, various signaling proteins and nucleus-associated RNA-binding proteins.

NMD as been well described in yeast, fruit flies and humans, but it still remains poorly characterized in plants. To identify new plant NMD factors we have analyzed UPF1-interacting proteins by affinity purification using a transgenic Arabidopsis line expressing tagged UPF1. Besides UPF2, UPF3 and SMG7 we have retrieved ribosomal and RNA-binding proteins, splicing factors, RNA helicases, subunits of eukaryotic initiation factor 3 and 4, and proteins involved in nuclear transport and proteolysis. We have focused on three families of RNA helicases and splicing factors. To investigate their involvement in NMD we have applied the VIGS assay (Virus-Induced Gene Silencing agroinfiltration transient NMD), which allows for quick and efficient testing of many potential NMD factors using transient transfection of N. benthamiana leaves. This approach was followed by analyses of endogenous NMD substrates in Arabidopsis T-DNA insertions mutant of factors under study. Beside we investigate co-localisation of novel NMD factors with UPF1 and UPF3 proteins in Arabidopsis protoplasts. Furthermore the new interaction between RNA helicases and UPF1 were confirmed by co-immunoprecipation in N. benthamiana. We will present results of these proteomic and functional analyses.

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526 The human homolog of a bacterial endonuclease is essential for mitochondrial gene expression

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Mammalian mitochondria contain a small genome encoding 13 polypeptides essential for oxidative phosphorylation (OXPHOS), which are translated by dedicated mitochondrial ribosomes. Additionally, the mitochondrial proteome includes approximately 1500 further proteins that are encoded in the nucleus and imported into the organelle, where they are involved in mitochondrial functions.

Many of these proteins are orthologs of bacterial proteins that have outlasted the evolutionary remodeling of the mitochondrial genome, suggesting that they have essential functions in mitochondria. One of them is the putative endoribonuclease C21ORF57/YBEY, whose bacterial counterpart is proposed to be involved in the maturation and quality control of ribosomal RNAs.

Here we show that human YBEY is involved in the post-transcriptional regulation of gene expression in mitochondria. YBEY-knockout cells exhibit impaired OXPHOS activity associated to severely decreased steady-state levels of the mitochondrial encoded OXPHOS subunits, ultimately leading to mitochondrial dysfunction. We observed a severe decrease of mitochondrial ribosomal RNAs, apparently resulting in a lack of actively translating mitoribosomes, although ribosomal RNA processing was not altered. Moreover, we identified a mitochondrial RNA-binding protein as a key interactor of YBEY, potentially mediating its functions.

527 Deadenylation suppresses cell death and immune genes to maintain liver homeostasis

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Deadenylation is the initial step of mRNA decay, though how it controls cellular mRNA levels is not fully understood. Here, we show that deadenylation affects both mRNA decay and transcription to regulate gene expression in liver. In liver, immune and cell death genes are expressed at low-to-intermediate levels, while metabolic genes are highly expressed. mRNA half-lives of immune and cell death genes, which the CCR4-NOT complex, a major deadenylase complex in mammals, strongly bind, are shorter than those of metabolic genes, suggesting that immune and cell death genes are rapidly degraded by its complex not to increase their expression levels. To investigate the role of the CCR4-NOT complex in the regulation of inflammatory and cell death gene expression in liver, we generated conditional knockout mice of Cnot1, a scaffold protein of this complex. Liver-specific disruption of *Cnot1* elongates bulk mRNA poly(A) tails from 70 to 250 nucleotides, indicating that deadenylation is disrupted in liver. Without Cnot1, expression of inflammatory and apoptotic/necroptotic genes is increased due to mRNA stabilization with increase of transcription as suggested by increment of precursor mRNA levels. The metabolic mRNAs expressed are also stabilized, yet their level is decreased, mainly due to inactive transcription as suggested by reduced precursor mRNA levels. The increase of immune and cell death genes and the decrease of metabolic genes cause lethal hepatitis. Thus, deadenylation suppresses cell death and immune genes to maintain liver homeostasis.

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528 A disordered C-terminus in the S. pombe decapping enzyme Dcp2 stabilizes an autoinhibited conformation

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5'-3' mRNA decay is critical for ensuring proper gene expression in eukaryotes and represents a tightly controlled process involving an intricate network of proteins. Many of these decay factors contain intrinsically disordered regions, including the decapping protein Dcp2 that is responsible for removing the 5' 7-methylguanosine cap from a mRNA transcript and committing it to degradation. In order to characterize the function of the disordered region in Dcp2, we reconstituted a construct of S. pombe Dcp2 containing a majority of the disordered C-terminus in complex with its obligate cofactor Dcp1. This extended construct of Dcp2 is autoinhibited relative to a Dcp1:Dcp2 complex consisting of only the structured core domains of Dcp2 and we identify two regions in the C-terminus responsible for autoinhibition. Additionally, we introduced a mutation in the catalytic domain of Dcp2 predicted to destabilize a catalytically incompetent conformation and show that it mitigates the negative effects of the C-terminus and largely bypasses the need for activation by the mRNA decay factor Edc3. Furthermore, we found this mutation quenches interdomain dynamics in the structured core of Dcp2, indicating conformational motions in Dcp2 primarily act to limit its activity. Our results suggest the C-terminal region of Dcp2 stabilizes an inactive conformation observed in solution and provides evidence for an additional layer of regulation that allows for exquisite control of 5'-mediated mRNA decay.

529 Pnrc2 Regulates 3'UTR-Mediated Decay of Cyclic Transcripts During Somitogenesis

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Vertebrate segmentation is regulated by the segmentation clock, a biological oscillator that controls periodic formation of embryonic segments. This molecular oscillator generates cyclic gene expression in the tissue that generates somites and has the same periodicity as somite formation. Molecular components of the clock include the *her/Hes* family of transcriptional repressors, but additional transcripts also cycle. Maintenance of oscillatory gene expression requires that transcriptional activation and repression, RNA turnover, translation, and protein degradation are rapid (one cycle is 30 minutes in the zebrafish). Little is known about post-transcriptional control of cyclic transcripts during somitogenesis and our work employs genetic and biochemical approaches to better understand rapid cyclic transcript turnover. We have shown that loss of Proline-rich nuclear receptor coactivator 2 (Pnrc2) in zebrafish causes accumulation of cyclic transcripts like *her1*, *deltaC*, and *deltaD*, and that the *her1* 3'UTR confers instability to otherwise stable transcripts in a Pnrc2-dependent manner. To begin to identify *her1* 3'UTR cis-regulatory elements critical for Pnrc2-mediated decay, we show here that the last 180 nucleotides (nts) of the 725 nt *her1* 3'UTR is sufficient to confer rapid instability. Additionally, we show that the 3'UTR of the *deltaC* cyclic transcript also contains destabilizing elements. We hypothesize mechanisms regulating cyclic transcript turnover are shared among cyclic transcripts and are currently identifying 3'UTR cis-regulatory elements that confer Pnrc2-mediated decay. Interestingly, cyclic protein levels do not accumulate in *pnrc2* mutants, suggesting that stabilized cyclic transcripts are not efficiently translated and that translation may be controlled by additional post-transcriptional mechanisms. Our work explores mechanisms regulating oscillation dynamics during vertebrate segmentation and will further our understanding of pathways controlling post-transcriptional gene regulation.

530 Withdrawn

531 Withdrawn

532 DND1 maintains germline stem cells via recruitment of the CCR4–NOT complex to target mRNAs

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Germ cells exhibit unique enrichment of tissue-specific RNA-binding protein (RBP) families, while the majority of RBPs are ubiquitously expressed. The losses of such germline-specific RBPs often causes infertility, indicating that these cells acquired unique RNA regulations for their biological functions. The vertebrate-conserved RBP, DND1, is one of such germline RBPs and required for survival of primordial germ cells (PGCs), as well as suppression of pluripotent testicular germ cell tumours (TGCTs) in mice. It has been proposed to protect specific mRNAs from repression by microRNAs. Here, we report that DND1 binds a UU[A/U] trinucleotide motif predominantly in mRNA 3' untranslated regions (UTR), and destabilizes target mRNAs through direct recruitment of the CCR4-NOT deadenylase (CCR4) complex. Transcriptomic analysis revealed that the extent of suppression is dependent on the number of DND1 binding sites. The DND1-dependent mRNA destabilization is required for survival of murine PGCs and spermatogonial stem cells (SSCs) by suppressing apoptosis. The target RNA spectrum includes positive regulators of apoptosis, inflammation, and modulators of signalling pathways regulating stem cell pluripotency including the TGF-beta superfamily, all of which are aberrantly elevated in *Dnd1*-deficient PGCs. We propose that the induction of the posttranscriptional suppressor DND1 synergizes with concurrent transcriptional changes to sharpen developmental transitions during cellular differentiation and maintenance of the germline.

533 Post-transcriptional regulation in glucose-stimulated insulin biosynthesis by the Ccr4-Not deadenylase complex in mouse pancreatic islets

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Glucose-induced insulin biosynthesis plays a pivotal role in the maintenance of blood glucose homeostasis. Accordingly, gene expression in glucose-induced insulin biosynthesis is tightly regulated, and its dysregulation causes metabolic disorders such as diabetes. Post-transcriptional regulation contributes to timely gene expression in response to external stimuli, such as elevated blood glucose just after food intake. Although post-transcriptional regulation also governs a multitude of physiological processes, its contribution to glucose-induced insulin biosynthesis remains largely unknown.

The Ccr4-Not deadenylase complex is involved in mRNA metabolism such as transcription and deadenylation, with mRNA persistence governed by removal of the poly(A) tail, leading to subsequent degradation of the mRNA. Thus, the Ccr4-Not complex is a key regulator of post-transcriptional control. Intriguingly, glucose-induced insulin biosynthesis is impaired in *Cnot7*-knockout (KO) pancreatic islets, in which the *catalytic* subunit of the Ccr4-Not deadenylase complex, *Cnot7*, is disrupted. Since the deadenylase activity of the Ccr4-Not deadenylase complex is suppressed in *Cnot7*-KO mice, gene expression by deadenylation of mRNAs might be altered in glucose-stimulated insulin biosynthesis.

Insulin is a hormone made in pancreatic β cells that contributes to glucose uptake into cells and subsequent reduced blood glucose. Insulin is synthesized as a single polypeptide called preproinsulin. The signal peptide of preproinsulin is removed in endoplasmic reticulum (ER), forming proinsulin, which is transported to the trans-Golgi network (TGN), where immature granules are formed. Mature insulin is composed of two peptide chains linked by disulfide bonds processed by cellular endopeptidases, PC1 and PC2, as well as CPE.

To investigate the role of CNOT7 in glucose-induced insulin biosynthesis, pancreatic islets isolated from *Cnot7*-KO mice were analyzed using genome-wide RNA-seq, proteomics and electron microscopy. Proteome analysis revealed that protein disulfide isomerase family A member (Pdia) is reduced in *Cnot7*-KO pancreatic islets. Pdia catalyzes protein folding and thiol-disulfide interchange reactions in ER. Moreover, morphological study using electron microscopy revealed that immature insulin granules containing proinsulin were increased in *Cnot7*-KO pancreatic β cells whereas mature insulin granules containing insulin were reduced. In summary, CNOT7 might contribute to glucose-induced insulin biosynthesis by regulating Pdia and subsequent formation of disulfide bonds between two peptide chains of insulin.

534 DDX5 partners with distinct coding and non-coding RNAs to control mucosal immunity

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The mucosal immune system in the gastrointestinal tract provides barrier protection against microbial challenges. Two main players of the system includes the continuous layer of intestinal epithelial cells and the adaptive immune cells patrolling in the underlying lamina propria. Recently, DDX5, a nuclear protein, and member of the DEAD box family of RNA binding helicase proteins, was reported to confer effector functions in a type of adaptive immune T cells in the gut called T helper 17 (Th17). Not only are Th17 cells responsible for tightly controlling commensal bacteria colonization, they also secrete inflammatory molecules that drives pathogenesis in mouse models of colon cancer. In colorectal cancer lesions from human patients, DDX5 is upregulated and contributed to the proliferation of the transformed epithelial cells. Similar to other RNA helicases, DDX5 has been reported to regulate transcription, mRNA splicing, and microRNA processing in various cellular contexts, however, little is known about its target RNAs in the cells of the intestinal niche. Additionally, the impact DDX5 has on the synthesis, stability, structure and function of its partner RNAs remains to be characterized. To address these questions, we employed the Enhanced Cross-Linked Immunoprecipitation (eCLIP) method and coupled it to high-throughput sequencing to define for the first time DDX5 binding sites on target RNAs in both intestinal epithelial cells and Th17 cells from mice.

Our genome-wide study showed that DDX5 binds to overlapping and distinct sets of coding mRNAs and non-coding RNAs in the different cell types. We found that DDX5 preferentially binds to introns of mRNAs in T cells and exons of mRNAs in epithelial cells. The majority of its target RNAs are marked by one or two DDX5 contact sites with notable cell-type-specific motif preferences. Gene ontology analysis of DDX5 bound mRNA transcripts revealed tissue-specific pathways downstream of DDX5. In summary, our study uncovered common as well as tissue-specific RNA partners of DDX5 in the intestinal mucosal immune system. Better understanding of the partnership between DDX5 and its associated RNAs may provide new targets for future immunomodulation and anti-cancer therapeutic development.

535 Functions of LARP4 RNA Binding Proteins in Vertebrate Development

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Background: The La-Related Proteins (LARPs) are a superfamily of RNA binding proteins with diverse roles across RNA metabolism. A duplication early in the vertebrate lineage yielded the LARP4 family (*LARP4A* and *LARP4B*), whose individual roles in development, homeostasis and disease are not yet understood. Whilst *in vitro* studies show both proteins regulate translation, postulated mRNA targets and disease associations differ, with *LARP4B* identified as a neurodegenerative disease-associated gene by our collaborators. Therefore, to understand the functions of the individual family members *in vivo*, and to assess the likelihood of *LARP4B* playing a role in disease, we investigated the functions of all LARP4 family members in zebrafish - characterising both expression across development and genetic loss of function models we generated.

Results: The zebrafish orthologs *larp4aa*, *larp4ab* and *larp4b* have distinct, spatiotemporally dynamic expression patterns throughout development. Whilst *larp4b* is ubiquitously expressed, *larp4aa* and *larp4ab* are expressed specifically in the visual system (retina and tectum) and myotomes. This suggests some tissue specific functions, with areas of overlapping expression indicative of partial functional redundancy. To model loss of gene function, each gene was knocked out using genome editing approaches. Whilst the loss of any individual *larp4* did not grossly impact morphology or larval survival, subtle morphological and behavioural phenotypes were observed. In depth analysis of the *larp4b* null alludes to a role for larp4b in the control of the cell division of neural progenitors and motor development, and provides the first comprehensive analysis of the impact of larp4b loss on a vertebrate transcriptome.

Conclusion: Our findings describe the dynamic expression of the *LARP4* family in vertebrates for the first time whilst our functional study indicates the LARP4 proteins have some distinct developmental functions in vertebrates.

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536 Disparate molecular mechanisms enable plasticity in RNA-protein interactions

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Post-transcriptional control of mRNA by RNA-binding proteins permeates biology. The molecular and structural underpinnings that enable diversification of RNA recognition are fundamental. RNA-binding specificity dictates which mRNAs are subject to regulation and is a key source of evolutionary plasticity in the configuration of mRNA regulatory networks. Pumilio and FBF are founding members of the PUF protein family, an exemplary system to study the divergence of eukaryotic RNA recognition. PUFs typically bind to mRNA in a modular fashion through the use of eight structural repeats. We focus on a pair of homologous Caenorhabditis elegans PUF proteins. The Pumilio homolog PUF-8 and FBF-2 bind eight- or nine-nucleotide sequences, respectively. We obtained the crystal structure of PUF-8 bound to an eight-nucleotide RNA element. Based on comparisons of this structure to that of FBF, we reasoned that mutations lining the RNA recognition surface would permit changes in binding element length. Through molecular genetics and structural analysis, we demonstrate that such mutants in FBF-2 preferentially bind the PUF-8 eight-nucleotide consensus element. Intriguingly, several of the mutants we describe occur naturally. Multiple mutant crystal structures confirm that curvature is not the sole mechanism responsible for changes in binding element length. Our results suggest a new model for specification of binding element length wherein amino acids that interact with RNA bases can dictate motif length independently of changes in backbone topology.

537 Withdrawn

538 Exploring functional complexes and disease networks within human RNA-binding protein interactomes

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RNA is subject to multiple processing steps under tight regulatory control by coordinated and dynamic interactions with hundreds of RNA binding proteins (RBPs). RBP-RNA and RBP-protein interactions at each stage of the RNA life cycle are disrupted in a growing number of human diseases. Recent approaches evaluating protein-protein interaction (PPI) networks for both nuclear and cytoplasmic RBPs in the absence or presence of RNase treatment have extended the human RBP catalog to include RBPs not identified by polyadenylated mRNA interactome-capture. Here we expand these efforts by conducting a large-scale RBP-centered, RNase-coupled proteomics approach. We use 3 complementary resources to affinity purify RBPs of interest from human cells and determine interactomes by quantitative mass spectrometry. First, we utilize a large RBP open reading frame (ORF) plasmid collection that allows constitutive expression of over 1000 RBPs all fused to standard affinity tags. This ORF collection also includes over 200 known disease associated RBP mutations for direct comparison with wild type controls, to establish how disease mutations perturb RBP interaction networks. Second, we use a CRISPR-Cas mediated approach to affinity tag RBPs of interest at their endogenous gene loci, allowing for stable and physiological expression of tagged baits. Third, we utilize our collection of ~300 IP validated commercial antibodies to purify endogenous RBPs and compare to tagged RBP interactomes. By overlapping these pull-down strategies over many biological replicates, we build a robust and predictive RBP PPI network for defining functional and disease-relevant higher order RNP complexes. This RBP PPI network also serves as a valuable resource for RBP discovery, by identifying RNA-mediated RBP interactors that are likely novel RBPs.

539 "High VaultAge" - Molecular studies on the vault RNA1-1 – p62 interaction

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Even though vault RNAs have been discovered more than three decades ago[1], the function of this abundant and broadly conserved RNA species remains largely elusive. We found that vault RNA1-1 binds the autophagy receptor p62/sequestosome-1 and affects p62-dependent autophagic flux in the human hepatocellular carcinoma cell line HuH-7 (see abstract by Horos et al.).

Here, we show that this interaction occurs in other human cell lines and also extends to the single mouse vault RNA and the mouse homolog of p62. We observe that the interaction of p62 with the autophagic protein LC3B changes upon acute (LNA mediated knockdown) as well as stable (CRISPR/Cas9 mediated knockout) vault RNA1-1 depletion. Furthermore, we identify vault RNA-dependent p62 interaction partners by mass-spectroscopy after immunoprecipitation of p62 from control versus vault RNA1-1 knockout cells, respectively. To further delineate the molecular and structural details of these interactions, we currently conduct *in vitro* mutational studies of both p62 and vault RNA1-1.

We suggest that vault RNA1-1 acts as a non-coding "effector RNA" which plays a functional role in the regulation of autophagy, and we characterize biochemical aspects of this RNA-protein interaction.

1) Kedersha, N.L., and Rome, L.H. (1986). *Isolation and characterization of a novel ribonucleoprotein particle: large structures contain a single species of small RNA. J. Cell Biol. 103, 699–709.*

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540 Exploring the characteristics and functions of RNA duplexes bound in vivo by Staufen

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The structure of RNA molecules is important for regulating post-transcriptional gene expression. This function is fulfilled largely through influencing the interactions between RNA and associated *trans*-acting factors, notably RNA binding proteins. Two techniques provide complementary insights into these interactions *in vivo* on a transcriptome-wide level. PARIS (psoralen analysis of RNA interaction and structure) maps cellular RNA duplexes on a global scale, while hiCLIP (RNA hybrid individual-nucleotide resolution UV cross-linking and immunoprecipitation) provides a targeted view of these structures from the perspective of an RNA binding protein of interest.

Here, we focus on the human double-stranded RNA binding protein, Staufen. Despite evidence for a role in mRNA localisation, stability and translation, its mechanism of action and the properties of its interactions with RNA duplexes remain poorly understood. hiCLIP has already found a preponderance of long-range intramolecular duplexes in the 3' untranslated regions. With new methodological insights into hiCLIP we have extended the bioinformatic analysis to identify hitherto undetected duplexes resulting in a more comprehensive and representative picture of Staufen binding.

Then, to gain mechanistic insight into Staufen binding and features conferring specificity, we perform the corresponding analysis of PARIS data, and thus place these Staufen-bound duplexes into the context of RNA duplexes cell-wide. Finally, to explore the potential functional implications of Staufen-bound duplexes, we integrate our results with orthogonal data sets, including 4sU-seq to study RNA metabolism profiling, ribosome profiling to examine translation and 3' end sequencing to explore polyadenylation. With these complementary approaches, we aim to deepen our understanding of the role of RNA structures bound by Staufen.

541 Single molecule study of the dynamics of RNA unfolding by protein S1: Conformational stability of the RNA matters most

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Conformational changes at the 5'-end of structured bacterial mRNAs regulate gene expression by modulating the formation of the translation initiation complex. Ribosomal protein S1 from E. coli is known to play important roles in the translation initiation step of many, if not all, mRNAs, particularly those with weak Shine-Dalgarno (SD) sequence or strong secondary structure at their 5' ends. In this work, we investigated the mechanistic details of the interaction between RNA and S1 using the well-characterized, highly structured pseudoknot from the Thermoanaerobacter tengcongensis (Tte) preQ1 riboswitch as a model. One particularly attractive feature of this pseudoknot is that, by virtue of being a riboswitch, its conformation and structural dynamics can be altered readily, without changes to the RNA sequence, by the addition of ligands with varying affinities, such as preQ1, guanine, and 2-6-diaminopurine (DAP). Using single molecule total internal reflection fluorescence microscopy (TIRFM) and a doubly-labeled RNA-pseudoknot, where changes in conformation of the pseudoknot can be monitored by changes in intramolecular fluorescence resonance energy transfer (FRET) between the fluorophores, we demonstrate that S1 binds to the pseudoknot and effectively stabilizes a partially unfolded conformation. Transition Occupancy Density Plots (TODPs) show that the fraction of molecules that display a reversible transition between fully folded (high-FRET, ~0.9) and partially unfolded (mid-FRET, ~0.7) conformations significantly increases in the presence of S1. Furthermore, competitive binding experiments reveal that S1 has little to no effect on pseudoknots that are more stably folded by binding the high-affinity ligand preQ1, but can interact considerably with and unfold the more weakly folded, DAP-bound pseudoknot. Complementary gel mobility shift assays show that ligand-induced conformational stability conversely disfavors the S1-RNA interaction, and additionally that S1 preferentially interacts with the less folded (i.e., ligand-free) form of the riboswitch. These mechanistic insights at the single molecule level delineate the scope and limitations of S1-mediated unfolding of structured RNAs.

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542 Lariat Debranching Enzyme Cleavage of Backbone Branched RNAs with Non-canonical Branch-point Residues

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During splicing, introns are removed as lariat structures in which an adenosine branch-point (bp) residue is linked at the 2'-position to the 5'-end of the RNA sequence that corresponds to the conserved residues of the intron. Subsequently, the lariat RNA with the adenosine bp is the substrate for lariat debranching enzyme (Dbr1p) that specifically cleaves the unique 2'-5'-phosphodiester bond in the lariat RNA. Following debranching, the introns are used in important cellular processes such as snoRNA and drosha-independent miRNA biogenesis among others, and accumulation of lariats can have significant negative consequences. While lariats with non-adenosine bp are known to accumulate at low levels, little is known about the ability of Dbr1p to recognize and cleave branched RNA with a non-adenosine bp. Here present data on the cleavage kinetics of synthetic branched RNAs with both adenosine and other non-canonical branch points by Dbr1p derived from *E. histolytica*. Dual fluorescently labeled backbone-branched RNAs (bbRNAs) with C, G and U besides A as bp residues enable real time kinetics assay of the Dbr1p cleavage reaction. From these assays we analyze the binding and cleavage activity of *E. histolytica* Dbr1p towards these bbRNAs with non-canonical branch-point residues. These data have implications for the design of Dbr1p inhibitors that may be significant in investigating the role of debranching in retrotransposition and retroviral infections.

543 Network of Splice Factor Regulation by Unproductive Splicing

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Nearly all human multi-exon genes undergo alternative splicing and approximately 20% of expressed genes introduce premature termination codons that lead to nonsense mediated mRNA decay (NMD). Some splicing factors regulate their targets' gene expression through alternative splicing that creates a premature termination codon, which in turn causes this unproductive transcript to be degraded by NMD. Some splicing factors are themselves regulated via alternative splicing coupled to NMD. We surveyed the literature to collect reported experiments that show splicing factor regulation via alternative splicing coupled to NMD. 34 instances are reported from 45 cases experimentally tested. Self-regulation is observed in one-third of them (13/34).

The reported regulatory experiments do not reveal the full extent of regulation via alternative splicing coupled to NMD, since only a few splicing factors were tested and none of these studies were performed on a genome-wide scale. We therefore undertook to explore the global prevalence of splicing factor regulation via alternative splicing coupled to NMD. This type of regulation requires binding of a splicing factor to the regulated mRNA. Using publicly available CLIP-seq data, we collected protein-RNA interactions between splicing factors. We find that splicing factors form a highly-connected network, where 60% (1153/1936) of all possible interactions between splicing factors and the transcripts encoding splicing factors are observed. Although not all interactions might represent regulatory events, this highly connected network suggests extensive cross-regulation among splicing regulators.

We compared the hierarchy of splicing factors to the hierarchy of transcription factors. We inferred hierarchies of transcription and splicing factors using ENCODE ChIP-seq and eCLIP data, applying the hierarchy metric of Gerstein et al. (Nature 2012 489:91-100). Our limited data show that the hierarchy of splicing regulators is different from that of transcription factors. Gerstein et al. plot networks in 3 distinct layers, with a top "executive" layer, a bottom "foreman" layer, and a middle layer in between. Unlike transcription factors, which concentrate at the extremes of the hierarchy metric, splicing factors form a network that has nearly uniform distribution of proteins across the hierarchy metric and thus less clearly defined separation into the three distinct layers.

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544 Sequence, Structure and Context Preferences of Human RNA Binding Proteins

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Regulation of RNA processing is principally driven by RNA-protein interactions, yet the specificity that determines how most RNA binding proteins (RBPs) select their targets among the complex cellular pool of RNAs remains incompletely understood. We developed a high-throughput version of RNA Bind-n-Seq (RBNS), an in vitro method capable of determining the sequence and RNA secondary structure preferences of RBPs in an unbiased fashion. In this assay, pools of randomized 20nt RNA fragments are synthesized and incubated with recombinant RBPs. RNA-protein complexes are isolated and subjected to sequencing yielding ~10-20 million unique reads preferentially bound to protein. RBNS assays resulting in over 6 billion protein-bound reads revealed detailed sequence specificity for a comprehensive set of ~80 human RBPs containing a variety of RNA binding domain types, of which a majority have not been previously characterized. We find that conservation of individual bases within RBP motifs relates to binding activity. That is, positions of a motif that are important for in vitro binding affinity tend to be more conserved in specific transcriptome regions, demonstrating a nucleotide-level link between affinity and conservation. Consistent with previous reports, most proteins assayed prefer unstructured RNA substrates; however, a subset of factors (e.g., PRR3 and ZNF326) preferentially bind structured or partially structured motifs. We report a set of factors that interact with discontiguous or split motifs characterized by multiple tandem cores interrupted by varying lengths of random nucleotides. We experimentally demonstrate that preference for split motifs is related to the orientation of the RNA binding domains. These sequence, structure and split motif specificities can often be observed in enhanced crosslinking and immunoprecipitation (eCLIP) and are predictive of regulation in cells. This work establishes binding preferences for a set of human RBPs and extends characterization beyond short linear motifs, highlighting the complex mechanisms of RNA-protein interactions that help determine target specificity in cells.

545 Investigating the Interactions of Splicing Factor SF3A1 with the Stem-loop 4 of U1 snRNA and RNA helicase UAP56

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The human splicing factor SF3A1 is one of three subunits of the SF3A trimer which interacts with the SF3B complex and the 12S U2 small nuclear ribonucleoprotein (snRNP) to form the mature 17S U2 snRNP; an essential component of the spliceosome. Previously, we have shown that SF3A1 interacts with stem-loop 4 (SL4) of the U1 snRNA to form a molecular bridge between the 5' and 3' splice sites during early spliceosome assembly (Sharma et al., Genes and Dev. (2014) 28:2518-31). Work in our lab also suggests that the RNA helicase UAP56 may interact with SF3A1 to stabilize the SL4-SF3A1 interaction, thereby mediating splice site pairing. However, the regions of SF3A1 responsible for binding to U1-SL4 or UAP56 are not known. The goal of this study is to map the SF3A1 domains required for interactions with U1-SL4 and UAP56. We are using an in-vitro HeLa cell free expression system to express SF3A1 deletion constructs and a combination of UV cross-linking/ SDS-PAGE to determine binding of truncated proteins to U1-SL4 RNA. Preliminary results indicate that U1-SL4 binding may be in the C-terminal region of SF3A1. To identify UAP56 interacting regions we will use a combination of GST-UAP56 pull-down and Western blotting. Characterization of the interactions of SF3A1 with U1-SL4 and UAP56 will contribute to a better understanding of the mechanism of splice site pairing, which is critical for formation of functional mRNAs and is an important target in the regulation of alternative splicing.

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546 Molecular basis of function of the RNA-binding protein PSF

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Polypyrimidine Tract-Binding Protein-associated **S**plicing **F**actor (PSF) is a ubiquitous RNA binding protein that regulates alternative splicing**,** a regulatory process generating distinct protein isoforms to impact cell physiology. To study mechanisms of signal-induced alternative splicing our laboratory uses the well-characterized model of CD45 pre-mRNA splicing in naïve versus stimulated T-cells. Previous work identified PSF as a major factor regulating CD45 pre-mRNA splicing. However, the molecular mechanisms by which signaling triggers changes in PSF to control splicing remain largely unknown. Investigations of these mechanisms will generate a model for allosteric regulation of RNA binding proteins while providing mechanistic insight into signal-induced alternative splicing.

In naïve T-cells, PSF is constitutively phosphorylated on a C-terminal threonine (T687), by **G**lycogen **S**ynthase **K**inase 3 (GSK3)**.** This phosphorylation promotes the binding of PSF to the **150**-kDa component of **T**hyroid hormone **R**eceptor-**A**ssociated **P**rotein complex (TRAP150), and PSF•TRAP150 interactions are known to occlude PSF•RNA binding. Upon T-cell activation GSK3 is inactivated, and newly translated PSF remains unphosphorylated. This PSF is not bound by TRAP150, and thus can bind the exonic splicing silencer motif (ESS) in CD45 RNA to alter splicing.

A partial crystal structure of the PSF dimer has been previously determined that includes two tandem RNA recognition motifs (RRMs). RRM2 was identified as the minimal binding domain for ESS, while a larger fragment of both RRMs and some flanking sequence is required to bind TRAP150. Intriguingly, PSF's RRM2 lacks motifs commonly associated with RNA-binding. I hypothesize that the interaction between RRM2 and ESS RNA likely represents a novel RRM-RNA interaction paradigm. Preliminary data has shown differential protection in RRM2 when bound to ESS-RNA, particularly in a charged loop connecting β-sheet-2 and β-sheet-3 (β2β3-loop) using Hydrogen-Deuterium Exchange coupled to mass spectrometry (HDX-MS). The β2β3-loop is also indicated in binding in mass spectrometry cross-linking experiments. Future work includes identifying the PSF•TRAP150 complex interface with HDX-MS experiments, as well as crystalizing and determining structures of PSF•TRAP150 and PSF•RNA.

547 Direct recruitment of eIF4G or death-associated protein 5 (DAP5) to the 5' untranslated region (UTR) of a subset of cellular mRNAs drives the cap-independent translation of these mRNAs

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During unfavorable cellular conditions (e.g., hypoxia, nutrient deprivation etc.), the canonical, cap-dependent translation initiation pathway is suppressed by sequestration of the cap-binding protein, eIF4E, by 4E-binding protein. Additionally, the expression levels of eIF4G and its cellular homolog, Death Associated Protein 5 (DAP5), are elevated. Under these conditions, a subset of cellular mRNAs, including many, such as the p53, HIF-1α and FGF-9 mRNAs, that encode proteins with important roles in human health and disease, is translated in a cap-independent manner. Despite their physiological importance, however, the molecular mechanisms underlying the cap-independent translation initiation of this subset of cellular mRNAs remain unknown. To address this gap in our understanding, we have used fluorescence anisotropy-based binding assays developed in our laboratories to demonstrate that an N-terminal truncated form of eIF4G that cannot interact with eIF4E (ΔN-4G) or DAP5 can directly bind to structural elements, known as cap-independent translation elements (CITEs), that are found in the 5' UTRs of this subset of cellular mRNAs. Specifically, we have measured the affinities with which ΔN-4G and DAP5 interact with the CITEs of four distinct members of this subset of cellular mRNAs and provide evidence that ΔN-4G and DAP5 bind with different affinities to these CITEs. Using a luciferase-based gene expression reporter assay, we further demonstrate that these same CITEs can promote translation initiation in an ΔN-4G- or DAP5-dependent manner in rabbit reticulocyte lysates in which cap-dependent translation has been largely abrogated. Collectively, our studies are providing quantitative binding data and in vitro translation data that are allowing us to elucidate how a subset of CITE-containing, cellular mRNAs switch from cap-dependent to cap-independent modes of translation initiation; how the CITEs in these mRNAs recruit eIF4G or DAP5 during cap-independent translation initiation; and how binding of eIF4G or DAP5 to the CITEs in these mRNAs enables the subsequent assembly of the translation initiation complex.

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548 Adenovirus Virus-associated RNA I mutations modulate PKR autophosphorylation

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Adenoviruses are icosahedral, dsDNA-containing viruses, sizing from 70-100 nanometers in diameter. They are among the largest group of non-enveloped viruses and primarily infect the membranes of the eyes and respiratory, gastrointestinal and urinary tracts of immuno-compromised children.

Evasion of host immunity by adenoviruses employs a number of different mechanisms, one of which involves the suppression of an interferon pathway protein, protein kinase RNA-activated (PKR). During infection, PKR recognizes and dimerizes on viral dsRNA, which leads to its autophosphorylation and kinase activation. Active PKR inactivates eukaryotic initiation factor 2 (eIF-2) by phosphorylating its alpha subunit, which halts global mRNA translation, thus reducing viral replication and spread.

Adenovirus encodes two ~160 nucleotide long, extremely abundant, highly structured noncoding RNAs termed Virus-Associated RNAs (VA-I and VA-II RNA). VA RNAs bind and inhibit PKR autophosphorylation. It has been reported that the more prevalent VA RNA, VA-I, folds into a three-domain structure containing an apical stem loop, essential for PKR binding; a central domain containing a pseudoknot, involved in PKR inhibition; and a terminal stem, presumably insignificant in PKR interaction.

We analyzed the functional importance of VA-1 structure on PKR inhibition through structure-guided mutagenesis. These findings led us to propose a model for how the apical and central domains of VA-I contribute to PKR capture and inhibition.

549 Structural basis of IMP3 RRM12 recognition of RNA

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The IMP family of RNA binding proteins, also named as insulin-like growth factor 2 (IGF2) mRNA-binding proteins (IGF2BPs), are highly conserved RNA regulators that are involved in many RNA processing stages, especially mRNA stability, localization and translation. There are three paralogs in the IMP family, IMP1-3, in mammals, all of which adopt the same domain arrangement with two RNA recognition motifs (RRM) in the N-terminus and four KH domains in the C-terminus. Here, we report the structure and biochemical characterization of IMP3 RRM12 and its complex with a short RNA that contains four cytosine nucleotides. These structures show that both RRM domains of IMP3 adopt the typical RRM topology with two α-helices packed on an anti-parallel four stranded β-sheet. The spatial orientation of RRM1 to RRM2 is unique compared with other known tandem RRM structures. In the IMP3 RRM12 complex with RNA, only RRM1 is responsible for recognizing a short pyrimidine sequence with modest affinity.

550 Genome-wide mapping RNA targets of *Enterococcus faecalis* Small Alarmone Synthetase RelQ using *in vivo* UV crosslinking

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The stress alarmone nucleotides guanosine pentaphosphate (pppGpp) and tetraphosphate (ppGpp) – collectively referred to as (p)ppGpp – are central players of the bacterial stringent response as well as key regulators of bacterial virulence, antibiotic tolerance and biofilm formation[1]. In addition to posttranscriptional regulation mediated by small signaling molecules such as (p)ppGpp, bacteria utilize an array of RNA-binding proteins (RBP) that directly interact with their RNA targets. We have recently shown that the Small Alarmone Synthetase (SAS) RelQ from the Gram-positive Pathogen *Enterococcus faecalis* combines both these mechanisms within a single protein: acting as an enzyme, it increases the levels of (p)ppGpp – and acting as an RBP, it binds single-stranded RNA which results in a sequence specific inhibition of RelQ's enzymatic activity[2]. While we proposed that RelQ:RNA interaction acts as a regulatory switch between the active and inactive states of the protein, the exact RNA targets and biological significance of the mechanism are unknown. Therefore, in order to identify the RNA targets of RelQ in live cells we have applied a combination of CRAC and CLIP functional genomics approaches.

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551 Structural basis of Dnmt2 stimulation by queuine tRNA modification

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A vast diversity of modifications on tRNA molecules has been identified in the recent years (Machnicka *et al*., 2013). Although several exist simultaneously on the same RNA molecule least is known about if and how they affect each other. Recently such an interplay of modifications was found in *S. pombe* for the S-adenosyl-methionine dependent methyltransferase Dnmt2 (spDnmt2) (Müller *et al*., 2015), which methylates cytosine at position 38 of tRNAs (Goll *et al*., 2006). Its activity on the substrate $tRNA^{Asp}$ was shown to strongly depend on queuine, a 7-deazaguanine derivative, which is incorporated by base exchange into the wobble base position 34 of the tRNA. We established an enzymatic assay to introduce Q34 modification into *in vitro* transcribed tRNA and could show that Q34 modification alone is able to stimulate spDnmt2 activity and did quantify the effect *in vitro*. Furthermore, we ought to understand the molecular details of this effect by determining the crystal structure of spDnmt2. For insights into tRNA binding by spDnmt2 we performed mass spectrometry-based analysis of the cross-linked protein-RNA complex. We observed a catalytically essential flexible loop of spDnmt2 in close contact with the tRNA. To gain insights into the enzyme substrate complex, we performed in silico docking of tRNA to the spDnmt2 crystal structure and obtained a reasonable model for the Dnmt2 tRNA substrate complex, which argues that the Q34 effect on Dnmt2 activity for $tRNA^{Asp}$ might not only be restricted to *S. pombe* but could also be a mechanism in other organisms and pathogens.

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552 In-gel probing to examine RNA structures within RNA-protein complexes of varying stoichiometry

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High-throughput SHAPE (selective 2'OH acylation analyzed by primer extension) has revolutionized RNA secondary structure probing. SHAPE reagents acylate the ribose 2'OH according to the flexibility of the backbone: where nucleotides are Watson-Crick paired the backbone is rigid and refractory to acylation; where nucleotides are single-stranded the backbone binds the SHAPE reagents readily. Primer extension assays are then used to map the relative amounts of SHAPE reagent bound at each nucleotide position along the backbone and this information is fed into modeling algorithms, alongside free energy parameters, to produce structural models. Capillary sequencing using fluorophore-labelled primers can be used to examine the primer extension assays making the technique high-throughput.

In 2013 we published a novel technique based upon high-throughput SHAPE: "in-gel SHAPE" (NAR 2013, Oct;41(18):e174). This technique separates different RNA conformers within a mixed sample using native PAGE, and then probes their structures whilst still inside the gel matrix, enabling analysis of each conformer individually. RNAs are recovered from the gel using electroelution and are then used in primer extension assays, as in the high-throughput SHAPE technique. We used in-gel SHAPE to examine the structures of the HIV-1 (human immunodeficiency virus type-1) packaging signal RNA in its monomeric and dimeric forms and to show that a structural switch occurred between the two.

We have now developed this technique further to examine the structures of RNAs in RNA-protein complexes of varying stoichiometry. Using HIV-1 RNA-protein interactions as a model we show that we can follow the RNA structural changes occurring as RNAs interact with each other and with successive protein molecules: HIV-1 monomeric packaging signal RNA is pushed towards a dimerization-competent structure by its protein ligand, Gag, whereas dimeric RNA is structurally stabilized. This provides valuable insights into the early packaging processes of HIV-1.

553 Systematic investigation of RNA-targeting determinants for CRISPR-Cas13

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The CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR-associated) systems arose in bacteria and archaea as an adaptive immune system to combat foreign genetic elements. Cas13 is a programmable CRISPR system guided by a short crRNA (or guide RNA) to bind and cleave RNA. The target RNA (or activator RNA) is recognized by Cas13 through base-pairing to the crRNA which activates the RNase activity of the higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domains. The activated HEPN-nuclease is a general RNase that robustly cleaves RNA in *trans* (or collateral RNA cleavage) and cleaves the activator-RNA in *cis* outside the crRNA complementary region. The sensitive and rapid activator-RNA dependent *trans*-cleavage by Cas13 has been harnessed for RNA detection as well as point-of-care diagnostics. In contrast, the activator-RNA dependent *cis*-cleavage has been leveraged as a tool for specific RNA targeting in mammalian, plant, and yeast cells.

Cas13 homologs show variability in their efficacy with a given guide and target RNA. Additionally, it is unclear how *trans*-cleavage by Cas13 is attenuated in cells, or to what extent *cis* and *trans* cleavage are separable activities. To address these outstanding questions and develop rules for predicting RNA-targeting outcomes with Cas13, we designed a synthetic oligo library to systematically investigate Cas13 RNA-binding and RNA-targeting *in vitro*. Nesting activator-RNA into an ensemble of secondary structures, we describe Bind-n-Seq data that comprehensively address how target RNA secondary structure and mismatches effect diverse Cas13 enzymes. Additionally, targeting the RNA library with catalytically active Cas13 and sequencing cleaved material produced profiles that describe HEPN-nuclease substrate preferences. Taken together, these data provide experimentally derived rules for predicting RNA-targeting outcomes with CRISPR-Cas13.

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554 Interactomic and Enzymatic Analyses of Affinity Isolated Human LINE-1 Retrotransposons

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LINE-1 (L1) retrotransposons are catalysts of evolution and disease, and L1 sequences compose a significant proportion of the human genome. Despite tremendous influence on genome composition, L1 ribonucleoproteins remain poorly characterized. Nevertheless, L1 RNAs are known to assemble with a combination of permissive host factors that are essential to their lifecycle, as well as repressive factors that constitute defenses against L1's mutagenic activity.

Building on our prior analyses, we have completed a series of experiments to create a multi-dimensional interactomic characterization of affinity isolated L1s as expressed in HEK-293T cells (Taylor et al. Elife 2018). Our results are consistent with the presence of multiple transposon-related macromolecules, likely poised at distinctive stages in the L1 lifecycle. In an effort to translate our findings to human health, we have turned to affinity proteomic analyses of L1s as expressed in resected human colorectal cancers (CRCs). While L1s are expressed in roughly half of all cancers, they are expressed in ≳90% of CRCs, suggesting that many CRCs evolve in the presence of L1. In contrast, ectopic L1 expression in L1-negative model cells, such as HEK-293T or HeLa, is toxic and leads to apoptosis. L1s in patient CRCs may therefore have different interactomes than L1s in model cells, and/or L1s expressed in CRCs may exist in a distinctive cellular context. We present our latest findings on L1 interacting proteins from these two systems.

555 Genome-wide identification of DEAD-box RNA helicase targets reveals roles for RNA secondary structure remodeling in transcriptional termination

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Accurate gene expression requires the coordination of RNA processing with assembly of messenger RNA-protein (mRNP) complex. RNA helicases are a class of enzymes that unwind RNA duplexes in vitro and have been proposed to remodel RNA structure in vivo. Herein, we provide evidence that the DEAD-box protein Dbp2 remodels RNA structure to facilitate efficient termination of transcription in S. cerevisiae. First, we find that Dbp2 associates with the 3' ends and near the translational start site of mRNAs genome wide. Using Structure-seq to map RNA secondary structure, we find altered secondary structures in dbp2Δ cells that overlap polyadenylation elements and correlate with inefficient termination. We also show that efficient termination requires Dbp2 helicase activity, and loss of stable structure in the 3' UTR bypasses the requirement for Dbp2. In addition, we identify the physical interaction of Dbp2 with termination factors Pcf11 and Sen1. This study reveals that DEAD-box RNA helicases remodel mRNA structure in vivo and that structural alteration is essential for proper gene expression.

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556 CRISPR-associated Argonautes: Tools for probing RNA biology

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Transcript abundance controls gene expression to drive cell type, tissue, and developmental specificity. Deregulation of transcript levels, alternative splicing and modification can lead to disease. Therefore, probing RNA biology *in vivo* is critical to understanding developmental processes and disease mechanisms, and can suggest new therapeutic targets. We are developing new technologies to track and manipulate specific single-stranded RNA (ssRNA) *in vivo*, to overcome challenges with present approaches based on oligonucleotide probes or on RNA binding proteins. Here we present mechanistic insights into *Marinitoga phiezophila* Argonaute (MpAgo)-guide RNA (gRNA) and ssRNA substrate binding. We demonstrate that using 5-bromo-2'-deoxyuridine (BrdU) as the 5' nucleotide of gRNAs stabilizes *in vitro* reconstituted CRISPR-associated MpAgo-gRNA complexes (MpAgo RNPs) and significantly improves their specificity and affinity for RNA targets. Using reconstituted MpAgo RNPs with 5'-BrdU modified gRNAs, we mapped the seed region of the gRNA that contributes to specific RNA substrate binding, and identified the nucleotides of the gRNA that play the most significant role in targeting specificity. We also show that these MpAgo RNPs can be programmed to distinguish between substrates that differ by a single nucleotide, using permutations at the 6th and 7th positions in the gRNA. Using these specificity features, we employed MpAgo RNPs to detect specific Adenosine to Inosine edited RNAs in a complex mixture. These findings demonstrate that MpAgo RNPs can be used as a highly-specific RNA-targeting platform to probe RNA biology.

557 RNA structures associated with protein binding sites in pre-mRNA splicing targets for the human RNA chaperones hnRNPA1 and DDX5

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Alternative pre-messenger RNA (pre-mRNA) splicing is a mechanism for controlling gene expression and determines protein isoform patterns in eukaryotes. Removal of introns can occur in distinct ways to generate different patterns of pre-mRNA splicing and distinct protein-coding mRNAs, which is determined by both RNA binding proteins and the structures of the nuclear pre-mRNAs. We have analyzed the pre-mRNA splicing targets for two RNA chaperone proteins, the heterogeneous nuclear ribonucleoprotein hnRNPA1 and the RNA helicase DDX5, both of which are known to modulate pre-mRNA splicing patterns and are implicated in cancer. Using RNA interference and RNA-seq in human K562 cells, we identified thousands of target transcripts for both proteins and quantitatively determined how their splicing patterns change upon RNAi knockdown using MISO and a new algorithm called the Junction Usage Model (JUM). RNA binding sites for both proteins in the nuclear transcriptome were determined using enhanced CLIP (eCLIP), an *in vivo* RNA binding assay. Finally, patterns of *in vivo* and *in vitro* reactivities of cellular RNA from SHAPE chemical RNA probing were used to determine and model RNA structures near exonic binding sites for hnRNPA1 and DDX5. In many cases, there appear to be highly structured RNA regions flanking the sites of hnRNPA1 and DDX5 binding to their RNA targets, suggesting an organization of the nuclear pre-mRNPs upon which the spliceosome forms to generate alternatively spliced pre-mRNAs. hnRNPA1 appears to bind single-stranded RNA regions, while DDX5 appears to bind both unstructured and structured regions of target RNAs.

558 Transcriptome-wide Identification and Validation of Interactions between the miRNA Machinery and HuR on mRNA Targets

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The 3′ UTR of mRNAs is the primary regulatory region that mediates post-transcriptional control by microRNAs and RNA-binding proteins (RBPs) in the cytoplasm. Aside from individual sequence-specific binding and regulation, examples of interaction between these factors at particular 3′ UTR sites have emerged. However, the whole picture of such higher-order regulatory modules across the transcriptome is lacking. Here, we investigate the interactions between HuR, a ubiquitous RBP, and Ago2, a core effector of the miRNA pathway, at the transcriptome-wide level. Using HITS-CLIP, we map HuR and miRNA binding sites on human 3′ UTRs and assess their co-occurrence. Additionally, we demonstrate global effects of HuR knockdown on Ago2 occupancy, suggesting a co-regulatory relationship. Focusing on sites of Ago2-HuR overlap, 13 candidates were screened in luciferase reporter assays. Eleven sites showed miRNA-dependent repression, as confirmed in Dicer-null cells. To test for HuR's role in co-regulation, we measured the reporters in HuR KO cells. Three of the miRNA sites demonstrated altered activities, indicating that HuR has an effect on miRNA repression at those sites. Our study presents an efficient search and validation system for studying miRNA-HuR interactions, which expands our understanding of the combinatorial post-transcriptional control of gene expression at the 3′ UTR.

559 RNA binding by the proline isomerase CypE

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Cyclophilin E (CypE, also known as Cyp33), comprised of an RNA-recognition motif (RRM) and a cyclophilin proline isomerase domain, regulates chromatin remodeling through interaction with the MLL1 complex[1-2]. As part of the chromatin remodeling function, the RRM domain is proposed to play dual roles by binding RNA with a consensus motif enriched at loci regulated by CypE and binding a peptide sequence found in the MLL1 complex through an overlapping binding interface. Surprisingly and potentially related to this dual role of the RRM domain, the cyclophilin isomerase domain was consistently found in a set of studies for non-canonical protein/RNA interactors spanning yeast to humans[3-5]. Remarkably, as a non-canonical RNA-binding domain, the cyclophilin domain binds randomized RNA more tightly than the RRM binds its consensus motif. Moreover, RNA activates the isomerase activity *in vitro* even in the absence of the RRM domain. Through RNA SELEX, we have identified RNA sequences specifically bound by the cyclophilin domain to help characterize the nature of the RNA interaction with the isomerase domain and clarify how RNA regulates its catalytic activity. RNA binding to and activation of the catalytic domain of CypE provides exciting avenues for new insights into the cellular functions of CypE and how RNA may play roles in the function of non-canonical RNA binding proteins.

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560 The effects of SF3B1 inhibitors on U2 snRNP

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The spliceosome is a macromolecular ribonucleoprotein (RNP) that removes introns from pre-mRNA. The spliceosome assembles from five U-rich small nuclear RNAs that associate with different proteins to form U snRNPs. Each snRNP joins the spliceosome in a specific order to recognize an intron and then create a catalytic center for splicing. U2 snRNP, which is composed of U2 snRNA, core proteins, and the SF3A and SF3B protein complexes, is involved throughout the splicing process. It participates in identification of the branch point sequence of the intron, and then positioning the branch point adenosine for splicing chemistry. SF3B1 is the largest SF3B subunit, and is of high interest because it is frequently mutated in cancer cells. Several natural products target SF3B1 and are able to block both early and later stages of the spliceosome, which correlate with U2 snRNA functional requirements. We hypothesize that compounds affect SF3B1 modulation of U2 snRNP structure. To begin testing this model, I used glycerol gradients to fractionate nuclear extract in low and high salt concentrations with and without the SF3B1 inhibitor pladienolide B (PB). Preliminary analysis of protein and RNA sedimentation indicates that PB promotes SF3B association with U2 snRNP. We are now testing whether other U2 snRNP associated proteins or ATP hydrolysis, which has been shown to modulate U2 snRNP conformation, are involved in the effects of the compounds. We expect that our results will provide mechanistic insight into the role of SF3B1 in U2 snRNP and help reveal how SF3B1 inhibitors interfere with the spliceosome.

561 Studying *in vivo* effects of *in vitro* selected antibacterial peptides targeting h31

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Expansion of the class of natural and synthetic antimicrobial peptides targeting the bacterial ribosome is one of the major approaches to address the development of bacterial resistance to currently available antibiotics. *In vitro* selection methods such as phage display are widely used to identify target-specific peptides for functionally important regions in the bacterial ribosome. A previous study in our lab identified several peptides, including some with moderate to high binding affinity towards helix 31 (h31) of the *E. coli* rRNA.[1] Assessment of the *in vivo* effects of these peptides remains a major challenge due to their poor cell penetration properties. In this study, we have utilized a chemically inducible plasmid-based expression system to generate h31-targeting peptides, inside bacterial cells and to assess their effects on cell growth. Our data show a time-dependent growth inhibition in *E. coli* expressing the h31-targeting peptides. These results will be compared to *in vitro* binding studies.[1] Our goal is to further study the interactions between these peptides and bacterial ribosome h31 within the cellular context.

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562 AMPK affects alternative splicing via SRSF1 phosphorylation

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AMP-activated protein kinase (AMPK) is a Serine/Threonine protein kinase that maintains cellular energy homeostasis by inhibiting anabolism and promoting catabolism. However, the function of AMPK is not fully elucidated. Thus, we purified proteins binding with AMPK using the Tandem Affinity Purification (TAP) assay and identified them by LC-MS/MS to find novel AMPK substrates. We identified Serine/Arginine-rich splicing factor 1 (SRSF1) as a candidate of AMPK substrate. SRSF1 belongs to the family of SR-proteins that regulate both constitutive and alternative splicing of pre-mRNA.

In vitro AMPK kinase assay showed phosphorylation of serine residue in the RNA recognition motif (RRM) of SRSF1. This phosphorylation of SRSF1 was induced in cells in which AMPK was exogenously expressed. Moreover, AMPK-inactivated cells showed reduction of the phosphorylation, suggesting that most of this phosphorylation was regulated by AMPK.

SRSF1 has two RRM (RRM1 and RRM2) and the site phosphorylated by AMPK is in RRM2, which is involved in alternative splicing by interacting with specific RNA sequences. Interestingly, it has been reported that the AMPK-phosphorylated serine residue is one of the important residues to recognize RNA. Indeed, its ability for binding with RNA was reduced by phospho-mimetic mutation and AMPK-induced phosphorylation *in vitro*.

Furthermore, we analyzed whether SRSF1 phosphorylation affected the pre-mRNA alternative splicing, such as exon skipping and inclusion. We found that inhibition of AMPK activity modulated SRSF1-dependent alternative splicing. Therefore, our results are the first to demonstrate that AMPK regulates alternative splicing via SRSF1 phosphorylation.

563 The C-terminus of DDX21 recognizes the 2' OH of loop nucleotides in RNA guanine quadruplexes

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Guanine quadruplexes can form in both DNA and RNA and influence many biological processes through various protein interactions. The DEAD-box RNA helicase protein DDX21 has been shown to bind and remodel RNA quadruplexes but little is known about its specificity for different quadruplex species. Previous reports have suggested DDX21 may interact with telomeric repeat containing RNA quadruplex (TERRA), an integral component of the telomere that contributes to telomeric heterochromatin formation and telomere length regulation. Here we report that the C-terminus of DDX21 specifically binds to TERRA. We use, for the first time, 2D saturation transfer difference NMR to map the protein binding site on a nucleic acid species and show that the quadruplex binding domain of DDX21 interacts primarily with the phosphoribose backbone of quadruplexes. Furthermore, by mutating the 2'OH of looped nucleotides we can drastically reduce DDX21's affinity for quadruplex, indicating that the recognition of quadruplex and specificity for TERRA is mediated by interactions with the 2'OH of loop nucleotides.

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564 Deciphering human ribonucleoprotein regulatory networks

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RNA-binding proteins (RBPs) control and coordinate each stage in the life cycle of RNAs. Although *in vivo* binding sites of RBPs can now be determined genome-wide, most studies typically focused on individual RBPs. Here, we examined a large compendium of 114 high-quality transcriptome-wide *in vivo* RBP-RNA cross-linking interaction datasets generated by the same protocol in the same cell line and representing 63 RBPs, which enabled a consistent, comparative analysis of target RNA class binding preference, sequence preference, and transcript region specificity. Furthermore, we identified potential posttranscriptonal regulatory modules, i.e. specific combinations of RBPs that bind to specific sets of RNAs and targeted regions. These regulatory modules encoded functionally related proteins and exhibited distinct differences in RNA metabolism, expression variance, as well as subcellular localization. This integrative investigation of experimental RBP-RNA interaction evidence and RBP regulatory function in a human cell line will be a valuable resource for understanding the complexity of post-transcriptional regulation.

565 hnRNP K interacts *in vitro* with the B and C repeat regions of Xist

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X chromosome inactivation (XCI), which occurs during mammalian female development, is essential for cell viability and completely dependent on the expression of Xist RNA[1]. In combination with a variety of protein binding partners, Xist acts in *cis* to transcriptionally silence the future inactive X (Xi). This process involves many protein partners that interact with different parts of this extremely large RNA[2]. Recent protein and RNA pull-down studies identified hnRNP K, which directly binds the B and C repeat regions of Xist *in vivo*[2,3,4]. This interaction is key in Xist's ability to recruit Polycomb repressive complexes 1 and 2 to Xi to elicit Polycomb-mediated silencing[4]. The B and C regions of Xist have separate and distinct sequences, yet hnRNP K directly binds to both.

Our work aims to better understand how hnRNP K interacts with and discriminates between these two regions of Xist. We find that hnRNP K interacts in a modular fashion with short motifs found in both the B and C repeat regions of Xist *in vitro* and redundantly uses combinations of domains to achieve high affinity binding. Interestingly, specificity for the B motif is conferred through the third KH domain of hnRNP K, but this domain is not required for high affinity binding. These results support a model whereby hnRNP K's multiple domains work in concert to achieve high affinity interactions with different RNA elements with a spectrum of specificity. In this model, overall protein-RNA interaction affinity is maintained as each hnRNP K domain contextually binds specifically or non-specifically depending on its partner RNA sequence and/or structure, thus allowing for robust binding to a subset of cellular RNAs with a rheostat of specificity.

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566 RNA Aptamers and HIV-1 Reverse Transcriptase: The Molecular Basis of Their Interface and Its Impact on the Reverse Transcriptase-Protease Interaction.

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Nucleic-acid aptamers that bind HIV-1 reverse transcriptase (RT) inhibit RT enzymatic activity in biochemical assays and they block HIV replication in cell culture. Because of RT sequence diversity across HIV clades and the complexity of the interaction network between RT and other viral proteins, the RT-aptamer complex is a suitable research model for exploring the molecular basis of protein-RNA and protein-protein interactions.

Several RNA aptamer structural families have been described, including family 1 pseudoknots (F1Pk) and UCAA bulge motif (UCAA). F1Pk aptamers are highly specific for RT encoding arginine at position 277, while UCAA aptamers inhibit RTs from diverse lentiviruses. It is likely that aptamers from each structural family make distinct contacts, although the molecular details are unknown. Therefore, we have defined some of the RNA-protein molecular interactions for F1Pk and UCAA aptamers. We found that the signature UCAA nucleotides appear to shape the aptamer's 3D structure and are crucial for aptamer-mediated inhibition. Separately, protected surfaces on RT caused by binding of UCAA and F1Pk aptamers were evaluated. Although protection was observed at the RNaseH domain of the p66 subunit for both aptamers, each aptamer showed a distinct pattern of protection/exposure upon binding by HDX/MS. With the RT-UCAA complex, we observed strong protection from positions 418 to 425 of the p51 subunit. The role of this region in RT-aptamer interactions was further investigated via alanine substitutions. During the viral life cycle, RT's p66 homodimer is cleaved by the viral protease (PR) to yield the p66/p51 heterodimer. Since the highly protected region was near PR cleavage site, we hypothesized that binding of UCAA aptamer to RT may alter RT-PR interaction. Surprisingly, the presence of UCAA aptamer enhanced protease cleavage and this enhancement was specific. The p66/p66 precursor develops different conformations along its maturation pathway. Our results suggested that interaction with UCAA aptamer might drive p66/p66 toward the productive conformation, allowing proteolytic processing to p66/p51.

Overall, our complementary structural information obtained from both the RNA and protein perspectives provides critical guidance for understanding RT-aptamer interface and studying the influence of nucleic acid binding on protein-protein interactions during biological processes.

567 Structural characterisation of RNase M5: insights into 5S rRNA binding

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In *E. coli*, the immediate precursor (pre) of 5S ribosomal RNA (rRNA) is cleaved by RNase E to yield the mature 5S rRNA. In many low G+C gram-positive bacteria, this cleavage is instead performed by RNase M5. The activity of RNase M5 is dependent on the small, ribosomal protein L18 that binds to the pre-5S rRNA prior to cleavage by RNase M5. The role of L18 in this reaction is yet unknown, but it is thought to induce conformational changes in the RNA to allow cleavage by RNase M5. Structures are available for L18 alone or in complex with 5S rRNA, but no structural data are available for RNase M5 and little is known about its interaction with 5S rRNA. Here, we present a 1.3 Å and 1.5 Å resolution X-ray crystal structures of the N- and C-terminal domains of RNase M5, respectively, and model a full-length structure guided by low resolution Small-Angle X-ray Scattering (SAXS) data. Interestingly, the C-terminal domain resembles a known protein-protein interaction domain, but in RNase M5 this domain functions as a protein-RNA interaction domain. Guided by the crystal structures and NMR data, we map the interaction with the 5S rRNA, which involves both of the protein domains. We further investigate the role of L18 in this binding and find an increased affinity of RNase M5 full-length and fragments towards 5S rRNA in the presence of L18. We are currently running crystallisation trials for the full complex of RNase M5:L18:5S rRNA, to gain further insights into the role of L18 and the mechanism utilised by RNase M5 in the cleavage of 5S rRNA.

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568 *circSamD4* promotes myogenesis

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Circular RNAs (circRNAs) lack free 5' and 3' ends and are thus believed to be quite stable. Given that they can regulate microRNA activity and the fate of interacting mRNAs and proteins, they have been proposed to be key players in diseases including cancer and neurological disorders. However, the impact of circRNAs on muscle differentiation is poorly understood. In this study, we compared the expression patterns of circRNAs during myogenesis using as a model mouse C2C12 myoblasts differentiating into myotubes. We identified 31 differentially expressed circRNAs with distinct subcellular localization (nuclear or cytoplasmic) in myotubes relative to undifferentiated C2C12 myoblasts and hypothesized that they may influence myogenesis. Validation by sequencing and by RT-qPCR analysis indicated that *circSamD4* and *circStern3* were preferentially expressed in the cytoplasm of differentiated C2C12 cultures. Silencing and overexpression interventions revealed that *circSamD4* enhanced the differentiation of human and mouse myoblasts. Among possible mechanisms under investigation, we tested if the association of *circSamD4* with RNA-binding proteins HuR, FUS or FMRP influenced *circSamD4* function. The interaction of *circSamD4* with FUS was particularly interesting, as a decline in FUS levels enhanced myogenic progression, and *circSamD4* reduced FUS levels in differentiating muscle cells. Together, our studies uncover *circSamD4* as a novel regulator of myogenesis, likely through its influence on FUS.

569 A nonsense-mediated mRNA decay factor interacts with CED complex and influences aggresome formation

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In mammals, premature termination codon (PTC)-containing mRNAs are subject to nonsense-mediated mRNA decay (NMD) for blocking expression of potentially harmful truncated polypeptides. Because NMD is translation-dependent mechanism, truncated polypeptides are generated inevitably which can be hazardous to cell. For seeking its degradation pathway, first we constructed PTC-containing FLAG-tagged GPx1 reporter (GPx1-Ter) and observed co-localization of GPx1-Ter aggresome and CFTR-ΔF508, the well-known aggresome target. Previously, we reported that misfolded polypeptides are selectively recognized and transported to aggresome via CTIF-eEF1A1-DCTN1 (CED) complex. We observed that each CED component co-localize with GPx1-Ter aggresome. Since mammals have developed highly sophisticated mechanisms for degradation of aberrant mRNA, we hypothesized that truncated polypeptides generated from aberrant mRNA will be degraded in connection with NMD mechanism. We found the co-localization of a NMD factor with CFTR-ΔF508 aggresome. We also confirmed that downregulation of a NMD factor inhibits aggresome formation and CED complex integrity. Altogether, we found that mRNA and protein quality control pathways are closely related via interaction of a NMD factor with CED complex.

*These authors contributed equally to this work.

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570 RNA recognition by the Glucocorticoid Receptor DNA-binding domain

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The Glucocorticoid Receptor (GR) is a well-studied sequence-specific DNA-binding transcription factor that produces ligand-dependent changes in gene expression. Recent reports surprisingly indicate that GR also participates in non-canonical RNA binding via its DNA-binding domain (DBD)[1]. Specifically, direct interaction with the long noncoding RNA Gas5 is proposed to be achieved by a short hairpin that mimics the DNA consensus and is critical for proper control of apoptosis during growth arrest[2]. Still, we lack a comprehensive understanding of RNA preference by the GR, as well as molecular-level details of the RNA-DBD interface.

To address this, we have characterized RNA binding by the GR-DBD *in vitro* using a combination of biochemical and structural approaches. Notably, we observe high affinity interaction with a wide breadth of RNA sequences. Refinement of the consensus binding motif suggests that the primary determinant of high affinity interaction is a hairpin loop, but that GR-DBD binds hairpins containing a spectrum of loop sequences. Analysis of DNA- and RNA-bound NMR spectra and protein mutagenesis studies indicate that the set of contacting residues only partially overlaps between DNA and RNA, suggesting separate modes of recognition. Our data provide new perspective towards the mechanism of dual DNA/RNA binding by a transcription factor domain and further the emerging role of RNA in direct transcriptional control.

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571 The RNA-binding protein ELAVL1 determines the efficacy of a nucleic acid pattern recognition induced innate immune response

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RNA-binding proteins (RBPs) regulate mRNA maturation affecting every stage of RNA, from co-transcriptional processing to their eventual decay. During highly dynamic and punctuated transcription events, like the innate immune activation of cells, RBPs can alter the levels of transcripts influencing gene expression by regulating the intensity and duration of the activated state of the cell. Embryonic lethal abnormal vision like protein 1 (ELAVL1) is an RBP reported to bind AU-rich elements on the 3' UTR of mRNA and can stabilize immune-relevant transcripts important in the initiation and the extent of an innate immune response. Conversely, knockdown of *ELAVL1* in macrophages led to an increase in expression of many immune transcripts promoting excess inflammation. The opposing observations of its role as a pro-inflammatory RNA stabilizing factor versus its potential anti-inflammatory role can be explained by distinguishing between direct RNA binding targets and indirect effects. These observations indicate that the presence of ELAVL1 is required for a proper innate immune response, although the exact mechanism of its action is not clear; there remains a clear need to better discern the RNA targets of ELAVL1 at baseline versus during an immune response. Neither the targets nor the regulatory impact of ELAVL1 is well understood in human immune cells. Innate immune activation of cells can be triggered through different pattern recognition receptor signaling pathways, each of which can lead to distinct transcriptional signatures. In this report, we discuss our progress of identifying and characterizing the direct RNA targets of ELAVL1 when the primary cytoplasmic nucleic acid sensing immune pathway in cells is stimulated. This high-throughput, nucleotide level data along with validatory biochemical assays will inform the specific mechanisms of ELAVL1-RNA targeting and function specifically during an INTERFERON REGULATORY FACTOR 3-dependent immune response.

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572 A conserved RNA-binding protein disorder-to-order transition required for embryonic cytokinesis

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RNA-binding proteins control the translation, stability, or localization of maternal mRNAs required for patterning decisions prior to zygotic gene activation. The *Caenorhabditis elegans* tandem CCCH zinc finger protein MEX-5 leads a cascade of RBP localization events that follow fertilization, ensuring correct anterior / posterior axis formation as well as germline / soma segregation. Using NMR, we discovered that the N-terminal zinc finger of MEX-5 is unfolded in the absence of RNA. To determine if this disorder-to-order transition contributes to MEX-5 function, we used molecular dynamics and mutagenesis to design a variant MEX-5 where both fingers are pre-folded. We show that the RNA-binding affinity and specificity is unchanged in this variant. To test the relevance of the unfolded state *in vivo*, we used CRISPR-hr to introduce this variant into the endogenous *mex-5* locus. Homozygotes are sterile, form massive uterine tumors within a few days of reaching adulthood, and often die by bursting. Heterozygotes are fertile but form tumors at advanced age. Tumors are derived from embryonic cells wherein nuclei divide, but not the cytoplasm, leading to giant polynucleated embryoid bodies in the uterus. Together, our results show that the unfolded state of MEX-5 is critical to its function *in vivo* by a mechanism distinct from its RNA-binding activity.

573 In vitro characterization of PKR activation by snoRNAs

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Protein Kinase RNA-activated (PKR) is an interferon-inducible kinase that is potently activated by long double stranded RNA (dsRNA), such as viral RNA replication intermediates, as part of the innate immune response. We recently identified small nucleolar RNAs (snoRNAs) as a class of noncoding RNA that associates with PKR under conditions of metabolic stress. In vitro experiments revealed that snoRNAs are capable of directly binding and activating PKR. Our results confirmed previous findings that indicated the extent of PKR activation by snoRNAs is likely reflective of RNA structure. While PKR activation by snoRNAs shows substrate inhibition, displaying the characteristic bell-shaped curve, the details of the mechanism of activation by snoRNAs remains unknown. Using SNORD113 as a representative snoRNA, we sought to understand the mechanism by which these small RNAs activate PKR. In vitro transcribed SNORD113 containing a 5'triphosphate is a robust activator of PKR, while synthetic SNORD113 containing a 5'hydroxyl does not activate PKR. Interestingly, in vitro transcribed SNORD113 treated with CIP, Antarctic phosphatase, or RNA 5′ Pyrophosphohydrolase, to remove the 5'triphosphate retains the ability to activate PKR. A bell-shaped curve of activation is commonly interpreted as evidence for formation of a PKR dimer. Substrate inhibition at high RNA concentrations is thought to occur by diluting individual PKR monomers onto distinct RNA molecules. To further interrogate the dependence of PKR activation on protein dimerization, we generated and purified two PKR mutants that disrupt a critical salt bridge in the kinase domain, PKR R262D and PKR D266R. Neither PKR R262D nor PKR D266R shows appreciable activation by in vitro transcribed or synthetic SNORD113, indicating that dimerization is required for PKR activation by snoRNAs containing a 5'triphosphoate or a 5'hydroxyl. Native PAGE analysis reveals that SNORD113 adopts multiple conformations, which show different propensities to activate PKR. We are currently pursuing studies to understand the structural properties of the SNORD113 conformers and how they impact PKR activation.

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574 Regulation of 2'-5'-oligoadenylate synthetase 1 (OAS1) by small double-stranded RNAs

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The innate immune system comprises a collection of critical intracellular and extracellular processes that limit viral infectivity. In order to provide its essential first line of defense against pathogens, the innate immune system must be able to accurately distinguish "self" from foreign molecules. Misregulation of the innate immune system can cause increased persistence and susceptibility to viral infection and human diseases, such as interferonopathies. The 2'-5'-oligoadenylate synthetase (OAS) family of enzymes are important innate immune sensors of cytosolic double-stranded RNA (dsRNA). Attesting to the importance of the OAS/ RNase L pathway, viruses have developed ways to evade OAS. Previous structural studies have revealed that dsRNA binding allosterically induces structural changes in OAS1 that reorganize the catalytic site to drive synthesis of 2'-5'-oligoadenylates (2'-5'-OA) from ATP. This 2'-5'-OA second messenger activates a single known target, the latent ribonuclease (RNase L). Active RNase L in turn degrades viral and cellular RNA to halt viral replication. Although X-ray crystal structures have given some insight into how OAS1 is activated by dsRNA, we still do not completely understand how specific features of the dsRNA contribute to the level of OAS1 activation. To address which specific features of dsRNA are required for potent OAS1 activation, we designed dsRNAs to test the impact of changes within a strongly activating RNA. Remarkably, while a single point mutation on one strand resulted in *complete loss* of OAS1 activity, the equivalent mutation on the opposite strand led to *increased* OAS1 activity. Despite these stark differences in ability to activate OAS1, both variants appear to bind OAS1 with similar affinity. Given these preliminary findings, I hypothesize that dsRNAs may contain competing OAS1 binding sites with remarkably different capacities to activate the protein in a context-dependent manner. However, the molecular signatures defining these sites as activating and non-activating are unknown. Clearly defining these features will enhance our understanding of host-pathogen interactions, such as how viruses might circumvent the OAS1/ RNase L pathway by masking activating RNA motifs to evade detection.

575 Global Identification of Non-Coding RNA Targets of 3' Processing Enzymes

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The 3' ends of non-coding RNA (ncRNAs) are dynamic battlegrounds of post-transcriptional regulation. Certain classes of exonucleases and polymerases are capable of shaping that 3' end in order to affect biogenesis, decay, and ultimately the proper functioning of ncRNAs in the cell. Identification of these trimming and extension events and the enzymes responsible for them is complex. Recent studies have focused mainly on known 3' events and searched for candidate enzymes responsible for them in a low-throughput manner. In this work, we use a next generation sequencing approach to assess the composition of the 3' ends of ncRNAs in the cell after knockdown of a 3' processing enzyme. ncRNAs that are altered in length after knockdown are identified as candidate targets for processing by that enzyme. This approach was applied to TOE1, a deadenylase we recently discovered to be responsible for trimming snRNAs to their mature length. Our experimental pipeline confirmed snRNAs as a strong hit. Using this assay, we hope to extend this pipeline to other 3' processing enzymes to find novel 3' modification events in the cell and further illuminate their complex biogenesis and regulation.

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576 Sub-cellular localization of *Eh*Rrp44 and its involement in pre-ribosomal RNA processing

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Abstract:

Exosome is a multiprotein complex which functions as a versatile RNA processing machine. It is found in the nucleus, cytoplasm and nucleolus and play some major functions which involve processing of precursors of stable RNA species, maturation and degradation of ribosomal RNA cryptic unstable transcripts and abberant RNAs. Exosomes are present in eukaryotes, archae as well as bacteria. Eukaryotic exosome core (Exo9) contains nine distinct proteins that are essential for growth. Ribonuclease activities of the eukaryotic exosome are attributable to two subunits that interact with Exo9, Rrp44 and Rrp6. Rrp44 is a member of the RNase II family of enzymes, it interacts with Exo9 in the cytoplasm and nucleus and has endonucleolytic activity associated with its PIN Domain. We have focussed on biochemical characterization of Entamoeba Rrp44 and Rrp6 by and mutating the conserved residues. Further we showed that the localization of EhRrp44 and Rrp6 changed form the nucleus to the cytoplasm upon serum starvation. Also, it has been shown in our lab that upon serum starvation the processing of pre- rRNA is inhibited with the accumulation of 5'ETS. Keeping this in mind we are characterizing the exoxome accessory protein Rrp44 and and Rrp6 to study their effect in the processing pathway in E. histolytica.

577 Antagonistic and cooperative AGO2-PUM interactions in regulating mRNAs.

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Approximately 1500 RNA-binding proteins (RBPs) profoundly impact mammalian cellular function by controlling distinct sets of transcripts, often using sequence-specific binding to 3′ untranslated regions (UTRs) to regulate mRNA stability and translation. Aside from their individual effects, higher-order combinatorial interactions between RBPs on specific mRNAs have been proposed to underpin the regulatory network. To assess the extent of such co-regulatory control, we took a global experimental approach followed by targeted validation to examine interactions between two well-characterized and highly conserved RBPs, Argonaute2 (AGO2) and Pumilio (PUM1 and PUM2). Transcriptome-wide changes in AGO2-mRNA binding upon PUM knockdown were quantified by CLIP-seq, and the presence of PUM binding on the same 3′ UTR corresponded with cooperative and antagonistic effects on AGO2 occupancy. In addition, PUM binding sites that overlap with AGO2 showed differential, weakened binding profiles upon abrogation of AGO2 association, indicative of cooperative interactions. In luciferase reporter validation of candidate 3′ UTR sites where AGO2 and PUM colocalized, three sites were identified to host antagonistic interactions, where PUM counteracts miRNA-guided repression. Interestingly, the binding sites for the two proteins are too far for potential antagonism due to steric hindrance, suggesting an alternate mechanism. Our data experimentally confirms the combinatorial regulatory model and indicates that the mostly repressive PUM proteins can change their behavior in a context-dependent manner. Overall, the approach underscores the importance of further elucidation of complex interactions between RBPs and their transcriptome-wide extent.

578 Withdrawn

579 GIGYF1-driven cooperation between ZNF598 and TTP in posttranscriptional regulation of inflammatory signaling

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In resting cells, inflammatory signaling is restricted through degradation and translational repression of cytokine mRNAs. A key factor in this regulation is tristetraprolin (TTP), an RNA-binding protein (RBP) that recruits RNA-destabilizing factors as well as the translation inhibitory complex 4EHP-GIGYF1/2 to AU-Rich Element (ARE)-containing mRNAs. Here, we show that the RBP ZNF598 contributes to the same regulatory module in a TTP-equivalent manner. Similar to TTP, ZNF598 harbours three proline rich motifs that bind the GYF domain of GIGYF1. RNA sequencing experiments showed that ZNF598 is required for the regulation of known TTP targets including IL-8 and CSF2 mRNA, as well as transcripts of non-inflammatory genes, such as KLF2. Furthermore, we demonstrate that ZNF598 binds to IL-8 mRNA, but not TNF mRNA, both *in vivo* and *in vitro*. Collectively, our findings highlight that ZNF598, in addition to its established role in ribosome quality control, functions as an RBP that exerts posttranscriptional control over a range of mRNAs. We propose that ZNF598 is a novel TTP-like factor that contributes to the regulation of the inflammatory potential of cytokine producing cells. Lastly, preliminary data indicates that the 4EHP-GIGYF complex is phosphorylated upon cellular stress, which not only alters the subcellular localization of the complex, but also impacts on which RBPs (e.g. TTP and ZNF598) are integrated into the complex.

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580 HnRNP profiling reveals long introns' pivotal impact on mRNA production resources in human cells

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Nascent pre-mRNAs co-transcriptionally recruit multiple factors, forming complexes (hnRNPs) in which mRNAs are processed. However, hnRNPs in cells remain poorly characterized. Here, we determined the composition and stoichiometry of hnRNPs in human cells, comprising >310 RNA-binding proteins, snRNPs, pre-mRNA processing- and transcription-factors. Profiling binding locations and amounts of key hnRNP proteins, snRNPs and splicing factors for >9,000 genes revealed unprecedented definition of gene-specific hnRNPs. Surprisingly, pre-mRNAs of the largest 20% of genes, which possess the longest introns, bound >50% of these resources, unexplained by their average mRNA productivity. This disproportionate sequestration was strongly reduced as normally-suppressed polyadenylation signals in long introns were activated, which causes their premature transcription termination. We suggest that massive hnRNPs that assemble on thousands of genes with long introns can serve as depots of mRNA production resources that can be rapidly deployed, without *de novo* synthesis, to acutely boost expression of other genes by transcription length regulation.

581 Human La binds mRNAs through contacts to the poly(A) tail

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In addition to a role in the processing of nascent RNA polymerase III transcripts, La proteins are also associated with promoting cap-independent translation from the internal ribosome entry sites of numerous cellular and viral coding RNAs. La binding to RNA polymerase III transcripts via their common UUU-3'OH motif is well characterized, but the mechanism of La binding to coding RNAs is poorly understood. Using electromobility shift assays and cross-linking immunoprecipitation, we show that in addition to a sequence specific UUU-3'OH binding mode, human La exhibits a sequence specific and length dependent poly(A) binding mode. We demonstrate that this poly(A) binding mode uses the canonical nucleic acid interaction winged helix face of the eponymous La motif, previously shown to be vacant during uridylate binding. We also show that cytoplasmic, but not nuclear La, engages poly(A) RNA in human cells, that La entry into polysomes utilizes the poly(A) binding mode, and that La promotion of translation from the cyclin D1 internal ribosome entry site occurs in competition with cytoplasmic poly(A) binding protein (PABP). Our data are consistent with human La functioning in translation through contacts to the poly(A) tail.

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582 Rbfox1 accomplishes extraordinary RNA specificityby stringent discrimination against non-cognate sequences

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Many RNA binding proteins (RBPs) have to distinguish between cognate and non-cognate binding sites in order to perform their biological functions. Although the molecular basis for RBP specificity is a fundamental question in RNA biology, we are only beginning to understand molecular mechanisms by which RBPs discern cognate binding sites from the often vast excess of non-cognate sites, that are frequently very similar to cognate sites. Here, we systematically examine substrate binding by Rbfox, an RBP involved in splicing regulation and other processes of RNA metabolism. Rbfox binds to a cognate sequence with 5 nucleotides. We measure equilibrium binding of Rbfox *in vitro* to all possible sequence variants of its binding site and one flanking nucleotide on each side, using a high-throughput sequencing approach (HITS-EQ) that allows us to simultaneously measure relative equilibrium binding constants of Rbfox to all 16,384 sequence variants. We find that Rbfox binds its cognate motif with affinities of more than three orders of magnitude higher than other sequence variants, including those with only a single changed nucleotide. We further observe that mutations in Rbfox alters the spectrum of affinities to its binding sites. While affinities to the cognate site change little, affinities to virtually all other sites increase significantly over those seen for wild-type Rbfox. The data reveal that the extraordinary binding specificity of Rbfox is not accomplished by optimizing affinity to its cognate sequence motif, but by stringent discrimination against non-cognate sequences. Our findings show that discrimination against non-cognate sites is an effective evolutionary strategy for RBPs to accomplish extraordinary high specificity.

583 How the Modular RelA Architecture Controls Bacterial Stringent Response

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The stringent response is a key bacterial stress response underlying pathogenicity and antibiotic resistance. RelA/SpoT-homolog proteins synthesize (p)ppGpp that mediates stringent response, enabling bacteria to adapt to environmental stress. RelA is activated on the ribosome by deacyl-tRNA, which accumulates during amino acid starvation. The mechanism of this regulation remains poorly understood. Cryo-EM structure of RelA have revealed that C-terminal of RelA is responsible for ribosome binding. To understand the specific roles of each regulatory domain, we have tested the activity of four truncation mutants of RelA: the catalytic NTD (N-terminal domain), NTD+TGS (ThrRS, GTPase and SpoT) domains, NTD+TGS+AH (alpha-helical) domains and NTD+TGS+AH+RIS (ribosome intersubunit domain). The N-terminal domain (NTD) is catalytically active, whereas the C-terminal domains are implicated in regulating RelA activity. We find that each of the C-terminal domain contributes a specific function in RelA autoinhibition or activation, explaining the conservation of domain architecture.

584 Therapeutic Effect of RECTAS for Familial Dysautonomia by Activating SRSF6 on IKBKAP Intronic Splicing Enhancer

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Familial dysautonomia (FD) is a neurodegenerative disease, in which aberrant splicing of IKBKAP exon 20 leads to reduced IKAP protein levels in neuronal tissues. IVS20+6T>C, responsible for >99% of FD cases, results in skipping of IKBKAP exon 20 due to a compromised donor site recognition by U1snRNP. Through chemical library screening, we have identified RECTAS, a small molecule compound that selectively restores IKBKAP exon 20 inclusion, with as high as 30-fold activity compared to previously reported compound, kinetin (Yoshida M et al. PNAS 2015). In the current work, we provide mode of action (MOA) of RECTAS in IKBKAP exon 20 inclusion.

By taking advantage of IKBKAP exon20 splicing reporter system with GFP/RFP dual fluorescence reporter (SPREADD system), we identified intronic splicing cis-element within intron 20, through which IKBKAP exon 20 splicing is balanced by two SR proteins, SRSF6 and SRSF8. The two SR proteins play distinct roles with SRSF6 promoting exon 20 inclusion while SRSF8 suppressing it. Subsequent analyses revealed that RECTAS, but not kinetin, selectively induces activation of SRSF6 over other SR proteins, demonstrating RECTAS restores IKBKAP exon 20 splicing by manipulating SR protein activities. Functional analysis with specific inhibitors further suggested cdc-like kinase (CLK), responsible for SR protein phosphorylation, is primarily involved in this process. Furthermore, above effects of RECTAS were also evident in FD patient fibroblasts, as well as induced pluripotent stem (iPS) cells. Finally, restoration of IKBKAP exon 20 inclusion was confirmed in dorsal root ganglia from IKBKAP humanized mice harboring IVS20+6T>C mutation following per os administration of RECTAS.

In conclusion, our current study demonstrates RECTAS as a promising therapeutic compound for FD, by activating SRSF6 to suppress IKBKAP splicing defect caused by IVS20+6T>C mutation.

585 Identification of subgenomic flaviviral RNA interactions with host proteins during infection

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Mosquito-borne flaviviruses are a global health threat infecting approximately 400 million people annually. These viruses include Dengue, West Nile virus, and Zika. During infection, these viruses produce non-coding fragments of their genome known as subgenomic flavivirus RNAs (sfRNAs). sfRNAs have been shown to be responsible for cytopathic effects in cell culture models of infection and for pathogenic effects in mouse models. sfRNA mutants are also attenuated and make promising vaccine candidates. sfRNAs have a high degree of RNA secondary and tertiary structure, with many evolutionarily conserved pseudoknot interactions. However, the molecular mechanism by which these RNA structures relate to sfRNA pathogenic effects is unclear. There are several proposed pathways that sfRNAs act upon, including RNA decay, interferon response, and RNAi pathways, all of which rely on direct binding of host proteins. Therefore, future biochemical and RNA structural studies of sfRNA elements require an understanding of sfRNA's protein binding partners during infection. We are adapting a method of RNA antisense purification-mass spectrometry to identify direct binding partners of sfRNAs during Zika virus infection. Initial results show that this approach has promise for purifying cross-linked sfRNA-protein complexes from infected cells.

586 Diverse Transcriptomic Response After Ischemic Stroke in Young and Aged Mice

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Brain ischemic injury is a leading cause of mortality in the aging population and a major healthcare and economic burden to the society in the developed countries. Stroke is a sexually dimorphic disease with age being the most important non-modifiable risk factor, yet a lot of research continues to examine only young male animal models. Moreover, despite the fact that molecular response to stroke is complex, many studies have focused on pre-selected pathways and/or on individual cell types only. To address these issues, we performed a comprehensive transcriptional analysis of brain ischemic injury in both young (3 months old) and aged (18 months old) female mice. We assessed differential gene expression across injury status and age, performed detailed pathway analysis and unsupervised co-expression analysis, identifying modules of genes associated with the various response to injury. We complemented these results with estimation of cell-type proportion changes using computational deconvolution techniques and assayed our results with findings from previous studies of similar design. We found disease signatures consistent with literature and extended these results with new findings. We show strikingly variable response of specific cellular pathways between young and aged ischemic animals, particularly related to immune response and neurodegeneration, likely owing to the altered cell-cell communication patterns. Together, these results paint a picture of ischemic stroke as a complex age-related disease and provide insights into interaction of age and stroke on cellular and molecular level.

587 Dissecting the role of RNA-Binding Proteins in Plant Immunity

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RNA-Binding Proteins (RBPs) are master regulators of gene expression and are involved in virtually all steps of RNA life from synthesis to decay. Moreover, RBPs can modulate the cellular transcriptome in response to changes in the physiological conditions or in response to environmental cues. The role of RBPs on the regulation of cellular processes has been extensively studied for metazoans, bacteria and viruses; however, very little is known about plant RBPs. Importantly, recent studies have pinpointed the crucial role of RBPs in plant immunity, although the identification of the RBPs involved in this process has been collected stepwise and comprehensive approaches are missing. Here, we have successfully applied 'RNA interactome capture' (RNA-IC) to mature *Arabidopsis thaliana* plants. In brief, RNA-IC employs UV light to promote crosslinking between RNAs and proteins that are in intimate contact. Next, the RNA–protein complexes are isolated under stringent conditions and the proteins quantitatively analysed by mass spectrometry. We identify more than five hundred RBPs in Arabidopsis leaves, many of which were previously unknown. We have further exploited this technique to determine the dynamic responses of the RNA-Binding Proteome (RBPome) to flg22 peptide, revealing dozens of RBPs with potential roles in plant immunity. In complementary approach, we performed a screen of Arabidopsis lines with mutations in candidate RBP genes and identified RBPs playing important roles in plant immunity.

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588 Cell Models for Molecular Mechanism and Discovery of Small Molecule Inhibitors of Repeat-Associated Non-AUG Translation

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Over two dozen neurological repeat expansion disorders are known, many of which are caused by expression and translation of repeat expansion RNA. To better understand molecular mechanisms and screen for molecules with therapeutic potential, we are engineering relevant cell based models. We have focused on a genetic form of frontotemporal dementia and amyotrophic lateral sclerosis called C9FTD/ALS. In this disorder, large GGGGCC repeat expansions in the first intron of the *C9ORF72* gene are transcribed into expanded tandem repeat-containing RNAs (xtrRNAs). These xtrRNAs are somehow translated through a non-canonical mechanism known as repeat-associated non-AUG (RAN) translation. Our cell based models aim to express GGGGCC repeats of varying sizes fused to mCherry to monitor expression. Repeats are driven by CMV or tetracycline-inducible promoters via canonical AUG or non-canonical CUG start codons. Target cell lines include human neural stem cells and common cancer cells. We are collaborating with the Stanford High Throughput Bioscience Center to screen chemical libraries and identify RAN translation inhibitors in these model cell lines. This study will generate model cells for repeat expansion disease research and may identify potential lead compounds for therapeutic development or RAN translation research.

589 The Impact of Antisense RNAs on Hepatocellular Cancer Development

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Some long non-coding antisense (AS) RNAs have been found to modulate the expression of protein-coding genes both in a transcriptional and in a post-transcriptional manner. As of today, the detailed expression profile of the AS RNAs and their functions in hepatocellular cancer (HCC) remains largely unclear. In order to shed light on this question, we conducted a strand-specific high-throughput RNA sequencing (RNA-Seq) for 6 matched pairs of tumor and non-tumor liver tissues. After data analysis, three AS RNAs (*HNF4A-AS*, *TMEM220-AS* -found downregulated in the HCC tissue- and *DLG5-AS* -upregulated in the HCC tissue-) were selected for further study considering the magnitude of its expression change from non-tumor to tumor and the relevance of the overlapping sense gene. TCGA expression data supported the gene expression trends identified in our set of samples, while survival data suggested a potential role of *TMEM220-AS* as tumor suppressor and of *DLG5-AS* as oncogene. The Rapid Amplification of cDNA Ends (RACE) revealed the full-length sequences of these three AS RNA, which were used to design silencing (shRNA) and overexpression constructs. The AS RNA-silencing assays carried out in the HCC cell line Huh7 showed an effective silencing of *TMEM220-AS* and *DLG5-AS* and a positively correlated downregulation of their counterpart sense associated genes *TMEM220* and *DLG5* respectively. On the other hand, the partial silencing of *HNF4A-AS* diminished the expression only of the subset of *HNF4A* isoforms originated from the *HNF4A* promoter 1 (P1) but not altered those derived from the *HNF4A* promoter 2 (P2). HNF4A is a well-known transcription factor and has a key role in liver development. *HNF4A* P1 and P2 isoforms are believed to have opposed differentiation roles and the P2/P1 isoform proportion is found increased in HCC. We measured the HNFA4-AS and HNF4A (P1/P2) expression levels during the three first weeks of liver development using an embryonic stem cell differentiation model. *HNF4A* P2 isoforms diminished through the liver differentiation progress while P1 isoforms and *HNF4A-AS* augmented. Overall, the obtained data suggests the participation of *HNF4A-AS* in the balance control of *HNF4A* P1/P2 isoforms during liver differentiation and HCC oncogenesis.

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590 Alternative mRNA polyadenylation modulates influenza A virus induced innate immune response

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Many viruses modulate mRNA and protein expression in infected cells, most extreme cases being transcriptional and translational host shut-off. We used a combination of -omics approaches to study the relationship between mRNA and protein expression levels in virally infected cells, focusing on various strains of influenza A virus (IAV). Interestingly, we detected a large number of post-transcriptional changes occurring in Influenza A infected cells.

Here we show that alternative polyadenylation (APA), characterized by the differential usage of polyadenylation sites, is an accompanying mechanism of previously described IAV host-shutoff mechanisms. Using a variety of Influenza A strains in vitro, we characterized the effect of viral proteins on APA of the host's mRNA pool. We functionally mapped the effect to a single amino acid residue of a viral protein. Conversely, using affinity purification followed by mass spectrometry and a comprehensive knock-down screen, we identified a host interactor that is required and sufficient to mediate APA.

Functionally, our data indicate that APA plays an important pro-viral role during IAV infection. Our study thus provides evidence that APA occurs in influenza A infected cells and serves as a significant determinant of viral pathogenicity.

591 Mouse Microsatellite Expansion Knockin Models for RNA-Mediated Disease

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The expansion of simple sequence repeats, or microsatellites, causes numerous neurological diseases. Several pathomechanisms have been proposed for these expansion disorders including haploisufficiency, RNA-mediated RBP sequestration and repeat-associated non-AUG (RAN) translation leading to the expression of toxic repeat peptides. To clarify the pathogenic roles of these various mechanisms, prior studies have attempted to model these diseases in mice using several strategies, including expression of uninterrupted and interrupted repeats in various transgene contexts, generation of human mutant allele BAC transgenics, AAV-based somatic transgenesis and the development of mutant gene knockin mice using conventional homologous recombination. However, these transgenics express relatively short repeats or require transgene amplification in bacteria, which leads to repeat contractions, or fail to recapitulate the spatiotemporal and expression level patterns of the affected allele. Here, we report the development of a combination of rolling circle amplification (RCA) and CRISPR/Cas9 genome editing to generate an allelic series of *Dmpk* CTG expansion (CTG^{exp}) mouse knockin models for the neuromuscular disease myotonic dystrophy type 1 (DM1). Importantly, wild type and expansion Dmpk RNAs are detectable at equivalent levels in multiple tissues while DMPK protein shows repeat length dependent decreases. Additional disease-associated features of these novel expansion knockin lines will be discussed.

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592 Circular RNAs in Aging and Alzheimer's Disease

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Circular RNAs (circRNAs) are naturally occurring RNA molecules that demonstrate enhanced stability and differential expression in various tissues and developmental time points, as well as and during aging. CircRNAs are formed predominantly through back-splicing and are highly expressed in the brain. During aging, circRNAs are upregulated in brain tissues, provoking questions about whether circRNA expression might be altered in age-related neurodegenerative diseases.

Alzheimer's disease (AD), the most common cause of dementia, is a progressive neurodegenerative disease characterized by cognitive declines and amyloid plaque and tau pathologies in the brain. Advanced age is the primary risk factor for developing AD. To determine whether circRNAs have altered expression in AD, we analyzed a publically available RNA-seq dataset from age-matched hippocampus tissue of patients with or without late-onset Alzheimer's disease (LOAD) (Magistri et al. 2015). After annotation and alignment to circRNA back-spliced junctions, we detected 4,363 circRNAs that originated from ~2,200 genes and ~120 intergenic regions. We observed that circRNAs are differentially expressed in the hippocampus between LOAD and control patients, with 130 circRNAs upregulated and just 20 downregulated in LOAD. We found that circRNA expression was largely independent from linear RNA expression, and also observed that many of the genes giving rise to circRNAs in LOAD also exhibited other LOAD-dependent splicing alterations.

These data indicate that circRNAs are upregulated in the hippocampus of LOAD patients, which may occur as a result of splicing alterations and slow turnover of circRNAs. Our future directions include circRNA analysis of tissues from a mouse model of AD to determine whether deregulation of circRNA expression occurs prior to, concurrent with, or after the formation of amyloid plaque pathology in the brain.

593 Disruption of Minor Splicing in Rare Inherited Developmental Disorders

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Biallelic mutations in the gene encoding the minor spliceosomal U4atac small nuclear RNA (snRNA), RNU4ATAC, have been identified in patients with two rare inherited developmental disorders, microcephalic osteodysplastic primordial dwarfism type I (MOPDI) and Roifman Syndrome. MOPDI is an autosomal recessive disorder characterized by growth retardation, severe neurological defects, cognitive delay, and malformation of the face, long bones, and joints. Roifman Syndrome patients share a similar but less severe phenotype that also includes immunodeficiency. Twelve separate mutations of RNU4ATAC have been identified in MOPDI patients. Roifman Syndrome patients have an additional ten distinct mutations and share several mutations with MOPDI patients. Current evidence suggests that RNU4ATAC mutations disrupt U4atac snRNA function and impair minor splicing. Mutations identified in MOPDI and Roifman Syndrome are often found in important structural domains of U4atac. Previous work has shown that MOPDI mutations in the U4atac 5' stem-loop result in impaired binding of essential protein components of the di-snRNP and reduced levels of the tri-snRNP were found in patient cells with the most common MOPDI mutation, 51G>A. We have continued to characterize the splicing activity and stability of uncharacterized MOPDI mutations and all of the Roifman Syndrome mutations. Using our orthogonal splicing assay, we demonstrate that in general MOPDI mutations exhibit a more considerable reduction in splicing when compared to the Roifman Syndrome mutations. MOPDI mutations clustered in the 5' stem-loop of U4atac have less than 30% splicing activity compared to wild-type, while Roifman Syndrome mutations clustered in stem II have nearly 50% splicing activity. Mutations in and near the Sm protein binding site are present in both diseases, and generally are very deleterious to U4atac activity, likely due to decreased abundance of the mutant snRNAs. The wide range in the severity of phenotypes in patients with RNU4ATAC mutations suggest there are likely multiple mechanisms that impair minor splicing in MOPDI and Roifman Syndrome patients.

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594 The role of riborepressor GAS5 lincRNA in glucocorticoid-mediated beta cell dysfunction in diabetes mellitus

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The long non-coding RNA Growth Arrest-specific 5 (GAS5) is a riborepressor that is involved in glucocorticoid signaling. Glucocorticoids (GCs) are highly-potent class of steroid hormones in the frontline of various clinical therapy procedures due to their anti-inflammatory and immunomodulatory properties. However, a widely recognized metabolic side-effect of GC therapy is steroid-induced diabetes mellitus (SIDM), in which rapid onset of hyperglycemia is observed in up to 80% of patients receiving high-dose GC treatment. Beta cell dysfunction, e.g. impaired insulin secretion, contributes in the development of diabetes. Here, using RNAseq we identified GAS5 as the most highly expressed lincRNA in the human pancreatic islets. We then used human islets and the human beta cell line, EndoC-βH1, to demonstrate the involvement of GAS5 in GC-mediated beta cell dysfunction. We showed the expression of major components of glucocorticoid signaling such as the glucocorticoid receptor (GR) and SGK1 to correlate with GAS5 expression. Knockdown of GAS5 using LNA gapmer resulted in downregulation of PDX1, NKX6-1 and SYT13 proteins that are important for beta cell function. Modulation of GAS5 levels using a “hormone response element mimic” in the human beta cell line alleviated the GC-induced insulin secretion defect demonstrating the potential of this non-coding RNA as a novel therapeutic target in rectifying GC-mediated beta cell dysfunction.

595 Identification of potential disease-driving molecules in mouse models of amyotrophic lateral sclerosis caused by mutant TDP-43

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Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease that leads to progressive loss of motor neuron function. It is almost always fatal and there is currently no curative treatment. The RNA-binding protein TDP-43 is frequently mislocalized in ALS patient motor neurons and is the major component of ubiquitinated aggregates that characterize ALS pathology. Moreover, a subset of ALS patients have mutations in TARDBP, the gene, encoding TDP-43. Numerous studies analyzing patient samples, as well as animal and cell-based disease models, strongly support altered RNA regulation by TDP-43 within motor neurons as a major cause of disease. Nevertheless, the specific molecular changes that actually trigger disease onset remain unclear. Here, we investigate molecular changes in motor neurons during the transition from the pre-symptomatic phase to disease onset in TDP-43-driven ALS. For this purpose, we applied Translating Ribosome Affinity Purification (TRAP) to established mouse models of ALS caused by mutant TDP-43. After a time-course with behavioral characterization, we collected spinal cords from mutant TDP-43 mice and matched controls at time points corresponding to pre-symptomatic and early symptomatic phases. Ribosome-associated mRNA from motor neurons was isolated via TRAP, followed by genome-wide high-throughput sequencing (RNA-Seq). Bioinformatic analysis of these samples revealed a number of mRNAs that are up- or down-regulated in motor neurons of ALS models specifically at disease-onset. We confirmed a subset of these via qRT-PCR in an independent TRAP experiment, supporting the validity of our genome-wide data. For some targets, we could also detect corresponding changes at the protein level by quantitative immunostaining of spinal cord samples. Importantly, we also saw these changes in motor neurons in an independent mouse model caused by a different patient mutant allele of TDP-43, suggesting that they may be generally important in ALS. In sum, we have identified new molecules that show altered regulation in motor neurons of mouse ALS models at the time when disease develops.

596 hnRNPC regulates alternative cleavage and polyadenylation profiles during colon cancer progression

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Alternative cleavage and polyadenylation (APA) greatly enhances the cellular repertoire of mRNA isoforms in eukaryotes. In humans more than half of all genes can undergo APA and deregulation of APA creates specific profiles that have been linked with many diseases including cancer progression. How cancer specific APA profiles are established and what their physiological consequences are, however, is largely unclear. Here we use a subcellular fractionation approach (1) to determine the nuclear and cytoplasmic APA profile changes that occur as colon cells transition to adenocarcinoma and then metastasised states. We identify that hnRNPC may play a critical role in the establishment of the APA profiles characteristic of the metastasised state by regulating poly(A) site selection. In particular elevated hnRNPC levels in metastatic derived colon cancer cells suppress the usage of an internal poly(A) site in *MTHFD1L* causing an increase in the full length mRNA isoform and protein production. *MTHFD1L* encodes a key component of the mitochondrial tetrahydrofolate cycle (mTHF) and overexpression of these enzymes accelerate the synthesis of one carbon units required for nucleotide and glycine synthesis, thereby abetting cell proliferation. Our results thus highlight hnRNPC as a critical regulator of APA events that can contribute to carcinogenesis by modulating expression of genes that impact cell proliferation. Finally, we find similar APA profile shifts including those for *MTHFD1L* using RNA-seq data obtained from patient derived tumour and normal colon cells (2).

(1) Neve et al. 2016. Genome Res. 26, 24–35

(2) Ongen, et al. 2014. Nature 512, 87–90.

597 Muscle-Specific Transcriptome Rearrangements During the Progression of Duchenne Muscular Dystrophy in *C. elegans*

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Duchenne Muscular Dystrophy (DMD) is a lethal, X-linked disease characterized by progressive muscle degeneration. The condition is known to be driven by mutations in the dystrophin gene that result in production of a truncated dystrophin protein. The resulting changes in muscle-specific genetic networks that take place in the absence of dystrophin remain unknown, as they are potentially obscured by the chronic inflammatory response elicited by extensive muscle damage in humans.

C. elegans have a mild inflammatory response that allows for the characterization of the transcriptome rearrangements affecting disease progression independently of inflammation. The two C. elegans strains dys-1(eg33) and dys-1(cx18) contain nonsense mutations in the dystrophin gene, which can be rescued with the wild type human ortholog, making them a suitable model system to study the effects of dystrophin deficiencies.

In effort to better understand the dynamics of transcriptome rearrangements during DMD progression, our lab has optimized an approach (PAT-seq) to isolate and sequence body muscle-specific transcriptomes from dys-1 strains at different stages of the disease. We have crossed these two dys-1 deficient strains (eg33 and cx18) with a strain expressing pab-1 fused to 3xFLAG under the control of a myosin heavy chain promoter, and characterized the extent to which these chimeric strains mimic the human version of DMD with survival curves, genomic DNA sequencing, and the muscle-specific RNA silencing of several members of the dystrophin-associated protein complex.

Taking into account the progressive nature of the condition, we divided both strains into two distinct developmental groups, termed pre-symptomatic and post-symptomatic, and performed muscle-specific RNA-IPs on each population to identify any differentially expressed genes in dystrophin dependent muscle degeneration.

We have identified novel altered gene networks implicated in disease progression, some of which are mirrored in the dystrophin deficient mdx mouse model. Among these, we found an upregulation of genes involved in muscle fiber repair in paralyzed worms, suggesting the presence of hidden compensatory mechanisms in place to counteract muscle degradation caused by loss of dystrophin.

Our results allow for the comprehensive identification of transcriptome rearrangements that could ultimately serve to identify new therapeutic targets for the treatment of DMD.

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598 A stable poly(A)-specific ribonuclease (*parn*) knockout zebrafish model

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Poly(A)-specific ribonuclease (PARN) is a regulator of RNA metabolism in eukaryotic cells by acting as a deadenylating enzyme that removes the poly(A) tails of the mRNAs and ncRNAs. It is localized in both the nucleus and cytoplasm. In the nucleus it is involved in the metabolism of ribosomal rRNAs and small ncRNAs like snoRNAs and miRNAs, whereas in the cytoplasm it targets a subset of mRNAs. Recently, we (Dhanraj et al, 2015) and others (REFs) discovered that mono/bi-allelic mutations in *PARN* gene cause telomere biology disorders (TBDs). PARN loss of function perturbs the maturation of the TERC level and in that way may influence the length of the telomeres in affected patients.

To further investigate the physiological role of PARN during the whole life span of an animal and the link between PARN deficiency and TBDs we have established a *parn* knockout zebrafish model, using a CRISPR/Cas9 genome editing strategy. We have so far generated three *parn* compound heterozygotes with the following genotypes: Fish G10 (p.Gln119fs/p.Ser118fs; p.(Ser562_Leu563del)); Fish H7 (p.Lys310fs/p.Glu311fs); and Fish G8 (p.Glu311del/p.Glu311fs). The compound heterozygous mutations in fish G10 and H7 will abolish Parn activity/expression whereas Parn activity/expression will be reduced in fish G8.

The telomere lengths of the compound heterozygous fish have been investigated and we have found that the G8 fish has short telomeres while the H7 and G10 fish have telomeres of normal length. We have subsequently crossed each of the compound heterozygotes with wild type zebrafish and generated heterozygous off-springs. The off-springs from the G8 fish retained short telomeres with variable lengths and in some cases the telomeres in the off-springs were even shorter than in the G8 fish. This telomere attrition correlates with the progressive telomere shortening that has been observed in successive generations of human families suffering from TBDs. We are currently characterizing phenotypes of the obtained *parn* deficient off-spring fish to study if *parn* deficiency in zebrafish induces phenotypes associated with TBDs in human patients.

599 The perfect storm: IGF2BP1 and MYCN form a self-sustaining oncogenic network in neuroblastoma

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Neuroblastoma is a tumour of the sympathetic nervous system and the most common cause of cancer-related death in infants. The amplification of the oncogene MYCN, observed in 25-30% of neuroblastoma, is an established prognostic marker that is used to stratify patients and countless studies have demonstrated that MYCN expression is an important factor in tumourigenesis and aggressive tumour-cell phenotype. How elevated MYCN expression is sustained at the post-transcriptional level remains largely elusive. The most frequent chromosomal alteration in neuroblastoma is the gain of chromosomal region of 17q21-ter (about 70% of all tumours). The insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) is located at 17q21.32 and upregulated expression of IGF2BP1 is observed in aggressive neuroblastoma. IGF2BP1 is an oncofetal mRNA-binding protein that is de novo synthesized in a variety of cancers. In neuroblastoma IGF2BP1 expression is an independent negative prognostic factor associated with elevated MYCN synthesis. Like its homologue MYC, MYCN promotes IGF2BP1 mRNA transcription. At the post-transcriptional level, IGF2BP1 interferes with the miRNA-directed downregulation of various factors including the prime let-7 miRNA targets LIN28B and HMGA2. Although decreased in MYCN-amplified neuroblastoma, let-7 miRNAs present one of the most abundant miRNA family in MYCN-amplified neuroblastoma. In MYCN-amplified tumour cells, IGF2BP1 impairs the downregulation of MYCN by let-7 miRNAs. In summary these findings suggest that IGF2BP1 and MYCN form a positive feed-back loop in aggressive neuroblastoma. MYCN drives IGF2BP1 transcription whereas IGF2BP1 interferes with the let-7 dependent downregulation of MYCN. Moreover, IGF2BP1 expression is positively correlated with the abundance of various MYCN target genes suggesting that the MYCN-IGF2BP1 axis act synergistically in driving oncogenic gene expression in aggressive neuroblastoma, the "perfect strom".

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600 MiR-105 is a central regulator of intermediate filaments associated with amyotrophic lateral sclerosis (ALS)

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Introduction: ALS is a neurodegenerative disease defined by progressive motor neuron death causing mortality 2-5 years after onset. Common pathological features of ALS include cytoplasmic inclusions of intermediate filaments (IFs) within motor neurons, where loss of IF stoichiometry due to changes in mRNA steady-state levels is thought to drive IF pathogenesis. In particular, *neurofilament light* (*NEFL*), *peripherin* (*PRPH*), and *alpha-internexin* (*INA*) are IFs that have drastically reduced mRNA levels within spinal motor neurons of ALS patients. While the mechanism by which IF mRNA levels are altered in ALS is largely unknown, it is critical to elucidate this pathway if we are to identify new therapeutic targets for ALS patients. Interestingly, microRNAs (miRNAs)-critical regulators of mRNA metabolism-are globally down-regulated in spinal motor neurons of ALS patients, indicating that disruption of IF mRNA steady-state levels may be caused by the dysregulation of miRNAs in ALS.

Hypothesis: Dysregulation of a specific pool of miRNAs in ALS disrupts mRNA steady-state levels of ALS-linked IFs (*NEFL*, *PRPH* and *INA*).

Methods and Results: Bioinformatic analysis identified 9 miRNAs that are predicted to regulate all three IFs of interest. Real-time PCR revealed only three of the identified miRNAs (miR-105, miR-140-5p and miR-9) were significantly down-regulated within the spinal cord of ALS patients. Fluorescent *in situ* hybridization analysis showed that these miRNAs are highly expressed within spinal motor neurons. Out of the three miRNAs identified, only miR-105 regulated the mRNA metabolism of all three IFs. In particular, miR-105 increased mRNA levels of a reporter gene when it contained the *NEFL* or *PRPH* 3'UTR, and suppressed mRNA levels when it contained the *INA* 3'UTR. Further, we show these changes in mRNA metabolism are dependent on miR-105 binding to the IF 3'UTRs.

Conclusion: We determined miR-105 to be expressed in spinal motor neurons, significantly down-regulated in ALS, and involved in regulating the mRNA metabolism of ALS-linked IFs-*NEFL*, *PRPH* and *INA*. Overall, the data indicates that miR-105 is a central regulator of IFs associated with ALS, and that the down-regulation of miR-105 in ALS plays a role in disrupting IF mRNA steady-state levels, potentially promoting IF pathogenesis.

601 Role of the p53 alternative spliced isoform in colorectal cancer cells

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p53 is a central sensor of cell signals and a master regulator of cell response to damage, so it acts as a powerful tumor suppressor in normal cells. TP53 gene encodes several different splice variants and distinct protein isoforms, but the functions of protein isoforms in tumor regulation have not been explored. One of p53 isoform is generated by the alternative inclusion of the exon containing a premature termination codon, which leads to a replacement of the entire p53 carboxy-terminal oligomerization domain with a short 10 amino acid tails. Surprisingly, the p53 isoform promotes tumorigenesis and induces molecular changes consistent with epithelial-mesenchymal transition, in contrast to tumor suppressive functions of the full-length protein. Interestingly, the p53 isoform strongly enhanced cell migration of colon cancer cells through the loss of E-cadherin and the induction of mesenchymal markers. Moreover, the p53 isoform localizes to the cytoplasm and regulates the translation of specific mRNAs involved in tumorigenesis. Mechanistic analysis of the p53 isoform-regulated tumor formation and progression will be discussed in more detail in the presentation. In summary, the p53 isoform induces tumorigenesis of colon cancer cells, providing the first evidence of a biological function for the metastatic effect of the p53 isoform.

602 Regulation of arachidonic acid metabolism by microRNAs and long non-coding RNAs in lung cancer cells

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One commonly dysregulated inflammatory pathway in cancer is the metabolism of arachidonic acid (AA) by cyclooxygenase-2 (COX-2) or 5-lipoxygenase (5-LO) and its activating protein (FLAP) into prostaglandins (PGs) and leukotrienes (LTs), respectively. Although COX-2, 5-LO, and FLAP inhibitors have shown promise for treating cancer, high toxicity and adverse side effects have limited their effective use in the clinic. Therefore, new methods of targeting these proteins are necessary. Our lab and others have shown that these molecules are regulated post-transcriptionally by microRNAs (miRNAs). miRNAs are small, non-coding RNAs that negatively regulate target gene expression through mRNA degradation or translational repression. They generally target multiple genes of similar biological function, making them attractive candidates for therapeutic use.

Here, we show that FLAP protein is overexpressed in lung cancer cells compared to normal lung cells. One miRNA in particular, miR-146a, is predicted to target FLAP via its 3' untranslated region (UTR). Previous work from our lab demonstrated that miR-146a directly regulates COX-2 expression through its 3' UTR. In this study, we have experimentally defined the specific regulation of FLAP by miR-146a. Transient and stable transfections of miR-146a in lung cancer cell lines repressed endogenous FLAP protein expression, and reporter assays indicated this regulation occurs through a direct interaction between miR-146a and the FLAP 3' UTR. This modulation of FLAP gene expression also resulted in decreased cancer cell LTB_4 production. We have also discovered that promoter CpG methylation contributes to miR-146a downregulation in lung cancer cell lines. This work suggests miR-146a can regulate both PG and LT production in lung cancer cells by directly targeting the 3' UTRs of COX-2 and FLAP, leading to dual inhibition of inflammatory eicosanoid production. Regulation of both arms of this metabolic pathway is significant in that it prevents shunting of AA, where blocking one pathway results in upregulation of the other due to more available substrate. Additionally, we have begun to investigate the role of specific long non-coding RNAs (lncRNAs) in AA metabolism. Overall, our data suggest an elegant mechanism of miRNA-mediated regulation of AA metabolism that further supports a tumor suppressive role for miR-146a in lung cells.

603 *RBFOX2 acts as a tumor suppressor in metastatic pancreatic cancer*

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RBFOX2 plays a key role in tissue-specific alternative splicing regulation. Aberrant expression of RBFOX2 in many cancers causes altered splicing of specific targets. Recent studies suggest that RBFOX2 is important in establishing an invasive phenotype through its regulation of mesenchymal-specific splicing events during EMT. In an attempt to investigate the role of RBFOX2 and its splicing targets in the development of metastatic pancreatic cancer we have established a model system using human primary pancreatic tumor samples (primary tumors) and human metastatic ascites cells (ascites) obtained from pancreatic cancer patients. The ascites was used to generate either solid tumors in mice (PDX tumors) or metastatic cell lines (PDX-derived cell lines) by xenografting. Using our model system, we found that RBFOX2 is down-regulated in the metastatic tumors compared to primary tumors. Overexpression of RBFOX2 in patient-derived metastatic cell lines induced cell death and inhibited cell migration in vitro and in vivo, while knockout of RBFOX2 promoted the tumorigenic abilities and migration of primary pancreatic cancer cells. Taken together, these findings suggest that RBFOX2 acts as a tumor suppressor in metastatic pancreatic cancer. Deep RNA sequencing of these cell lines revealed both known and novel RBFOX2 regulated alternative splicing targets. Bioinformatics analysis of these splicing targets showed enrichment of targets in the Rho and Hippo signaling pathway, pathways known to have a role in migration. Modified RNA oligonucleotides, to induce either skipping or inclusion of a specific exon, will be used to manipulate the splicing of these targets in vitro and in vivo. Understanding the biological importance of RBFOX2 and its involvement in pancreatic metastatic tumors has the potential to aid early diagnosis and shed light on novel approaches for treatment.

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604 Cyclic naphthyridine dimers as therapeutic agents for fragile X-associated tremor/ataxia syndrome.

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder caused by a limited expansion of CGG repeats (pre-mutation; 55-200) in the 5'-untranslated region of the *FMR1* gene encoding fragile X mental retardation protein (FMRP). The patients show specific manifestation of late-onset clinical symptoms that include intention tremor, gait ataxia, cognitive deficits and brain atrophy. Pre-mutated *FMR1* transcripts sequester a number of proteins such as hnRNP A2/B1, PURα and DGCR8. Nevertheless, the most recent data indicate that the degeneration of neuronal cells is primarily triggered by accumulation of toxic polyglycine (FMRpolyG), a by-product of translation initiated with near-cognate start codons upstream to the repeats. Specific aims of our work included: (1) estimating if cyclic naphthyridine dimers could be used to block production of FMRpolyG in cells transfected with vectors carrying CGG repeats and in primary neuronal cultures from an inducible mouse model for FXTAS, and (2) testing whether this potentially therapeutic intervention could be achieved without affecting generation of FMRP translated with FMRpolyG from the same *FMR1* transcript. The latter could be of particular importance as loss of FMRP caused by hypermethylation of the promoter due to longer expansion (full mutation; >200) results in fragile X syndrome (FXS), an early-onset disease characterized by alterations in physical appearance, intellectual disability and autism. We demonstrate that cyclic naphthyridine dimers efficiently block expression of FMRpolyG without affecting the overall RNA content of transcripts carrying expanded CGG repeats. Despite the parallel downregulation of the FMRP content, we show that the treatment can be balanced to achieve optimal reduction of FMRpolyG and preservation of the FMRP function.

605 *H. Sapiens* tRNase Z^L (Elac2) Mutations Associated with Hypertrophic Cardiomyopathy Reduce Mitochondrial Pre-tRNA 3'-End Processing and Translation

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tRNase Z is a widely conserved enzyme responsible for endonucleolytic 3' end processing of pre-tRNAs. The long form of tRNase Z (tRNase Z^L), encoded by ELAC2 and dually targeted to the *H. sapiens* nucleus and mitochondria, cleaves both nuclear encoded pre-tRNAs and mitochondrial (mt) tRNAs from the primary, polycistronic transcripts. Recently, we showed that Mendelian mutations within the nuclear ELAC2 gene associated with hypertrophic cardiomyopathy reduce mt tRNA 3' end processing (Haack et al., AJHG 93:211, 2013). Five patients from three independent families harbour three substitutions (in the amino domain, linker and carboxy domain) which reduce the efficiency of mt tRNA processing relative to wild-type. Two more mutant tRNase Z^Ls were analysed based on another recent report (patient 27 from Taylor et al., JAMA, 312(1), 68, 2014), and mutations from 10 additional patients (in collaborations coordinated by MM) were included in the analysis. Substitutions are amenable to enzymatic analysis, while splice junction mutations do not lend themselves to such evaluation. The two most impairing substitutions are close to basic residues which clamp the substrate acceptor stem proximal to the scissile bond; another is found toward the end of a long carboxy-terminal α-helix, in the region where substrate enters the active site. Establishing pathogenicity of novel variants remains challenging and functional studies, aided by careful interpretation of crystal structures when available, should be central to the evaluation, complementing the increased utility of genetic testing and targeted exome sequencing. Combined with detection of precursor in Northern blots and the deficiency of mitochondrial translation from patient cell lines, the impairment of mt tRNA processing suggests a molecular role of tRNase Z^L mutation in mitochondrial disease.

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606 Using transcriptomics to determine the impact of low dose radon on lung epithelial cells

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Tobacco smoke and radon are Group 1 carcinogens, and the leading causes of lung cancer. Tobacco smoke exposure preferentially results in G to T transversions, while radon emits high energy alpha-particles that randomly mutate DNA. The establishment of transformation upon tobacco smoke exposure has begun to be elucidated; several genes thought to drive this transformation have been identified in various lung cancer types. Much less is known, however, regarding the molecular mechanisms which drive radon-associated transformation. Such studies are also complicated by evidence that exposure to low dose radon can actually be beneficial to cells, potentially through inducing upregulation of DNA repair processes. The objective of this study was to first determine if low doses of radon, much lower than those currently considered safe, influence gene expression, and if so, to characterize all transcriptomic changes induced using RNA-seq. Gene expression changes induced by radon were then compared to those induced by tobacco smoke, to provide an indication of transformation risk, and cannabis smoke, a non-carcinogenic comparator. We determined that even low doses of radon did significantly impact the transcriptome of human lung epithelial cells, and that changes were observable following as little as one week of exposure. In addition, the radon-induced expression changes gradually reflected those associated with tobacco-smoke exposure, suggesting that continuous exposure to low-dose of radon may promote transformation over time. Overall, our findings provide the first evidence of the transcriptomic effects of low dose radon exposure and highlight that exposure time to a constant low concentration of radon is an important consideration for radon-risk evaluation. Furthermore, the high degree of similarity between the radon-induced gene expression changes and tobacco-induced changes suggests that the early cellular processes by which both of these carcinogens influence transformation are similar. Future studies are thus needed to further characterize the molecular effects of low dose radon exposure, and the relationship between radon and tobacco smoke exposure outcomes.

607 Splicing regulation by CLK1 during cell cycle affects cancer progression

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About 95% of human genes are alternatively spliced, and alteration of alternative splicing (AS) is closely associated to human cancers. Previously we have identified hundreds of cancer-specific AS events, many of which are enriched with functions in regulating cell cycle. In addition, we discovered that AS of more than a thousand of genes showed periodic oscillations during mitotic cell cycle, providing a novel mechanism for temporal control of gene function in cell division. Mechanistically, we found that CLK1, a SR protein kinase, controls a large fraction of periodic splicing events, presumably by phosphorylating SR proteins that are major class of splicing factors.

To further determine the splicing regulatory pathways controlled by CLK1, we have used a combination of three approaches to systematically identify splicing factor substrates of CLK1. (1) We conducted Bio-ID experiment to identify potential proteins that are interacted with CLK1, including the protein with transient interaction; (2) We also used phosphoproteome to search for proteins whose phosphorylation status is affected by CLK1 inhibition; (3) In addition, we used RNA-seq data of synchronized cells to identify RNA binding proteins that undergo periodic expression during cell cycle. The genes identified in all the three methods will most likely to be splicing factors regulated by CLK1 and functioned in controlling periodic AS in cell cycle. One of the candidate genes identified is SF3B2, a key component of U2 snRNP that recognize 3' splicing site. We have further identified endogenous AS events regulated by SF3B2 using RNA-seq in cells with SF3B2 knockdown. Consistently, the SF3B2 regulated AS events showed significant enrichment in cell cycle related functions. SF3B2 and SF3B1 form a tight complex required in splicing reaction, and mutations in SF3B1 were found as key cancer driver mutations especially in leukemia. Our study suggests that SF3B complex could affect cancer pathogenesis via cell cycle regulation, leading to more detailed mechanistic analyses of the links between cell cycle regulation, splicing control and cancer pathogenesis. This regulatory pathway involving CLK1 and SF3B complex may result in a new unconventional route of therapy though modulating splicing events that are critical for cancer cell cycle.

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608 Tumor suppressive role of small nucleolar RNAs in the control of lipid metabolism

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H/ACA small nucleolar RNAs (snoRNAs) are responsible for converting hundreds of specific uridine residues to pseudouridine within the ribosome and are found altered in numerous cancers. However, it remains unknown whether H/ACA snoRNA expression and function is modulated to alter the pseudouridine landscape of the ribosome as a means to orchestrate cellular transformation. To our surprise, we have uncovered that specific subsets of H/ACA snoRNAs, guiding modifications within distinct regions of ribosomal RNA (rRNA), display marked coordinated regulation during the earliest cellular responses to oncogene activation. To address whether individual snoRNAs play a role in tumor suppression, we assessed whether a reduction in distinct H/ACA snoRNAs may alter cellular senescence, a critical barrier to oncogenic transformation. Interestingly, we observed that a reduction in distinct H/ACA snoRNAs including SNORA24, which guides pseudouridine modifications within 18S rRNA, leads to the bypass of oncogene-induced senescence. Furthermore, we demonstrate that reduced expression of SNORA24 *in vivo* leads to bypass of senescence induced by oncogenic RAS and promotes the development of liver cancer. Histological analysis of tumor nodules revealed a dramatic increase in lipids that closely resembles human steatohepatitic hepatocellular carcinoma, a rare liver cancer variant characterized by lipid deposition. Employing pharmacological inhibitors of distinct metabolic pathways, we uncovered that SNORA24 modulates specific phospholipases thereby directly impinging on fatty acid and lipid production. Currently, we are defining the molecular mechanisms by which a single H/ACA snoRNA alters the expression and activity of genes involved in lipid metabolism at the post-transcriptional level in response to oncogene activation. From a clinical perspective, hepatocellular carcinomas with low SNORA24 expression exhibit increased lipid content and are associated with poor patient survival. Altogether, this study provides a previously unappreciated link between H/ACA snoRNAs, lipid metabolism, and cancer and suggests that the pattern of rRNA modifications is dynamically regulated to maintain a tumor suppressor program thus providing a new layer of ribosome-mediated control in safeguarding the genome against oncogenic insult.

609 miR-708-5p regulates lung cancer cell phenotype through suppression of pro-oncogenic eicosanoid signaling

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Our objective is to define dysregulated post-transcriptional mechanisms in the arachidonic acid pathway relevant to cancer progression and tumor microenvironment (TME) composition. Many cancers maintain an inflammatory microenvironment to promote their growth. Besides being its own hallmark of cancer, inflammation influences other characteristics, such as proliferation, invasion, angiogenesis, and immune evasion. Lung cancer is of particular importance, as it is the deadliest cancer. Moreover, $11.9 billion is spent on lung cancer treatments in the United States annually, yet 5-year survival rates are exceedingly low. Currently, many cancer treatments focus on cancerous cells only, without considering the off-target effects on cells within the TME. Hence, resolving how inflammation is dysregulated in cancer may provide fresh opportunities for therapeutic development to more comprehensively treat tumors.

One inflammatory pathway commonly dysregulated in cancer is the metabolism of arachidonic acid by Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO) into potent lipid-signaling molecules called eicosanoids. Eicosanoids promote proliferation, invasion, and angiogenesis through autocrine and paracrine signaling to cancer cells and the tumor stroma. Additionally, eicosanoids stimulate an immunosuppressive response, specifically by modulating T-cell and macrophage recruitment and polarization in solid tumors. While the arachidonic acid pathway is commonly upregulated in cancer, the mechanisms governing this deregulation are not well understood. One profound regulator of expression are microRNA (miRNA), which act post-transcriptionally to suppress target mRNAs through transcript degradation or translational stalling. miRNAs are commonly misexpressed in cancer and can perform oncogenic or tumor suppressive functions. One miRNA in particular, miR-708-5p, is commonly underexpressed in cancer and has been shown to repress oncogenic signaling pathways. Interestingly, our preliminary data indicate miR-708-5p suppresses both COX-2 and 5-LO expression in lung cancer cells.

We show miR-708-5p does indeed directly target both COX-2 and 5-LO 3' UTRs in lung cancer cells, decreasing their mRNA and protein levels. This direct targeting of COX-2 and 5-LO decreased oncogenic eicosanoid production, resulting in decreased proliferation of lung cancer cells *in vitro*. We are continuing to extend our research to phenotypic effects of miR-708-5p on lung cancer cells and other non-cancerous cell types commonly found within the TME.

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610 TDP-43 modulates translation of specific mRNAs linked to neurodegenerative disease and cell-specific factors determine the impact on protein levels

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The RNA-binding protein TDP-43 is heavily implicated in the neurodegenerative diseases Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Numerous patient mutations have been identified in TARDBP, the gene that codes for TDP-43, suggesting a causal link between altered TDP-43 function and disease. Data from cell and animal models strongly suggest that altered RNA regulation by TDP-43 in motor neurons drives disease. However, it remains unresolved which effects on gene expression actually drive disease. TDP-43 is primarily a nuclear protein, but increased levels in the cytoplasm in disease suggest an important role in this cellular compartment. Here we used ribosome profiling of motor neuron cell lines and primary cortical neurons to identify mRNAs whose translation is altered by expression of a TDP-43 patient mutant protein. This revealed a small number of translational target mRNAs for mutant TDP43, including some affected in both cell types. For several, we could validate increased ribosome density by polysome profiling and demonstrate direct TDP-43 binding by UV crosslink-IP. Two target mRNAs encode proteins directly linked to ALS and other neurodegenerative diseases. For one TDP-43 mRNA target, we performed a detailed analysis of the impact of altered translation on levels of the encoded protein. Strikingly, this revealed effects that were cell compartment-specific, varied with cell type, and depended on levels of a specific interaction partner for the encoded protein. Our results uncover a previously unappreciated role for TDP-43 as an mRNA-specific translational enhancer and suggest that this function contributes to disease. They also reveal dynamic, cell-specific regulation of how translational enhancement by TDP-43 affects protein levels.

611 Characterization of human disease-causing mutations affecting U12 snRNP function

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In recent years, several human diseases have been described that result from mutations in the unique snRNA (U12, U4atac) or protein components of the minor spliceosome (U11/U12-65K, ZRSR2) (1). With the notable exception of the U4atac snRNA, these factors associate with the U12 snRNP and/or the U11/U12 di-snRNP, the intron recognition complex of the minor spliceosome. The U11/U12-65K protein is a structural component of the U11/U12 di-snRNP, where it interacts with the U12 snRNA and the U11-59K protein, connecting the U11 and U12 snRNPs into a di-snRNP. Previously, we reported that the compound heterozygous mutations P474T and R502X in the C-terminal RRM of the U11/U12-65K protein result in isolated growth hormone deficiency (IGHD) with pituitary hypoplasia (2). More recently, a mutation in the RNA binding partner of the 65K protein, the U12 snRNA, was shown to cause early-onset cerebellar ataxia (3). Here, we present a detailed biochemical and structural characterization of IGHD-causing U11/U12-65K mutations (4), as well as preliminary work characterizing the ataxia-causing 84C>U U12 snRNA mutation.

We have introduced the U12 snRNA 84C>U mutation to several human cell lines using CRISPR/Cas9. Our preliminary results have verified the reported splicing defect (3) and we are currently in the process of determining the mechanistic consequences of this mutation. With the U11/U12-65K mutations, we used NMR to demonstrate that the P474T mutation leads to defective folding of the C-terminal RRM, which assumes a molten globule-like structure. Additionally, both mutants bind to U12 and U6atac snRNAs with reduced affinity and show defects in U11/U12 di-snRNP formation. Futhermore, in patient lymphoblastoid cell lines, a subset of 65K mRNAs carrying the R502X mutation are targeted for NMD in isoform-specific manner, where the long-3'UTR 65K isoform is retained in the nucleus and thus escapes NMD. We propose a model in which NMD-mediated decrease in U11/U12-65K protein levels and defective folding of the P474T mutant reduce the formation of the U11/U12 di-snRNP, leading to a defect in U12-type intron recognition.

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2. Argente (2014) *EMBO Mol Med* 6(3):299-306
3. Elsaid (2016) *Ann Neurol* 81(1):68-78
4. Norppa (2018) *RNA* 24(3):396-409

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612 Musashi 1 - The double-edged Keeper of Stemness

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The RNA-binding protein Musashi (MSI1) controls the mRNA translation of various target transcripts to promote cell cycle progress. It is highly abundant in stem cells where it supports stem cell renewal and inhibits differentiation. Furthermore, MSI1 is upregulated in various cancers where it is presumed to promote an aggressive stemness-like tumor cell phenotype. Recent studies confirmed MSI1 to modulate the fate of glioblastoma multiforme (GBM) derived tumor cells and iCLIP studies indicated various novel candidate target mRNAs.

Although MSI1 is considered to mainly control mRNA translation, we speculated that it regulates also mRNA turnover. Candidate target mRNAs regulated by MSI1 were determined by MSI1 depletion in GBM-derived KNS42 cells using RNA-sequencing. These analyses identified the surface molecule CD44 as novel candidate target mRNA. Moreover, we could confirm this finding in GBM cancer stem cells derived from primary GBM tumor.

This study identifies MSI1 as regulator of stemness and spots mRNA stability control as a new function for the well described translational modulator in human GBM Cancer stem cells.

613 The ribosome associated helicase IGHMBP2 linked to distal spinal muscular atrophy type 1 regulates translation initiation

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Mutations in the immunoglobulin μ-binding protein 2 (IGHMBP2) gene cause the autosomal recessive motor neuron disease distal spinal muscular atrophy type 1 (DSMA1). IGHMBP2 encodes a ubiquitously expressed member of the helicase superfamily 1 that associates with ribosomes suggesting a functional link to translation. Here we show that IGHMBP2 becomes recruited to mRNA via its initial binding to the large ribosomal subunit. Ribosomal and polysomal association requires different domains of IGHMBP2 and is selectively affected by DSMA1 causing missense mutations. Ribosome profiling revealed that upon reducing IGHMBP2 to pathological ribosomes stall at the start codon of a subset of transcripts. These transcripts have characteristic features in their 5'UTR and a high GC content in the vicinity of the start codon. IGHMBP2 paucity results in down-regulation of a defined group of proteins, many of which are linked to muscle and neuron specific functions. Our studies identify IGHMBP2 as a translation factor that selectively impacts on the expression of a subset of proteins. The data also provide insight of the etiology of DSMA1 and may help to find therapeutic targets.

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614 ALS-associated FUS mutations affect U7 snRNP activity and the expression of replication-dependent histone genes in neuronal cells

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Mutations in the FUS gene, which encodes a nuclear RNA-binding protein of the hnRNP family, were identified in patients with an inherited form of Amyotrophic Lateral Sclerosis (ALS). Most reported FUS-linked ALS causing mutations are missense mutations clustered in the nuclear localization signal. They lead to almost abolished or significantly reduced nuclear import of FUS and to cytoplasmic accumulation of FUS aggregates in neurons and glial cells of ALS patients. Mislocalized FUS mutants sequester RNA-binding proteins and U snRNPs, resulting in extensive pre-mRNA processing defects in cells (1-5).

We have recently reported that FUS interacts with U7 snRNP and acts as a positive regulator of replication-dependent histone gene transcription and 3'end processing of their pre-mRNAs during the S phase of the cell cycle (5). Furthermore, we suggested that FUS might be involved in U7 snRNP-dependent repression of histone gene expression outside of S phase, thereby preventing the synthesis of extra histones that would be harmful to the cell (6).

In this work we confirm that FUS influences the expression of histone genes both in undifferentiated neuroblastoma cells and in terminally differentiated neuron-like cells. Interestingly, in proliferating cells FUS plays a role as a positive regulator of histone gene expression whereas in terminally differentiated neuron-like cells it acts as a repressor. In both kind of cells ALS-linked FUS mutations cause cytoplasmic re-localization of U7 snRNP and affect histone gene expression.

We conclude that in both proliferating cell as well as in terminally differentiated neurons ALS-linked FUS mutations can lead to destabilized transcription control mechanism due to U7 snRNP mislocalization. As a consequence, disturbed repression or activation of histone gene expression resulting in genome instability or toxic effect of excess of histones may be the molecular mechanisms underlying altered motor neurons homeostasis in ALS.

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615 NMD as a Potential Mechanism of Oncogenesis in Spliceosomal-mutant MDS

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Recurrent heterozygous somatic mutations in the spliceosomal proteins SF3B1, SRSF2, and U2AF1 represent the most common class of genetic variations in patients with myelodysplastic syndromes (MDS), and are present in ~60% of MDS patients. Since the discovery of these mutations, published data suggested that mutations in SRSF2 and U2AF1 alter their normal RNA binding and splicing preferences in a sequence-specific manner, whereas mutations in SF3B1 promote selection of cryptic 3' splice sites. Several mRNA isoforms promoted by the various splicing-factor mutants harbor a premature termination codon, and are therefore potential targets of nonsense-mediated mRNA decay (NMD). Some of the genes with altered splicing include important regulators of hematopoietic differentiation, with consequent pathological defects in hematopoiesis, leading to MDS. Although links between alternative splicing and NMD have been proposed, evidence for a specific role of mutant splicing factors in the NMD pathway was lacking. Previously, we showed that overexpression of certain individual SR proteins enhances NMD. We hypothesized that in addition to altering splicing, SRSF2 mutants might have a direct role in NMD. Combining different biochemical and functional approaches, together with genomics, proteomics, and bioinformatics, we uncovered differential roles of SRSF2 mutants in the NMD-regulatory pathway, compared to wild-type SRSF2, and determined the molecular basis of this altered regulation. Our findings point to NMD as a potential pathway of oncogenesis, and provide relevant information towards developing RNA-based targeted therapies to correct splicing errors in MDS.

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616 Hereditary cancer genes are highly susceptible to splicing mutations

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Substitutions that disrupt pre-mRNA splicing are a common cause of genetic disease. On average, 13.4% of all hereditary disease alleles are classified as splicing mutations mapping to the canonical 5′ and 3′ splice sites. However, splicing mutations present in exons and deeper intronic positions are vastly underreported. A recent re-analysis of coding mutations in exon 10 of the Lynch Syndrome gene, *MLH1*, revealed an extremely high rate (77%) of mutations that lead to defective splicing. This finding is confirmed by extending the sampling to five other exons in the *MLH1* gene. Further analysis suggests a more general phenomenon of defective splicing driving Lynch Syndrome. Of the 36 mutations tested, 11 disrupted splicing. Furthermore, analyzing past reports suggest that *MLH1* mutations in canonical splice sites also occupy a much higher fraction (36%) of total mutations than expected. When performing a comprehensive analysis of splicing mutations in human disease genes, we found that three main causal genes of Lynch Syndrome, *MLH1, MSH2,* and *PMS2,* belonged to a class of 86 disease genes which are enriched for splicing mutations. Other cancer genes were also enriched in the 86 susceptible genes. The enrichment of splicing mutations in hereditary cancers strongly argues for additional priority in interpreting clinical sequencing data in relation to cancer and splicing.

617 THE SNORNA U3 ALTERS THE CELLULAR PHENOTYPE OF CHONDROCYTES IN OSTEOARTHRITIS

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Osteoarthritis (OA) is a debilitating joint disease involving cartilage, with a reduced anabolic and increased catabolic state and an altered chondrocyte hypertrophic cellular phenotype (increase *COL10A1, MMP13* and *ALP,* decrease of *COL2A1* and *ACAN)*. SnoRNAs play a role in the processing of pre-ribosomal RNA (pre-rRNA) and thus cellular anabolic state. We hypothesize that snoRNAs play a role in the OA chondrocytes' reduced anabolic state and shift towards a catabolic hypertrophic phenotype via altered processing of pre-rRNA.

Cartilage for microarray analysis was obtained from human OA patients or healthy donors (n=10). Total RNA was extracted and hybridised onto Affymetrix miRNA 4.0 arrays to determine differentially expressed snoRNAs with a probe set for *Homo sapiens*. Ribosomal RNA and OA phenotype-associated gene expression in healthy and OA human articular chondrocytes (HAC) was measured using qRT-PCR and cellular translational capacity was assessed using SUnSET assays. U3 snoRNA knockdown in SW1353 cells was undertaken using antisense oligonucleotides (ASO) and chondrocyte phenotypic markers and rRNA intermediates were determined by qRT-PCR.

Microarray analysis showed reduced expression of several snoRNAs in OA chondrocytes compared to healthy individuals, including U3; involved in early endoribonucleolytic pre-rRNA processing. Compared to healthy chondrocytes *in vitro* OA HAC cultures demonstrated a decrease in U3 as well as mature rRNA levels, accompanied by an increase in precursor-rRNA. Additionally, overall translational capacity in OA chondrocytes was reduced. ASO-mediated knockdown of U3 snoRNA expression in SW1353 chondrocytic cells resulted in p53 protein stabilization, indicative for ribosomal stress. U3 knockdown further revealed a reduction in mature rRNAs with an accumulation of 47S pre-rRNA intermediates compared to scrambled controls. Following U3 snoRNA depletion, the chondrocyte phenotype shifted towards a more hypertrophic/catabolic chondrocyte phenotype.

Our results show the differential expression of several snoRNAs in OA compared to healthy cartilage, including U3. OA chondrocytes demonstrate reduced translational capacity. The reduction in levels of U3 leads to downregulation of rRNA expression in SW1353 cells. U3 depletion may accommodate a chondrocyte hypertrophic shift towards a more OA-like phenotype. We conclude that besides its role in 47S pre-rRNA maturation, altered U3 expression can also result in cell phenotypic changes relevant for osteoarthritis development.

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618 Nuclear export pathways of disease-associated repeat expansion RNA in C9FTD/ALS

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The expansion of microsatellites is associated with over two dozen neurological diseases. A common denominator among the majority of these disorders is the expression of expanded tandem repeat-containing RNA, referred to as xtrRNA, which can mediate molecular disease pathology in multiple ways. To understand disease mechanisms we are focusing on xtrRNA export in C9FTD/ALS, a model repeat expansion disorder. In C9FTD/ALS, the repeat expansion occurs in the first intron yet xtrRNA is somehow exported and translated into toxic repetitive polypeptides. We hypothesize that xtrRNA escapes into the cytoplasm by hitchhiking with a fraction of intron-retained mRNA. Two main mRNA export pathways may be utilized: nuclear RNA export factor 1 (NXF1)-mediated and chromosome region maintenance (CRM1)-mediated export. We will knock-down nucleocytoplasmic transport factors in these pathways, including ALY/REF, NXF1, TREX, CRM1 and UPF1, and monitor xtrRNA nuclear focal aggregation, translation and cellular distribution. Results and progress will be presented. Identification of the pathway or specific proteins responsible for C9FTD/ALS xtrRNA export should shed light on disease mechanisms and potential strategies for therapeutic intervention.

619 Circulating microRNAs as a biomarker of Ectopic Pregnancy in South Indian population

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Objectives:

To evaluate serum microRNAs (miRs) as a potential diagnostic marker to discriminate between women with Ectopic Pregnancy (EP) and normal pregnancy.

Materials & Methods:

This study included 280 pregnant women between 4 – 10 weeks of gestation, 140 women with EP and 140 women with viable intrauterine pregnancy as controls. Venous blood samples were obtained from these women; total RNA including miRs was extracted from the serum samples and converted to cDNA. Relative gene expression of miRs was determined by real time PCR. The PCR data was analyzed by SAS 9.1 software to estimate ΔΔCT and its p value. A computational analysis to identify the gene targets and pathways was performed.

Results:

Of the 8 miRs evaluated in this study, four miRs were differentially down regulated (hsa-miRs-141,218, 519d and 873) and four up regulated (hsa-miRs-223, 517a, 523 and 323-3p) in serum samples of women with EP. The expression fold change was statistically significant ($p<0.05$) except for hsa-miR-141 and 218. Pathway interaction predicted by miRBase and DIANA tools – mirPath were: 1) Prokineticin dysregulation in fallopian tube by PROKR2 gene influenced by miRs 141, 218, 517a and 873; 2) Intracellular communication between trophoblast and immune cells mediated by miRs 141 and 519d present in extracellular vesicles. For the miRs 223, 523 and 323-3p whose expression is significantly altered in EP, the pathway interaction could not be deduced based on the above database.

Conclusion:

Early prediction of an EP could rely on estimation of circulating miR levels in the sera of pregnant women. This can be used to screen women presenting with a clinical picture suggestive of EP and determine appropriate treatment modalities at the earliest.

Keywords: biomarker, ectopic pregnancy, microRNA, serum

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620 Modeling microcephaly using MOPD I patient-derived iPS cells and cerebral organoids to study neuro-developmental defects

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Microcephalic **O**steodysplastic **P**rimordial **D**warfism type I (MOPD I) is a rare autosomal recessive genetic disorder characterized by severe intrauterine and postnatal growth retardation including microcephaly (mean occipital frontal circumference -7.0 SD, range -4.0 to -9.5 SD). MOPD I is the first human disease which has been linked to biallelic mutations in one of the RNA components of the U12-dependent spliceosome, U4atac snRNA. U4atac snRNA is essential for the formation of the U4atac/U6atac.U5 tri-snRNP complex and functions as a molecular chaperone to sequester the activity of U6atac snRNA. The majority of the point mutations associated with MOPD I are located in the 5' stem loop region of U4atac snRNA and there is evidence demonstrating that these mutations interfere with RNA-protein interactions essential for the formation of the tri-snRNP complex. So far there is no clear genotype-phenotype correlation which leads us to hypothesize that different mutations may be contributing to the difference in severity of the disease via fine tuning the expression of different subsets of downstream U12-dependent intron containing genes. To address this, we are in the process of performing RNA-sequencing on RNA isolated from iPS cells homozygous for the severe MOPD I G51A mutation (patient cells) compared with isogenic cells expressing an exogenous wild type U4atac snRNA (corrected cells). Also, in order to study the neuro-developmental defects associated with the G51A mutation, we have generated 3D cerebral organoids. Interestingly, the organoids formed from the patient iPS cells were smaller in size as compared to those formed from the corrected iPS cells during the early stages of organoid development. Additional phenotypic studies are on ongoing and will be described.

621 Activation of *HOXA* transcriptional networks is associated with *NUP98/NSD1* fusions in pediatric AML

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NUP98/NSD1 fusions occur in 3-5% (Ostronoff *et al.* 2017) of pediatric AML patients. 82% of *NUP98/NSD1* fusion+ patients also harbour *FLT3/ITD*, a known driver of treatment resistance (Ostronoff *et al.* 2017). Patients with both genetic alterations had a much lower rate of complete remission induction (27% vs 69% for *FLT3/ITD* patients without *NUP98/NSD1*; Ostronoff *et al.* 2017). Although the co-occurrence of these two events is associated with a low rate of patient response to therapy, the mechanisms by which the co-expression of *FLT3/ITD* and *NUP98/NSD1* induces innate treatment resistance are not well understood.

We hypothesize that the co-occurrence of *FLT3/ITD* and *NUP98/NSD1* can result in downstream disruptions of regulatory networks. To address this hypothesis, we analysed rRNA-depleted RNA and miRNA sequencing data generated for 1,055 pediatric AML cases as part of the Children's Oncology Group AAML1031 clinical trial (Aplenc *et al.* 2016). RNA expression was quantified using Sailfish (Patro *et al.* 2014) and Ensembl annotations. miRNA expression was quantified using previously annotated miRNAs in miRBase (version 21; Kozomara *et al.* 2014). Transcription factor (TF) networks were inferred through grouping co-expressed genes sharing common TF binding motifs. Such TF networks were assessed for activity across all samples. Results revealed a distinct set of patients, apparently enriched for *NUP98/NSD1* fusion+ cases but independent of *FLT3/ITD* status, with high expression of *HOXA6*, *HOXA3* and their downstream target genes. We noted that 26 out of 30 *NUP98/NSD1* fusion+ patients showed high *HOXA3* activity and 22/30 showed high *HOXA6* activity. Integrative miRNA:RNA expression correlation analysis revealed that the expression of hsa-mir-196b (MIMAT0001080) was significantly highly correlated with *HOXA6* expression (Spearman's rank correlation coefficient=0.791, BH-adjusted p-value = 0). *HOXA6* also harbours putative binding sites for hsa-mir-196b as predicted by both TargetScan and miRanda.

Our results indicated that innate treatment resistance may be conferred through the dysregulation of transcriptional networks and that non-coding RNAs could have roles in mediating the process. This study may thus provide insights into possible mechanisms of treatment resistance in pediatric AML.

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622 The human MAPT locus generates human-specific circular RNAs

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The microtubule-associated protein Tau, generated by the MAPT gene is involved in dozens of neurodegenerative conditions ("tauopathies"), including Alzheimer's disease (AD) and frontotemporal lobar degeneration/frontotemporal dementia (FTLD/FTD). The pre-mRNA of MAPT is well-studied and aberrant pre-mRNA splicing of tau exon 10 causes frontotemporal dementia and is also implicated in sporadic AD.

Using RNA from adult human brain tissues, we found that the MAPT locus generates two classes of circular RNAs through a backsplicing mechanism from exon 12 to either exon 10 or 7, as well as a backsplicing from exon 4 to exon 1 that includes a novel exon. These circRNAs are human-specific, possibly because they rely on Alu elements for their formation.

We constructed minigenes with shortened introns that still contain Alu elements and defined the necessary cis-elements to generate MAPT circRNAs. Using transfection studies, we found that proteins regulating the alternative usage of exon 10 in the linear MAPT RNA such as clk2, SRSF7/9G8, PP1 (protein phosphatase 1), SRPK1 and NIPP1 (nuclear inhibitor of PP1) reduce the abundance of the circular MAPT exon 12->10 backsplice RNA, suggesting that its formation can be controlled by the cell. Importantly, mutations that activate exon10 and lead to frontotemporal dementia change the effect of these trans-acting factors, suggesting that a deregulation in MAPT circRNAs contribute to hereditary frontotemporal dementia.

MAPT circular RNAs are localized in the cytosol and contain open reading frames encoding Tau protein fragments that could form polymers if translated in a 'rolling circle' mechanism. When ectopically overexpressed from a tRNA intron, MAPT circRNAs change alternative splicing of reporter genes, suggesting that they can influence gene expression. The expression of MAPT circRNAs is changed in different Braak stages of Alzheimer's disease, and could thus contribute to the pathology.

In summary, we report the identification of new bona fide human-specific brain circular RNAs produced from the MAPT locus. These may be a component of normal human brain Tau regulation and, since the circular RNAs could generate high molecular weight proteins with multiple microtubule binding sites, and since they influence pre-mRNA processing, they could contribute to tauopathies.

623 eCLIP-Seq reveals differential recognition of RNA targets by mutant SRSF2 in stem cell derived hematopoietic progenitor-like cells

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Recurrent point mutations in components of the U2 spliceosome machinery including SRSF2, U2AF1, and SF3B1 are found in >60% of patients with myelodysplastic syndromes (MDS), a bone marrow failure disease caused by mutations in hematopoietic stem cells. Although these mutations commonly occur, it is still not well understood how such mutations mechanistically alter the protein's normal function in the cell. We used CRISPR/Cas9 to model the heterozygous SRSF2 P95L point mutation in human induced pluripotent stem cells (iPSCs). We also introduced an allele specific 3X FLAG tag on the mutant allele in heterozygous cells, and on one wild-type allele in normal cells to allow for specific immunoprecipitation of the mutant and wild-type proteins. To determine if the mutation affects RNA binding, we performed eCLIP-Seq on cells differentiated into CD34+/CD45+ hematopoietic progenitor-like cells. We found a preference for the wild-type protein to bind GAAG-rich sequences while the mutant preferred G/C rich sequences. These differential motif preferences correlate with the small subset of exons that are alternatively spliced in cells containing the mutation. This work has characterized, alterations in the specific binding profile of SRSF2 directly linked to a disease-relevant mutation that is affected in patients. Future work will follow up on candidate binding events that are specific to the mutation to determine the direct causal effects of individual events on disease phenotypes.

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624 Development of an inhibitor against Musashi-1 and its use in glioblastoma therapy

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We identified the RNA binding protein Musashi1 as a major driver of glioblastoma and medulloblastoma. High Musashi1 expression is prevalent in high-risk groups and correlates with poor prognosis. Biological and functional genomic analyses determined that Musashi1 affects numerous cancer relevant processes such as apoptosis, cell cycle, proliferation, adhesion, invasion, migration, DNA-repair and radio-resistance and it does so by modulating the translation and stability of several oncogenic factors. Moreover, Musashi1 is possibly involved in tumor initiation as indicated by the negative impact on the growth of "tumor initiating cells" and expression of stem cell related genes. So, we propose to use Musashi1 targeting as an alternative to treat glioblastoma patients. The RNA sequence recognized by Musashi1 is very specific and well-characterized. Taking advantage of that, we have designed a biochemical fluorescence polarization-based platform which allows us to assay compounds for binding inhibition of a specific fluorescent-target RNA oligomer to purified Musashi1 protein. We screened circa 25,000 compounds. 41 hits were identified and later tested by NMR to confirm interaction with Musashi1. A flavonoid named luteolin produced the best results and was selected for future analysis. To assure that luteolin inhibits Musashi1 in vivo, we first checked its effect on the expression levels of Musashi1 targets (PDGFRα, IGF1R, EGFR, CCND1 and CDK6)-these targets are positively regulated by Musashi1. In all cases, we observed a reduction in protein levels. Next, we used a luciferase reporter containing the 3'UTR of PDGFRα. In normal circumstances, co-transfection with an Musashi1 expressing plasmid increases luciferase activity. Addition of luteolin abolished this effect. Subsequently, we tested the impact of luteolin on glioblastoma cells. Luteolin inhibited the proliferation of U251 and U343 cells but didn't affect astrocyte cultures. Similarly, we showed that luteolin decreases GBM cells' viability, colony formation, migration and invasion. Luteolin also affected the growth of glioma stem cells and tumor-organoids. Finally, combined treatments with PARP inhibitor or ionizing radiation showed synergistic effects. In summary, our results show that luteolin functions as an inhibitor of Musashi1 and establishes a case for its potential use in glioblastoma therapy.

625 Structural insights into the RNA methyltransferase domain of METTL16

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N^6-methyladenosine (m^{6}A) is an abundant modification in messenger RNA and noncoding RNAs that affects RNA metabolism. Methyltransferase-like protein 16 (METTL16) is a recently confirmed m^{6}A RNA methyltransferase that methylates U6 spliceosomal RNA and interacts with the 3'-terminal RNA triple helix of metastasis-associated lung adenocarcinoma transcript 1 (MALAT1). Here, we present two X-ray crystal structures of the N-terminal methyltransferase domain (residues 1-291) of human METTL16 (METTL16_291): an apo structure at 1.9 Å resolution and a post-catalytic *S*-adenosylhomocysteine-bound complex at 2.1 Å resolution. The structures revealed a highly conserved Rossmann fold that is characteristic of Class I *S*-adenosylmethionine-dependent methyltransferases and a large, positively charged groove that likely represents the RNA-binding site. This putative RNA-binding site is comprised of structural elements unique to METTL16, suggesting this region may contribute to the RNA substrate specificity of METTL16 that is distinctly different from the major m^{6}A RNA methyltransferase complex, METTL3/METTL14. In contrast to the METTL3/METTL14 heterodimer, full-length METTL16 forms a homodimer and METTL16_291 is a monomer based on size-exclusion chromatography. These results indicate that the C-terminal domain facilitates protein dimerization. Furthermore, a native gel-shift assay shows that METTL16 binds to the MALAT1 RNA triple helix but monomeric METTL16_291 does not. Our results provide insights into the molecular structure of METTL16, which is distinct from METTL3/METTL14.

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626 Crosslinking of segmentally isotope labeled RNA and MS/MS on large multi-molecular complexes and different RNA-binding domains

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Deciphering the interaction sites on RNA and protein provides the basis for a profound understanding of ribonucleoprotein (RNP)-mediated gene expression and its regulation in health and disease. **C**ross**l**inking of segmentally **i**sotope labeled **R**NA and tandem **m**ass **s**pectrometry (CLIR-MS/MS) is a new, efficient technique to pinpoint direct protein-RNA contacts with single amino acid and single nucleotide resolution. Applying CLIR-MS/MS on the complex of Polypyrimidine Tract Binding Protein 1 (PTBP1) with an IRES-derived RNA, enabled the precise positioning of all four RNA recognition motifs (RRM) of PTBP1 on the RNA, thereby substantially revising the previous model proposed for this complex[1,2]. Following up on this proof of principal example, we are aiming at optimizing the method and applying it to other RBP containing different kinds of RNA binding domains (zinc fingers and double-standed RNA binding domains) and *in vitro* reconstituted multicomponent RNPs, including U1 snRNP in complex with alternative splicing factors. The high spatial resolution of RNA-protein interaction sites achieved by CLIR-MS/MS will provide distance restraints that could significantly contribute to the generation of 3D atomic models of these complexes by integrative structural modelling.

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627 Determining RNA target binding specificities of *Marinitoga piezophila* Argonaute (MpAgo) using a structural approach

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Argonaute (Ago) proteins are conserved in all domains of life. Previous studies have shown that Argonautes play a pivotal role in RNA interference pathways in eukaryotes and are linked to host defense in bacteria and archaea. However, the specific role of Ago proteins in prokaryotes has not been elucidated. Kaya et al. investigated prokaryotic Ago proteins found to be encoded in CRISPR-cas operons. They focused on one of these proteins, MpAgo, from *Marinitoga piezophila*. They found that MpAgo has specificity for 5'-hydroxylated (5'-OH) guides, unlike previously studied Argonautes. As a whole, their study represents the discovery of a new CRISPR-associated Argonaute subclass capable of cleaving single stranded DNA and single stranded RNA when programmed with a 5'-OH guide RNA (gRNA) complementary to such targets. This confers bacterial MpAgo great potential as a tool for exploring eukaryotic RNA biology for several reasons. One of them is that its specificity for 5'-OH guides allows it to be neither capable of recognizing the endogenous RNA interference machinery nor of binding endogenous 5' phosphorylated gRNAs. More importantly, bacterial MpAgo does not target double stranded DNA and can be made catalytically inactive for RNA cleavage through mutagenesis, while retaining RNA binding. Our lab has recently been working on characterizing MpAgo's interactions with its guide and target RNA in order to develop a new RNA-guided RNA targeting tool using MpAgo. Although previous studies have elucidated MpAgo's structure, both with a gRNA and with a single stranded DNA target, no previous study has revealed how MpAgo interacts with target RNA. Therefore, we are working on taking a structural approach to investigate the structural features that determine MpAgo's target binding specificity. For this, we have expressed and purified catalytically inactive MpAgo and loaded it with a 5'-BrdU gRNA. Once the RNP is assembled, it is loaded with a target RNA and purified using chromatography. We are currently working on determining the binding properties of this tandem target RNA RNP complex and the most optimal purification steps for it in order to prepare it for structural experiments such as crystallography and cryo-EM.

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628 Mutational studies of the Adenoviral Virus-Associated RNA I support a functionally important pseudoknot

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Adenoviruses are dsDNA viruses that infect the upper respiratory system and gastrointestinal and urinary tract. The Adenoviral Virus-Associated RNA_I (VA-I), expressed in high levels late in viral infection, has been characterized as an inhibitor of a number of enzymes involved in innate immune response. One target, Protein Kinase RNA-activated (PKR), recognizes long double-stranded RNAs expressed during viral infection and activates eIF2a to halt global translation. VA-I binds to PKR to prevent its autophosphorylation and activation. VA-I RNA consists of a highly structured apical stem, central domain, and terminal stem. The secondary structure of VA-I has been extensively studied and is thought to contain several distinct features important for PKR inhibition. One feature is a highly conserved, four base pair stem termed tetrastem. Mutations in the tetrastem drastically reduce the RNAs ability to inhibit PKR and converts it into a PKR activator. Additionally, VA-I has been proposed to contain a pseudoknot within the central domain. Mutations that disrupt the pseudoknot also negatively impact VA-I activity, while compensatory mutations that restore the pseudoknot rescue VA-I activity. Point mutations of nucleotides thought to further stabilize the pseudoknot also significantly impact VA-I activity. Insertion of a bulge in the apical stem prevents VA-I from activating PKR. Deletion of the terminal stem, however, does not reduce PKR inhibition by VA-I. Taken together, the effects of point mutations and other alterations in VA-1 RNA support the existence of a 3-bp pseudoknot in the central domain and its crucial role in mediating the inhibitory activity of PKR.

629 An atomic structure of the yeast Ribonuclease P

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Ribonuclease P (RNase P) is a universal ribozyme in all three kingdoms of life responsible for processing the 5' end of precursor tRNA. Here we report the first atomic structure of eukaryotic RNase P holoenzyme from Saccharomyces cerevisiae at a resolution of 3.48 Å. The RNA component of yeast RNase P adopts an extended single-layered conformation that maintains a central helical core, but lacks most of the long-range RNA-RNA interactions that are essential for structural stability in bacterial RNase P. Remarkably, the protein subunits together form a hook-shaped architecture that tightly wraps and stabilizes the RNA by extensive tertiary protein-RNA interactions. Consistent with the structure, molecular dynamics simulation reveals an obvious stabilizing effect on the RNA by the proteins. Overall, our results provide a valuable evolutionary model depicting how the structural roles of bacterial key peripheral RNA elements are delegated to the protein components in RNase P from higher organisms.

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630 Structural architecture of human RNase P holoenzyme by cyro-EM

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Ribonuclease P is a ubiquitous ribozyme in practically all organisms, required for the maturation of the 5’ end of pre-tRNA. Here we provide the first insight into the structural organization of human RNase P holoenzyme by cryo-EM at a resolution of 3.9 Å. It exhibits an elongated flat triangular-shaped configuration, with the H1 RNA mainly on one side of triangle whereas the proteins concentrated on the other. The H1 RNA packs against with an observed characteristic three coaxially helical stems in bacterial RNase RNAs, resulting in an extended single-layer configuration. Four structural modules composed of ten proteins of human RNase P are intimately connected with each other to from an architecture resembling a right-hand-shaped clamp, which tightly grabs the H1 RNA. The C domain of the H1 RNA snugly fits into the narrow space between the palm and fingers of the protein clamp. In contrast, the S domain of H1 resides outside of the hand-shaped clamp, forming the sharp corner of the RNase P triangle. Unexpectedly, we identity a newly feature, K-turn, of the S domain, which we design as CR-VI. Our work recovers the mechanism of the assembly of active human RNase P and helps understand the structure and function of ribonucleoprotein complexes with a catalytic RNA core.

631 Structural Insight into the Catalytic Mechanism of Yeast RNase P

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Ribonuclease P (RNase P) is one of the first ribozyme discovered and it is responsible for processing the 5' end of precursor tRNA in all phylogenetic groups. Here we report the atomic structure of a *Saccharomyces cerevisiae* RNase P holoenzyme in complex with its substrate pre-tRNA^{Phe} at an overall resolution of 3.48 Å by single particle, electron cryo-microscopy (cryo-EM). The *S. cerevisiae* RNase P consists of one large RNA molecule Rpr1 responsible for catalysis and nine protein components formed a hook-shaped architecture. The recognition of pre-tRNA by RNase P occurs through the specific intermolecular base-stacking interactions between the CR-II, CR-III in S domain of Rpr1 and the pre-tRNA TΨC and D loops, and stem P15 in C domain of Rpr1 and the 3' end of pre-tRNA. Notably, the largest protein subunit Pop1 also plays an important role in pre-tRNA binding to be a pre-catalytic state. Remarkably, two magnesium ions existing in the active site indicates that RNase P employs a two-metal ion catalytic mechanism, similar to other large ribozymes. And the molecular dynamics simulation data further confirmed this observation. Overall, our structure provides a molecular scenery for understanding the mechanism of catalysis by eukaryotic RNase P.

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632 Defining an exon commitment window in Exon Definition: insights from Designer Exons

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Previously we used simplified exons of our own making, Designer Exons, to construct a biophysical model for splicing in an Exon Definition system. We focused on the first irreversible reaction in the splicing process: commitment. In the model, we allowed both co-transcriptional as well as post-transcriptional commitment. Using a three-exon minigene to assess splicing in HEK293 cells, we evaluated the effects of different parameters of the middle exon, such as length and ESS/ESE content, on inclusion. These data were used to generate the biophysical model; further tests were performed to verify its predictive power. This model surprisingly suggested that post-transcriptional commitment played no role in determining the outcome, characterizing commitment as exclusively co-transcriptional.

Here, we have searched for the end of a commitment window using two competing donor splice sites at the end of the middle exon: a somewhat weak donor site (proximal site, relative to the body of the middle exon) and a very strong competing donor site (distal site) placed at different distances downstream of the proximal site. Without the competing splice site the proximal site yielded ~60% inclusion; when the distal site was used alone, 100% inclusion was observed at all distances tested. When these two splice sites were made to compete, the strong distal site should "steal," shortly after its synthesis, whatever molecules were not committed to splicing to the proximal site. Therefore, changing the distance of the strong distal site relative to the fixed proximal splice site allows "snapshots" of the commitment process to be taken. Using this tool, a series of individual "snapshots" were assembled to generate a "*commitment curve*" for the weak donor site.

Our results show the end of the commitment window at only ~220nt downstream of the proximal site confirming our hypothesis of co-transcriptional commitment. Importantly, they also show that commitment does not start until ~120nt of the intron have been synthesized. This surprising result defines a *pre-commitment window*. The *commitment window* thus defined, which we estimate to be only ~2s long, has implications for understanding important features of splicing like processing of cryptic splice sites and splicing of long introns.

633 Structure of the Post-catalytic Spliceosome from *Saccharomyces cerevisiae*

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Precursor messenger RNA (pre-mRNA) splicing is executed by the spliceosome. Removal of an intron in the pre-mRNA requires two steps of transesterification, resulting in the ligation of two exons in the post-catalytic spliceosome (known as the P complex). Here we present a cryo-electron microscopy (cryo-EM) structure of the P complex from *Saccharomyces cerevisiae* at an average resolution of 3.6 Å. At the active site center, three bases at the 3'-end of the 5'-exon remain anchored to the loop I of U5 small nuclear RNA (snRNA). The nucleotide at the 3'-end of the 5'-exon is covalently linked to the nucleotide at the 5'-end of the 3'-exon, with three ensuing nucleotides of the 3'-exon clearly identifiable in the active site. The two conserved nucleotides AG of the 3'-splice site (3'SS), which have just been released in exon ligation, are specifically recognized by the invariant adenine of the branch point sequence, the guanine base at the 5'-end of the 5'-splice site and an adenine base of U6 snRNA. The 3'SS is placed between the ligated exon and the intron lariat and stabilized by the 1585-loop of Prp8. This structure represents an important contribution to structural and mechanistic elucidation of the complete splicing cycle.

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634 Characterization of regulatory mechanisms for *BIM* exon 3 alternative splicing

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BCL-2-interacting mediator of cell death (BIM) is a proapoptotic member of the BCL-2 protein family. BIM upregulation is required for tyrosine kinase inhibitor (TKI)-mediated cell death in many kinase-driven cancers including chronic myeloid leukemia (CML) and epidermal growth factor receptor mutated non-small cell lung cancer (EGFR NSCLC). Previously, a 2.9 kb deletion polymorphism in BIM intron 2 was found to contribute to TKI resistance in CML and EGFR NSCLC patients. The deletion allele switched splicing from BIM exon 4 (E4) to exon 3 (E3) in a mutually exclusive manner, generating isoforms lacking the proapoptotic BCL2-homology domain 3 (BH3) encoded by E4. This suggests that modulating BIM splicing to exclude E3 might resensitize the polymorphism containing cancer cells to TKI. In this project, the mechanisms of BIM E3 alternative splicing regulation were studied. To identify the cis-acting splicing elements regulating BIM E3, a series of sequential 10 nucleotide deletions throughout E3 and upstream intronic region were generated in two BIM minigenes with and without the polymorphic fragment. By comparing the E3/E4 ratio of the deletion mutants to that of the full-length minigenes by quantitative real-time RT-PCR, putative splicing enhancers and silencers regulating BIM exon 3 were defined. The exonic enhancers were subsequently verified by point mutations and heterologous minigene analysis. The length of the BIM E3 poly-pyrimidine tract, which is an essential sequence within 3' splice sites was varied, and it was found that an unusually long minimum of 16 uridines was required for maximal E3 inclusion.

The splicing factor, SRSF1 was confirmed to promote BIM E3 inclusion by knockdown/overexpression. An exonic splicing enhancer in E3 was found to act via SRSF1. In addition minor alleles for four annotated SNPs were found to potently alter E3 splicing in BIM minigenes and the allele combinations showed additive effects. These annotated SNPs are being tested for their effects on TKI responses. The splicing regulatory elements elucidated in our study may reveal therapeutic targets for sensitization of TKI responses in cancers dependent on BIM expression for drug sensitivity.

635 Novel self-splicing introns discovered in genomes of ubiquitous, but little-known Candidate Phyla Radiation bacteria

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Culture-independent analyses of microbial communities have revolutionized our understanding of the metabolic capabilities of microbes, and our understanding of the tree of life. Genome-enabled phylogenetic studies have made it apparent that approximately 50% of bacterial diversity is represented by a recently recognized group referred to as the Candidate Phyla Radiation (CPR). The extent of the genomic diversity within the CPR indicates that these organisms employ biochemical strategies that have yet to be discovered. Extensive genomic analyses have shown that the CPR is comprised of organisms with small genomes and substantial metabolic limitations that indicate an obligate symbiotic lifestyle. CPR bacteria are ubiquitous, are sometimes numerically dominant members of microbial communities, and exhibit high replication rates under certain conditions. However, nearly all have resisted isolation. Interestingly, the rRNA genes of CPR bacteria frequently encode introns. Many of these introns are predicted to self-splice using mechanisms common to group I or group II introns, or to be enzymatically cleaved, which results in fragmented rRNA. However, others likely self-splice using novel mechanisms. To identify novel self-splicing introns, we used a metatranscriptome assembly method to identify unclassified introns associated with intact rRNAs, thus excluding those that are enzymatically processed. Based on structural modelling and phylogenetic analysis, we identified a novel clade of introns, which we confirmed to be self-splicing in vitro. In a survey of groundwater metagenomes, we find that these novel introns are primarily associated with members of the CPR and a similar radiation in the archaeal domain known as DPANN. However, they were also encoded in the genomes of Rokubacteria and Bacteroidetes.

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636 In search of kinetic changes in splicing in response to mutations in SF3b1 and Prp8

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Eukaryotic pre-mRNA must be processed by the spliceosome to remove non-coding introns and ligate together coding exons. This process, splicing, is largely concurrent with transcription of the pre-mRNA by RNA polymerase and perturbations of transcription rate can activate alternative splice sites. Many changes in splicing are associated with human diseases, yet identifying splicing outcomes does not reveal what went "wrong" during splicing. We are taking an *in vivo* approach that examines the precursors, intermediates and products of splicing through single molecule intron tracking (SMIT) and long-read sequencing (LRS) of nascent RNA, which quantify splicing progression as a function of Pol II position[1]. Focusing on recurrent mutations in SF3b1 and Prp8 that have been identified in myelodysplastic syndromes (MDS), I am determining changes in the dynamics of splicing that could help explain mechanistically how these outcomes arise. Additionally, I will determine the impact of splicing factor mutations and natural splice site variation on splicing in budding yeast. These studies investigate the roles of SF3b1 and Prp8 in co-transcriptional splicing, while contributing novel insights into human disease.

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637 mRNA processing regulates the unfolded protein response.

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During the unfolded protein response (UPR), protein-folding stress in the endoplasmic reticulum activates the transmembrane kinase/endoribonuclease Ire1, which processes the *HAC1* pre-mRNA by endonucleolytic cleavage of its intron. The exon products of cleavage are subsequently ligated, and mature *HAC1* mRNA is translated, leading to the expression of hundreds of stress-response genes. *HAC1* mRNA cleavage is thought to be the rate-limiting step of UPR activation. We have identified two mRNA processing steps *between* mRNA cleavage and ligation that negatively regulate the UPR. First, we show that *HAC1* pre-mRNAs likely undergo multiple rounds of 5′-splice site cleavage and rejoining by RNA ligase and propose that this "futile cycle" prevents UPR activation while simultaneously poising *HAC1* mRNA for further rounds of cleavage. Second, we show that Xrn1-mediated decay of the cleaved 3′-exon competes with its ligation to suppress UPR activation. Together, these novel mRNA processing events divert substrates from productive mRNA ligation and suppress the UPR.

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638 A global binding map of hnRNP A2/B1

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Correct splicing of exons with weak splice sites depends on a tight balance between positive and negative splicing regulatory elements (SREs). A large fraction of disease-causing mutations disrupt mRNA splicing by decreasing splice site strength or disrupting/creating SREs. We used iCLIP (individual-nucleotide resolution crosslink and immunoprecipitation) and targeted RNA immunoprecipitation (tRIP) combined with next generation sequencing to create an in vivo binding map for the splicing regulatory protein hnRNP A1/B2. This map can be used to identify important SREs that function through hnRNP A1/B2 binding, and which can potentially serve as binding sites for splice switching oligonucleotides (SSO) in order to modulate splicing.

HeLa cells with inducible expression of T7-tagged hnRNP A1/B2 were UV irradiated generating irreversible crosslinks between RNA and RNA binding proteins allowing stringent purification of the bound RNA. The iCLIP and tRIP libraries were subjected to next-generation sequencing. We correlated the hnRNP A1/B2 binding map with hnRNP A1/B2-regulated exons identified by hnRNP A1/B2 knockdown and RNA-seq to identify regulated exons and the responsible SREs. Furthermore we employed Surface Plasmon Resonance imaging (SPRi) to validate the hnRNP A2/B1 binding sites discovered by iCLIP and tRIP, as well as elucidating the RNA-protein interactions by investigating the kinetics of protein association and dissociation with RNA oligonucleotides of interest.

The heterogeneous nuclear ribonucleoprotein particle (hnRNP) A2/B1 has been widely associated with human disease, including several cancers and amyotrophic lateral sclerosis (ALS). Our project may therefore generate new knowledge, which can be important for development of new treatments for these severe diseases.

639 Identification of protein components in *Trypanosoma cruzi* spliceosome

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Trypanosomes are unicellular parasites with high economical and medical importance, causing disease in more than 20 million people around the world. *Trypanosoma cruzi* is the causative agent of Chagas disease, for which there's still no cure or vaccine. These parasites lack usual transcriptional control as observed in other eukaryotes. Genomes are composed of polycistronic units and mRNAs are individualized and processed by insertion of a capped spliced-leader (SL) RNA in their 5' end by a *trans*-splicing reaction. Trans-splicing is mediated by the spliceossome, a large and dynamic machinery composed by five small nuclear RNA (U1, U2, U4, U5 and U6) and small nuclear ribonucleoproteins (snRNPs). Control of this process might therefore be critical to regulate gene expression and the parasite adaptation to both mammalian and arthropod hosts during its complex life cycle. We aimed at identifying *T. cruzi* spliceosome components by using tandem affinity purification of a canonical core component, SmD1; and a putative U5 specific protein, Cwc21. Analyses of co-purified proteins by mass spectrometry identified 68 proteins. Among these, we detected all Sm and LSm proteins and putative proteins specific to snRNPs. We also identified proteins with no specific annotation but harboring domains related with RNA interactions like RRM and WD-40 domains, indicating their close association to the spliceosome complex. We are currently performing functional analysis to confirm protein associations to the complex. These results will be important to define specific targets to parasite and to develop new forms of disease treatment.

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640 Characterizing the role of Prp16 in spliceosome dynamics and disease

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Splicing, the removal of introns from pre-mRNA, is catalyzed by the spliceosome through two transesterification reactions: branching and exon ligation. Both reactions are catalyzed in the spliceosome's single catalytic core, necessitating remodeling of substrate-spliceosome interactions prior to exon ligation. This remodeling is initiated by Prp16, a DEAH-box helicase whose ATPase activity is necessary for progression into the exon ligation conformation after branching. Intriguingly, some pre-mRNA substrates, such as *TER1* pre-mRNA in several yeast species, contain substitutions at the 5' splice site consensus sequence (5'SS) that terminate splicing just after branching, which is required to generate mature telomerase RNA. While Prp16's importance in the branching-to-exon ligation transition during canonical splicing is well-known, how substitutions in the 5'SS or disease-correlated mutations in Prp16 affect this transition is less understood.

Two out of three known disease-correlated mutations in DHX38, the human ortholog of Prp16, occur in the N-terminal domain. Though the N-terminal domain of Prp16 was thought to be poorly conserved, I have identified a highly-conserved motif within the N-terminal domain in which these disease-causing DHX38 mutations lie. Additionally, while the N-terminal domain is thought to be important for *S. cerevisiae* Prp16's ability to bind the spliceosome, mutations in this N-terminal motif do not impede its ability to complement a second, ATPase-defective copy of Prp16 *in vivo*. Here, I characterize the impact this conserved N-terminal motif has on Prp16 function.

Additionally, though Prp16 repositions the branch site prior to exon ligation, it is unclear whether Prp16 is also responsible for 5'SS remodeling. Structural data suggests that a heteropurine interaction, formed between position A3 of the 5'SS and residue G50 of U6 snRNA in the branching conformation, is disrupted in the exon ligation conformation, possibly to allow mutually-exclusive interactions that stabilize 3'SS binding in the exon ligation conformation. Notably, a naturally-occurring substitution (A3c), seen in *TER1* introns from yeasts that terminate splicing after branching, changes this heteropurine interaction to a Watson-Crick base pair and causes an exon ligation defect in *S. cerevisiae*. Here, I show A3c blocks exon ligation in a Prp16-independent manner, likely by slowing entry into the exon ligation conformation.

641 Investigating the Role of DDX41 in pre-messenger RNA Splicing and Leukemogenesis

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DEAD-box RNA helicases are a highly conserved family of proteins involved in nearly all aspects of RNA metabolism, including several members with crucial roles in pre-messenger RNA (pre-mRNA) splicing. Whole exome sequencing has identified *DDX41*, a member of this family, as commonly mutated in myelodysplastic syndrome (MDS) patients. MDS is a bone marrow neoplasm that results from a buildup of precursor cells, or blasts, giving rise to subsequent defects in the myeloid lineages. Germline frameshift *DDX41* mutations have been identified in numerous families with histories of MDS, potentially causing *DDX41* hemizygosity at birth. Interestingly, approximately half of the individuals with *DDX41* germline mutations will acquire a recurrent, somatic, missense mutation, resulting in the conversion of amino acid arginine 525 to histidine (R525H) in the highly conserved helicase domain of the second *DDX41* allele. Biochemical assays in vitro with recombinant DDX41^{R525H} displayed slightly tighter binding of double stranded RNA and a 3-fold decrease in RNA duplex unwinding compared with DDX41^{WT} protein; this suggests a hypomorphic function. DDX41 co-immunoprecipitation experiments coupled with mass spectrometry have identified that many known proteins involved in the catalytic spliceosomal complex associate with DDX41. Previous work suggested that DDX41 was a component of the catalytic spliceosome. Analysis of RNA cross-linking-immunoprecipitation-high-throughput-sequencing (CLIP-seq) data demonstrated that DDX41 binds preferentially to exons and splice sites of pre-mRNAs. Furthermore, DDX41 binds to both major and minor class spliceosomal snRNAs. RNA-seq analyses of DDX41^{WT} and DDX41^{R525H} over-expression or DDX41 knockdown human cells have revealed subtle-yet global-effects on pre-mRNA splicing. These data support our hypothesis that DDX41 is a component of the spliceosome and plays an important role in pre-mRNA splicing. Mutations and/or deletions of *DDX41* may result in the aberrant splicing of key leukemic drivers, such as tumor suppressor genes and/or proto-oncogenes; in turn, this results in leukemogenesis of the myeloid lineage.

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642 Structural and Functional Characterization of the Spliceosomal Protein Prpf39

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Splicing is an essential step in RNA processing. It is catalyzed by the spliceosome which consists of five core components, the U1, U2, U4, U5 and U6 snRNPs. The spliceosome is assembled in a stepwise manner and must accurately recognize each splice site as a single mistake can result in the production of a nonfunctional protein. This complex process needs to be finely regulated and to this purpose many additional proteins are involved.

Prpf39 is a largely unstudied protein that came to our attention because it is alternatively spliced in a differential manner in murine naive vs memory T-cells and it has been proven to be essential in cell lines tested. Earlier studies in yeast have shown that Prp39 forms a heterodimer with Prp42 which is associated to the U1snRNP. Stable association of the U1snRNP to the pre-mRNA could only be shown in the presence of Prp39.

The objective of this project was to analyze mammalian Prpf39 on both a structural and functional level to further increase understanding of how this protein is involved in the splicing cycle. Interestingly, outside of the yeast system there is no Prp42 homolog which could act as a dimerization partner leading to the question of how Prpf39 is associated with the spliceosome in higher eukaryotes. Furthermore, understanding the functionality of Prpf39 could help us understand its connection to T-cell differentiation states.

Here we present the crystal structure of murine Prfp39 at 3.3 Å resolution. The protein is largely α-helical and the structure shows the protein to be organized as a dimer with three distinct subdomains. The dimerization is also observed in solution with SEC-MALS and the mode of dimerization shows significant similarity to the heterodimerization of Prp39 and Prp42 in yeast. The dimerization interface shows a high conservation within higher eukaryotes in its residues implying a functional relevance for the Pprf39 homodimerization. Based on this, structurally guided point-mutations were made to disrupt the dimer. The mutation of a single residues of Prpf39 is enough to completely abolish dimerization giving us a basis for further functional studies exploring the impact of Prpf39 dimerization on splicing.

643 Using Rapid Nuclear Depletion of Srp2 in *Schizosaccharomyces pombe* with Targeted Sequencing of Splice Junctions from Nascent RNA to Better Understand the Role of SR Proteins in Splicing Regulation

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While it is generally understood that members of the highly-conserved Splicing Regulator (SR) protein family modulate the capacity of the spliceosome to assemble on distinct splice sites by binding to mRNA sequences and directing interactions with the spliceosomal machinery, a detailed understanding of their mechanism(s) of action remains elusive. Although genome-wide views of the binding locations for many SR proteins have been ascertained through RNA-binding assays, such techniques fall short at distinguishing between binding events that are essential for efficient splicing from those reflecting spurious interactions and/or roles in processes outside of splicing. Moreover, the sometimes-redundant function of the 12 SR protein family members in mammals can conceal molecular phenotypes arising from targeted SR protein manipulation, complicating the interpretation of experiments designed to understand mechanisms of protein function.

To better characterize and understand the role of SR proteins in spliceosomal assembly and activation we have turned to the fission yeast, *Schizosaccharomyces pombe*, an organism which exhibits a splice site degeneracy similar to higher eukaryotes, and for which mammalian-like alternative splicing has been demonstrated, yet which encodes just a single, essential, *bona fide* SR protein, Srp2. I have constructed an inducible system using the 'anchor-away' approach that allows for the rapid depletion of Srp2 from the nucleus. By combining protein sequestration with rapid metabolic labeling via 4-thiouracil, I have isolated a time-resolved set of nascent RNAs which were synthesized in an environment lacking Srp2. Using a targeted RNA sequencing approach, I have monitored the genome-wide splicing status of all transcripts, enabling the identification of those events which are dependent upon Srp2 for accurate and efficient splicing. Sequences that are enriched in sensitized transcripts are being identified and tested via transplantation experiments to determine their necessity and/or sufficiency for conferring Srp2-dependency, proving a framework for a more detailed mechanistic analysis of the role that SR proteins play in splicing regulation.

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644 Yeast to human: Yeast U1 snRNP as a model to understand human alternative splicing

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Splicing of pre-mRNA is essential for eukaryotic gene expression and is catalyzed by the spliceosome, a large protein-RNA complex composed of U1, U2, U4, U5, and U6 small nuclear ribonucleoproteins (snRNPs) and numerous non-snRNP related proteins. Despite the abundance of recent structural data, we have limited understanding of the initial intron recognition and early spliceosomal assembly. U1 snRNP is the most abundant snRNP and is critical for the recognition of the pre-mRNA 5′ splice site (ss) through base pairing with the 5′ end of U1 snRNA. Due to this role, U1 snRNP is often targeted by alternative splicing factors to assist or prevent U1 snRNP binding to the 5′ ss. In contrast to the human 250kD U1 snRNP, the Saccharomyces cerevisiae (yeast) U1 snRNP is nearly 800kD and much more complex. The yeast U1 snRNP is composed of a U1 snRNA roughly 3.5-fold larger than the human U1 snRNA and has seven additional stably associated auxiliary proteins (Luc7, Nam8, Prp39, Prp40, Prp42, Snu56, and Snu71). Most of these auxiliary proteins have human homologs which are weakly associated with the human U1 snRNP and are implicated in alternative splicing.

We have recently determined the cryo-EM structure of the yeast U1 snRNP at 3.6Å resolution. The foot-shaped yeast U1 snRNP contains a core in the "ball-and-toes" region architecturally similar to the human U1 snRNP. All auxiliary proteins are in the "arch-and-heel" region and connected to the core through the Prp42/Prp39 paralogs. We present here crystallographic and biochemical analyses of the human PrpF39 (homolog of the yeast Prp42/Prp39 paralogs) and its interaction with human U1C, TIA1, and Luc7L (the homolog of yeast Yhc1, Nam8, and Luc7, respectively). Our results suggest that the yeast U1 snRNP serves as a valuable model for how human alternative splicing factors bind and recruit U1 snRNP.

645 When the Arabidopsis *sr45-1* mutant meets environmental challenges

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The pre-mRNA splicing process is a crucial step for gene expression control and it affects many biological mechanisms. Many mature RNAs can rise from a single gene through alternative splicing known to drive proteomic diversity. Among splicing factors, Serine/Arginine-rich (SR) proteins are essential regulators of both constitutive and alternative splicing. They belong to a conserved family in eukaryotes and are characterized by a modular structure consisting of one or two RNA recognition motifs (RRMs) in the N-terminus and a C-terminal domain rich in arginine-serine dipeptides repeats (RS). The phosphorylation state of this domain is known to affect protein-protein and RNA-protein interactions, subcellular localization and nucleocytoplasmic dynamics. Our previous results showed that SR45, a unique SR protein possessing one RRM flanked by two RS domains, presents a shuttling activity through the involvement of the nuclear export XPO1. The homozygous Arabidopsis *sr45-1* null mutant is viable, but exhibits diverse phenotypic alterations, including delayed root development, late flowering, shorter siliques with fewer seeds, abnormal silique phyllotaxy, narrower leaves and petals, and unusual numbers of floral organs. In this study, we demonstrated that some *sr45-1* abnormalities are tied to alteration in metal homeostasis. Data about the characterization of the mutant are further presented.

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646 Crystallographic snapshot of the pre-catalytic state of the spliceosomal DEAH-box ATPase Prp2 in complex with RNA

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In higher eukaryotes the vast majority of non-coding intron sequences present in precursor mRNAs are excised via the spliceosome, a multi-megadalton molecular machine composed of numerous protein and RNA components. One key player during pre-mRNA splicing is the DEAH-box ATPase Prp2, which is critical for the catalytic activation. In contrast to other spliceosomal DEAH-box helicases, Prp2 seems not to function as an RNA-unwindase but rather as an RNA-dependent RNPase [1]. Recent crystal structures of the spliceosomal DEAH-box helicase Prp43 and the closely related RHA helicase MLE in complex with an ATP-analog and RNA have contributed to a better understanding of how RNA-binding and processivity might be achieved in this helicase family [2,3]. In order to shed light onto the divergent way of functioning of Prp2 we crystallized an N-terminally truncated construct of the *Chaetomium thermophilum* Prp2 in the presence of ADP-BeF_3 and a poly-U_{12} RNA. The refined structure revealed a virtually identical conformation of the helicase core compared with the ADP-BeF_3- and RNA-bound structure of Prp43 and only a minor shift of the C-terminal domains. Interestingly, the four most 3' nucleotides show an almost identical conformation in both structures, but at the subsequent position the RNA exhibits different conformations. While in Prp2 this nucleotide follows the straight RNA-backbone path of the previous four nucleotides, in Prp43 the nucleotide at this position introduces a kink in the RNA-backbone, which is stabilized by interactions of the base with residues of a nearby loop in the C-terminal domain. These residues are conserved among DEAH-box members but in Prp2. Instead, Prp2 exhibits an additional insertion in this region, which is responsible for an alternate conformation of this loop, stabilized by an extensive interaction network with surrounding residues. We postulate that this different loop conformation hinders the RNA to interact with the C-terminal domains thereby impeding effective helicase activity.

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647 Characterization of a group IIc intron with its intron encoded maturase protein

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Group II introns are self-splicing RNA elements found in the organelles of yeast and plants and in bacterial genomes. They are classified into group IIa, IIb or IIc intron based on sequence identity, tertiary interactions and host organism and have a conserved secondary-structure organization which is divided into 6 domains. Within the fourth domain resides an intron encoded protein (IEP), or maturase, which promotes splicing activity while demonstrating both reverse transcriptase and retrohoming functions as well. The intron can be removed by a branching reaction or by hydrolysis where removal of intron proceeds through two transesterification reactions. In the first step, the branchpoint adenosine within domain 6 acts as the nucleophile which attacks the 5' splice-site (water acts as the first-step nucleophile in the hydrolysis reaction). The second step proceeds where the free 5' intron-end attacks the 3' splice-site resulting in spliced exons and the release of lariat (or linear) intron. Here we report the characterization of a class IIc intron from Eubacterium rectale, a gut microbe found in humans. We have found conditions that afford optimal branching activity which include choice of pH, monovalent and divalent cation. The intron requires maturase activity for splicing by the branching pathway and it is noted that hydrolysis activity, although nominal, is not affected by the presence of the IEP. We hope these studies provide insight as to how the intron RNA and IEP co-evolved to promote efficient splicing and retrohoming activity within an isolated system.

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648 Investigating alternative RNA processing during mammalian germ cell development

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Alternative RNA processing is a critical factor in increasing transcriptome complexity and post-transcriptional gene regulation during cell differentiation and tissue development. Remarkably, more alternatively processed RNAs are expressed in spermatogenic cells compared to most whole tissues, even exceeding the brain. While it has been known that alternative RNA splicing and polyadenylation are prevalent in the testis, its regulation and biological impact remains unclear. Here, we demonstrate that both alternative splicing and alternative polyadenylation are highly regulated in a stage-specific manner during postnatal spermatogenesis.

Recently, we revealed an essential function for the tissue-restricted RNA binding protein, Ptbp2, in regulating alternative splicing during spermatogenesis. We found that Ptbp2 controls a network of genes involved in cell adhesion, migration, and polarity via exon repression. Importantly, we showed splicing regulation by Ptbp2 is necessary for cellular crosstalk during postnatal germ cell development.

To understand the importance of alternative polyadenylation during spermatogenesis, we generated polyA-seq libraries from purified male germ cells. Using this approach, we characterized stage-specific polyA site usage. Consistent with previous reports, we observed that the majority of alternative polyadenylation occurs as germ cells enter differentiation with significant 3'UTR shortening in post-meiotic cells. Interestingly, 3'UTR shortening did not correlate with expression of *trans*-acting factors or *cis*-element enrichment, suggesting against co-transcriptional regulation. To determine if these alternative isoforms are being differentially regulated at the post-transcriptional level, we are currently using polysome analysis and 3' mRNA-seq to investigate if alternative polyadenylation impacts the translational fate of mRNAs during spermatogenesis. We are also actively studying if certain alternative isoforms are preferentially degraded or exported.

Collectively, our data demonstrate the existence of an alternative splicing regulatory network essential for spermatogenesis, the splicing factor that controls it, and its importance in germ-somatic cell communication during mammalian gametogenesis. While previous studies have investigated polyA site selection and cleavage/polyadenylation machinery during spermatogenesis, our efforts will provide new insights on whether cell-type specific alternatively polyadenylated transcripts are differentially translated, stabilized, and/or exported, which have not yet been characterized.

649 Evidence Suggesting Prp22 Activates 3' Splice Sites Sequestered in RNA Secondary Structure

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Pre-mRNA splicing is an essential step in eukaryotic gene expression that requires three intronic elements: the 5' splice site (5'ss), the branch site (BS), and the 3' splice site (3'ss). Alternative usage of these sites gives rise to different mRNA isoforms which are essential for proteome plasticity, tissue-specific function, and development. In the case of 3'ss selection, cis-acting protein factors, core splicing components, and pre-mRNA secondary structure have been shown to regulate 3'ss selection. Focusing on the latter, previous studies from Plass *et al.* and Meyer *et al.* have highlighted the importance of RNA secondary structure to allow for skipping of sequestered 3'splice sites to allow for the usage of distal 3'splice sites. These findings led to the model that the effective distance rather than the gross distance from the branch site to the 3'ss is an essential component of 3'ss selection. Importantly, these findings revealed that 3' splice sites sequestered through secondary structures remain unspliced, highlighting the importance of 3'ss accessibility. Toward the understanding of 3'ss regulation by RNA secondary structure, we provide evidence suggesting the DEAH-box ATPase, Prp22, can activate sequestered 3'splice sites in an ATP-dependent manner. We showed this using *in vitro* splicing assays in which ATPase competent Prp22 or ATPase deficient Prp22 was tested against RNAs with varying degrees of secondary structure. We found that sequestered 3'ss could only be activated in the presence of wild-type Prp22 whereas ATPase deficient Prp22 was unable to activate sequestered 3' splice sites. These findings suggest the ATPase activity of Prp22 is required to activate sequestered 3'splice sites. Building on previous work from our lab and others, these data point toward a mechanism by which Prp22 pulls the substrate to disrupt the RNA secondary structure interactions thereby activating the sequestered 3'ss.

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650 Growth temperature phenotypes of chimeric yeast/human HSH155/SF3B1 genes uncover a relationship between thermo-stability and Pladienolide B-resistance in HSH155/SF3B1 function.

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We are interested in how the intron branchpoint sequence (BPS) is chosen during pre-spliceosome assembly, and how differences in BPS recognition occur in different organisms. In yeast, BPSs are highly constrained with strong base pairing to U2 snRNA, whereas in humans a wider variety of BPSs bind to the identical U2 sequence. Recent cryoEM models of pre-catalytic spliceosomes from both organisms show the branchpoint helix is held by the highly conserved SF3B1/HSH155 protein. HEAT repeats 15 and 16 of HSH155/SF3B1 play an important role in the binding of the branchpoint helix, although how degenerate human BPSs are accommodated is not clear. This region also contains the amino acid residues whose substitution leads to resistance of spliceostatin-like splicing inhibitors such as pladienolide B (PB). In humans, SF3B1 R1074H confers resistance to this class of drugs. Although yeast HSH155 also has arginine at the homologous position 743, it is naturally more resistant, suggesting that non-conserved features of this region of the protein may mediate drug-sensitivity. Alignment of SF3B1 to HSH155 revealed 20 of the 73 amino acids of HEATs 15 and 16 are not conserved. To test the function of HEAT repeats 15 and 16, we replaced yeast HSH155 HEATs 15 and 16 with the corresponding HEATs from human SF3B1, thus "humanizing" HSH155 ($HSH155^{Hum15\text{-}16}$). We made a second replacement including the PB-resistant R to H substitution ($HSH155^{Hum15\text{-}16}$ R743H). We introduced these constructs into yeast, selected for cells in which humanized HSH155 was the only source of the protein, and tested for growth at various temperatures. Both constructs support growth at 30°C. Surprisingly, $HSH155^{Hum15\text{-}16}$ yeast cells are unable to grow at 37°C, and the $HSH155^{Hum15\text{-}16}$ R743H suppresses this temperature-sensitive phenotype (in the absence of drug). This suggests that the PB-resistance mechanism and some thermal characteristics of the protein's function may hinge on the same underlying dynamics of HSH155/SF3B1. We plan to test this hypothesis by characterizing branchpoint selection and drug sensitivity using these and additional humanizing mutations in the yeast splicing apparatus.

651 The role of Npl3p in pre-mRNA splicing in yeast

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To better understand the mechanism of the 5'SS selection during pre-mRNA splicing we have carried out a genetic screen for alleles that improve splicing of 5'SS-G5a introns defective in spliceosome assembly and the first step of splicing. The screen yielded predominantly alleles in *npl3* and *mtr10*, genes that encode two functionally linked proteins. Mtr10p is a karyopherin that mediates nuclear localization of Npl3p – a primarily nuclear, shuttling SR-like mRNA binding protein implicated in many aspects of mRNA biogenesis (e.g. splicing and export).

The majority of selected *mtr10* mutations introduce stop codons, generating truncated proteins. The identified *npl3* allele carries two mutations: L219S point mutation within the RRM2 domain, and an in frame deletion of 7 (out of 15) Arg-Gly repeats within the RGG domain. The identified *mtr10* and *npl3* suppressors, as well as additional *npl3* mutants generated to test various deletions in the RGG domain, disrupt nucleocytoplasmic shuttling of Npl3p. We have also carried out screens to identify mutants in an export factor, *mex67*, that similarly improve splicing of suboptimal introns. All these mutant alleles result in decreased nuclear Npl3p levels and improved splicing of suboptimal introns.

Our data suggest that reduced levels of nuclear Npl3p delay export of unspliced pre-mRNAs to the cytoplasm (as evidenced by RNA-FISH), giving them more time to assemble spliceosomes and undergo splicing. We consider two different mechanisms to explain these results. First, destabilized Npl3-Mtr10 interactions (caused by *npl3* and *mtr10* mutants) lead to inefficient re-import of shuttling Npl3 to the nucleus. Second, defective interactions of Npl3 with the export machinery (caused by *npl3-RGG* and *mex67* mutants) lead to reduced mRNA export. Both these mechanisms may provide more time for the spliceosome assembly on suboptimal introns before their export to the cytoplasm.

Our results highlight the previously suggested similarities between Npl3 and metazoan SR proteins, and reveal a role of Npl3 in modulation of splicing fidelity.

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652 Conserved regulation of RNA processing in somatic cell reprogramming

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Along with the reorganization of epigenetic and transcriptional networks, somatic cell reprogramming brings about numerous changes at the level of RNA processing. These include the expression of specific transcript isoforms and 3' untranslated regions. A number of studies have uncovered RNA processing factors that modulate the efficiency of the reprogramming process. However, a comprehensive evaluation of the involvement of RNA processing factors in the reprogramming of somatic mammalian cells is lacking.

Here, we used data from a large number of studies carried out in three mammalian species, mouse, chimpanzee and human, to uncover consistent changes in gene expression upon reprogramming of somatic cells. We found that RNA processing factors undergo a modest, but general upregulation in induced pluripotent stem cells compared to parental cells. A core set of nine splicing factors have consistent changes across the majority of data sets in all three species. Most striking among these are ESRP1 and ESRP2, which accelerate and enhance the efficiency of somatic cell reprogramming by promoting isoform expression changes associated with mesenchymal-to-epithelial transition. We further identify genes and processes in which splicing changes are observed in both human and mouse. Our results provide a general resource for gene expression and splicing changes that take place during somatic cell reprogramming. Furthermore, they support the concept that splicing factors with evolutionarily conserved, cell type-specific expression can modulate the efficiency of the process by reinforcing intermediate states resembling the cell types in which these factors are normally expressed.

653 New Insights into Spliceosome Activation using CoSMoS

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The spliceosome is an extremely complex and highly dynamic molecular machine. It carries out the pre-mRNA splicing process with the coordinated action of five small nuclear ribonucleoprotein particles (snRNPs): U1, U2, U4, U5 and U6. Each snRNP contains a U-rich RNA and several snRNP-specific proteins. The splicing cycle proceeds through a series of intermediate complexes, E→A→**B**→**B^{act}**→B*→C→C*→P→ILS. snRNPs and numerous other splicing factors assemble, rearrange and/or dissociate during each step of splicing. A huge conformational change occurs in the spliceosome complex during the activation step (**B→B^{act}**) as U1 and U4 snRNPs are released. Recent high resolution cryo-EM structures of B and B^{act} complexes have suggested a possible pathway for the B to B^{act} transition [1; 2]. In this work, we examine the activation process with respect to release of the U6 snRNP LSm2-8 ring using Colocalization Single Molecule Spectroscopy (CoSMoS). LSm2-8 is a heptameric protein ring that is loaded on to the 3' end of the U6 snRNA and is a stable component of U6 snRNP, U4/U6 di-snRNP, U4/U6.U5 tri-snRNP, and the spliceosome B complex. Using CoSMoS, we observe the dissociation order of the LSm2-8 ring respect to the release of the U4 and the arrival of the other proteins. Our data suggest the presence of an intermediate spliceosome complex between B and B^{act} that contains the LSm ring but not the U4 snRNP. This observation signifies an extended role of the LSm2-8 ring in the spliceosome activation.

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654 Antisense oligonucleotides correct the familial dysautonomia splicing defect in IKBKAP transgenic mice.

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Familial dysautonomia (FD) is a rare inherited neurodegenerative disorder caused by a point mutation in the IKBKAP gene that results in defective splicing of its pre-mRNA. The mutation weakens the 5' splice site of exon 20, causing this exon to be skipped and changing the translational reading frame, thus introducing a premature termination codon. Though detailed pathogenesis mechanisms are not yet clear, it may be possible to treat FD by correcting the splicing defect in the relevant tissue(s), which would thus express normal levels of the full-length IKAP protein. Using a two-step screen with antisense oligonucleotides (ASOs) complementary to sequences within exon 20 or the adjoining intronic regions, we identified a lead ASO that fully restored exon 20 splicing in patient fibroblasts. Also, we characterized the corresponding cis-acting regulatory sequences controlling exon 20 splicing. When administered into transgenic mice with a mutant human IKBKAP allele, the ASO promoted expression of full-length IKBKAP mRNA and IKAP protein levels in several tissues tested, including the central nervous system. These findings provide insights into the mechanisms of IKBKAP exon 20 recognition, and pave the way for the clinical development of a targeted therapy for FD.

655 NOVA2-mediated regulation of circRNAs during mammalian neurogenesis

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Circular RNAs (circRNAs) are an abundant, recently appreciated class of RNAs with largely uncharacterized functions. CircRNAs typically emanate from protein-coding genes and are formed by backsplicing, which involves a downstream splice donor of a circularizing exon joining an upstream splice acceptor. Interestingly, circRNAs are enriched in fly, mouse, and human brain tissue. In addition, circRNAs are upregulated during *in vitro* neural differentiation and *in vivo* synaptic maturation. However, the factors underlying the regulation of neural-expressed circRNAs remain unknown, and only a handful of RNA binding proteins to date have been shown to regulate circRNA expression. NOVA2 is a neural-enriched RNA binding protein with well-characterized roles in alternative splicing and alternative polyadenylation. To elucidate the potential role of NOVA2 in regulation of circRNA biogenesis, we analyzed publicly available total RNA-seq data from embryonic *Nova2-null* mouse cortex (Elife, e14371, 2016) using the CIRI2 algorithm. Our analysis uncovered a global bias toward the downregulation of circRNAs in NOVA2-deficient mouse cortex vs. wild-type samples. In total, we identified 54 and 22 circRNAs that were significantly down or upregulated, respectively, in NOVA2-deficient mice (fold change > ±2). We further probed this trend by knocking down *Nova2* in the immortalized mouse neuroblastoma cell line, N2a, and in primary cortical neurons using shRNA knockdown and quantifying circRNA abundance. Together, our findings suggest that NOVA2 might serve as a regulator of circRNA biogenesis in the brain and likely contributes to their regulation during neurogenesis.

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656 Knock out of human variant U2 snRNAs affects alternative splicing

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snRNAs are essential components of the spliceosome, which catalyzes the removal of introns from nascent pre-mRNA transcripts. The spliceosome comprises five small nuclear RNAs (U1, U2, U4, U5, and U6 snRNAs), each of which binds a specific set of core proteins to form stable small nuclear ribonucleoproteins (snRNPs). In metazoans, many pre-mRNA transcripts are alternatively spliced, increasing the diversity of mRNAs and number of functional splice sites. The proper identification of splice sites is cell-type specific and developmentally regulated. Although base pairing between snRNAs and the pre-mRNA substrate is important for splice site identification, positioning the substrates in the active site, and coordinating catalytic magnesium ions, it is currently unclear how spliceosomes accommodate the high variability of substrates found in vertebrates. Whereas snRNAs are known to be highly and ubiquitously expressed (10^5-10^6 molecules/nucleus), their expression and regulation are not well understood.

Early characterization of snRNAs showed sequence heterogeneity of vertebrate snRNAs, and it was suggested that expression of snRNA variants could regulate alternative splicing. Since sequencing of the human genome, the repetitive nature of snRNA gene loci has become evident. However, almost all current RNA-Seq protocols actively select against the sequencing and alignment of snRNAs. Therefore, the diversity of variant snRNAs expressed has been inadequately addressed.

We have discovered previously undescribed U2 snRNA variants that are expressed and assembled into snRNPs. Spliceosomes assembled with variant U2 snRNAs are expected to have altered affinity for splice sites based on nucleotide variation in sequences that form Watson-Crick base pairs with the pre-mRNA substrate. These U2 snRNA variants are differentially expressed in hematopoietic cell lines suggesting variant U2 snRNAs contribute to cell-type specific alternative splicing. Additionally, these snRNAs have altered snRNP assembly kinetics, likely due to sub-optimal spliceosomal protein binding sequences. We have utilized CRISPR/Cas9 to generate cell lines with single U2 snRNA variants knocked out. RNA sequencing of these cell lines has identified introns sensitive to modulation of U2 snRNA variants. The expression and regulation of U2 variant spliceosomes may help explain changes in splicing that take place during development and disease that are currently not understood.

657 A novel multi-domain protein CCAR-1 regulates transcription and co-transcriptional splicing in *C. elegans*.

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Accumulating evidence indicates that pre-mRNAs are co-transcriptionally spliced. However, mechanisms or factors affecting the fidelity of co-transcriptional splicing in living organisms are yet to be determined. Here, we report a novel protein CCAR-1, which regulates transcription and alternative splicing.

Fourteen *ccar-1* alleles were isolated in a screen for mutants defective in neuron-specific selection of exon 7a of an *unc-32* splicing reporter. Unexpectedly, double- and triple-inclusion of mutually exclusive exons *unc-32* exon 4a/4b/4c occurred in the *ccar-1* mutant. mRNAseq analysis revealed tens of alternative exons regulated by CCAR-1. Among such target exons was *ccar-1* exon 5, indicating that CCAR-1 negatively regulates its own expression at the pre-mRNA splicing level like many other typical splicing factors. The intron downstream from *ccar-1* exon 5 begins with evolutionarily conserved GA sequence instead of GT, and exon 5 was no longer skipped even in the wild-type worms when the GA site was changed into GT or GC in the splicing reporters. Nuclear run-on analysis revealed that co-transcriptional excision of *ccar-1* intron 4 and intron 5 was facilitated in the *ccar-1* mutant. These results indicate that CCAR-1 represses co-transcriptional splicing of exons with weak splice sites, which is critical for proper regulation of alternative splicing.

mRNAseq also suggested that CCAR-1 represses abundance of its own mRNA, which was confirmed by upregulation of a *ccar-1* transcriptional fusion reporter in the *ccar-1* mutants. Chromatin immunoprecipitation (ChIP)-qPCR analyses of the wild-type and *ccar-1* mutant strains suggested that CCAR-1 is associated with its own promoter region to pause RNA polymerase II (Pol II) in the wild type. ChIP-seq analysis revealed that CCAR-1 is also associated with gene bodies of expressed genes like CTD-phosphorylated RNA Pol II, which led us to find that CCAR-1 and Pol II can be co-immunoprecipitated.

These results indicate that CCAR-1 is a novel class of factors that associates with Pol II and represses transcription of its own pre-mRNA as well as co-transcriptional splicing of exons with weak splice sites to affect alternative splicing events in a variety of genes.

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658 Esrp1 splicing regulation is required for tight junction integrity through epithelial Arhgef11 isoforms that activate RhoA and myosin phosphorylation

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The epithelial-specific splicing regulators Esrp1 and Esrp2 are required for mammalian development, including the establishment of epidermal barrier functions. Esrp ablation leads to a disruption of epithelial tight junction (TJ) integrity in vivo and in vitro, indicating that alterations in TJs underlie epidermal permeability defects in Esrp KO mice. TJ located at stratum granulosm regulates the passage of water and other molecules between epithelial cells and integrity of TJ is a biophysical parameter of skin diseases such as atopic dermatitis or psoriasis. One example of a splicing change in Esrp KO epidermis occurs in the Rho GTP exchange factor Arhgef11, which maintains tight junctions via RhoA activation and myosin light chain (MLC) phosphorylation. Esrp1/2 ablation inhibits MLC phosphorylation and expression of the epithelial, but not mesenchymal, the isoform of Arhgef11 rescued MLC phosphorylation in Esrp KO epithelial cells. The Esrps induce skipping of a C-terminal exon in Arhgef11 transcripts that is required for binding of p21-activated kinase 4 (PAK4), which inhibits RhoA activation. These results illustrate how cell type-specific splicing can be functionally and mechanistically linked to disease related phenotypes that are associated with impaired functions of splicing regulators.

659 Dissecting the mechanism of H3K36 methylation in regulating RNA splicing

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Pre-mRNA splicing is catalyzed by the spliceosome, a large RNA-protein complex composed of five snRNPs and many associated protein cofactors. In the nucleus, pre-mRNA splicing is tightly coordinated with transcription. Spliceosome assembly and the subsequent catalytic steps take place in the context of a dynamic chromatin environment. Posttranslational modifications of histones play an important role in regulating chromatin state. However, the precise role of histone modifications in regulating pre-mRNA splicing have remained unclear. Histone H3K36 methylation (H3K36me) is a well-studied mark found in the body of actively transcribed genes, making it a strong candidate for the regulation of RNA processing. Here, we investigate the role of the histone methyltransferase Set2 and its associated chromatin mark, H3K36me, in determining splicing outcomes. Through high-throughput RNA-sequencing, we identify transcripts whose splicing is strongly dependent on the presence of Set2. Moreover, these same genes show defective splicing when the H3K36 residue is mutated to prevent its methylation. To understand the mechanism by which this histone modification affects splicing, we have analyzed molecular events downstream of H3K36me, and have identified a histone binding protein as a mediator of the interaction between the splicing machinery and H3K36 methylation.

660 Withdrawn

661 Cryo-EM structure of the yeast prespliceosome

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The spliceosome catalyzes the excision of introns from pre-mRNA in two consecutive steps, branching and exon ligation, and is assembled from five small nuclear ribonucleoprotein particles (snRNPs; U1, U2, U4, U5, U6) and numerous non-snRNP factors. In the beginning of the pre-mRNA splicing, the U1 and U2 snRNPs recognizes the 5' splice site (5'SS) and the branch point sequence (BPS) to form the prespliceosome. The U4/U6.U5 tri-snRNP subsequently joins the prespliceosome to form the complete pre-catalytic spliceosome. In the poster, we report the cryo-electron microscopy structure of the yeast *Saccharomyces cerevisiae* prespliceosome at near-atomic resolution. In the prespliceosome, the U1 snRNP associates with the U2 snRNP through two small contacts, leaving its tri-snRNP-binding interface fully exposed. The structure also reveals an induced stabilization of the 5'SS in the U1 snRNP. The results suggest mechanisms for 5'SS transfer to the U6 ACAGAGA region within the assembled spliceosome and for its subsequent conversion to the activation-competent B complex spliceosome. The data provide a working model to investigate the early steps of spliceosome assembly.

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662 Coupled Yeast transcription and splicing *in vitro*

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The eukaryotic gene expression pathway encompasses transcription, mRNA processing, export of the mature mRNAs to the cytoplasm, translation, and mRNA turnover. In the past few decades, these processes were studied extensively but independently. It was not until recently that different steps of the gene expression pathway are known to impact on each other. Detailed mechanistic dissection of such a "coupling" process, however, proved to be challenging, owing to a lack of a robust *in vitro* system that can recapitulate the interconnecting events. Since then, several *in vitro* systems using HeLa cell extract were reported. Glaringly missing on this experimental landscape is a corresponding *in vitro* system from the budding yeast, arguably the most versatile model organism that affords a synergistic approach of combined genetics and biochemistry. Here we report our effort in establishing a DNA-based *in vitro* system using yeast exact that can perform transcription and splicing efficiently. Using intron-containing DNA templates driven by either *TDH3* or *ACT1* promoter, we showed that the newly transcribed pre-mRNAs can be observed within 5 min and the spliced mRNAs can be detected within 10 min. In addition, this system recapitulates the splicing phenotypes caused by *cis*-mutations reported previously. Most importantly, we showed that the nascent RNAs were predominantly associated with the affinity-purified DNA templates, suggesting that splicing is co-transcriptional in our robust system. Taken together, we have developed a robust and reproducible *in vitro* system that can conduct transcription and splicing in a "one-pot" reaction, which will allow us to determine the key factors responsible for functional coupling of transcription and splicing in the near future.

663 Estimating the Genome-wide Prevalence of SF3A1-dependent Splice Site Pairing

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During splicing of pre-mRNA, the 5' and 3' splice sites are brought within proximity by cumulative interactions between the U1 and U2 snRNPs which is followed by the recruitment of the tri-snRNP to assemble the mature spliceosome required for intron removal and exon ligation. These splice site pairing interactions must occur with a high degree of specificity to produce functional mRNAs; their modulation is therefore also a target for regulation of alternative splicing. Previously, we identified an interaction between the stem-loop 4 (SL4) of U1 snRNA and the U2 snRNP specific protein SF3A1 that is critical for splice-site pairing (Sharma et al., Genes and Dev. (2014) **28**:2518-31). This contact was found to be ATP-dependent and occurred during the E to A complex transition in early steps of spliceosome assembly. Further studies have shown that the stem-loop 3 of U1 snRNA also plays an important role by recruiting the DExD/H-box helicase UAP56 that mediates the interaction between SF3A1 and U1-SL4. However, the genome-wide prevalence of U1 snRNA-SF3A1 interaction-dependent introns is still not known and, how UAP56 may regulate this interaction to produce alternatively spliced transcripts is also not known. We are using a combination siRNA mediated knock-down of SF3A1/UAP56 and RNA-seq analysis to estimate the fraction of introns that are paired by SF3A1-U1 snRNA dependent mechanism. The data obtained will reveal how extensively this network of interactions regulates constitutive and alternative splicing of pre-mRNA during generation of the cellular transcriptome.

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664 Roles of Fission Yeast SR Protein Phosphorylation During Stress

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The essential process of pre-mRNA splicing is carried out by the spliceosome, which is composed of 5 snRNPs as well as many non-snRNP proteins. In addition, splicing can be influenced by post-translational modification of spliceosomal proteins. Using a chemical-genetic approach, it was determined previously that the conserved SR protein kinase Dsk1 (Srpk1 in humans) in the fission yeast *Schizosaccharomyces pombe* is required for the efficient splicing of ~40% of the ~5,000 total introns. In addition, systematic identification of direct Dsk1 substrates through analog sensitive kinase mutation revealed many core splicing factors as substrates of Dsk1. Preventing phosphorylation in these Dsk1 substrates under permissive conditions identified a minor splicing defect with the two SR proteins in fission yeast Srp1 and Srp2. Given the importance of SR proteins in humans for regulated splicing, we are focusing on how SR protein phosphorylation by Dsk1 is differentially required under a variety of stress conditions. Initial work has been focused on screening stress conditions with SR protein phosphorylation mutants to analyze growth defects using oxidative stress induced by H_2O_2. A global transcriptional response to a variety of stresses, including H_2O_2 has been identified previously in fission yeast and we are analyzing if SR protein phosphorylation could be more important for constitutive splicing of induced mRNA during periods of stress. Other well-established stress conditions such as DNA damage, osmotic stress and temperature stress will follow in our analysis. With only two SR proteins and predominantly constitutive splicing in fission yeast, we feel this will allow us to more firmly establish how phosphorylation of SR proteins is differentially required for splicing in suboptimal stress conditions.

665 Yeast protein Cwc2 and the N-terminal domain of Prp8 stabilize the spliceosomal catalytic center in the first-step conformation.

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Prp8 forms a number of important interactions with the spliceosomal components of the catalytic center and encloses the snRNA catalytic core, yet its impact on structural rearrangements between the catalytic steps is poorly understood. Mutant allele *prp16-302* is defective in the transition between the first-and-second steps of splicing. We previously identified a class of *prp8* alleles that improve second-step catalysis, however, none of them suppressed *prp16-302* defects. We have now performed a genetic screen for *prp8* alleles that suppress cold-sensitivity of *prp16-302*. We identified two clusters of *prp8* mutations. The first one is located in proximity of U6-ISL and the 5' splice site in complex C, whereas the second one is located at the interface of Prp8, Cwc2, and U6 (pos. 41-43). Together, these results suggest that destabilization of contacts between U6, Prp8, and Cwc2 promotes transition out of the first step and thereby suppresses *prp16-302* defects.

Independently, we have also identified *cwc2* alleles that suppress *prp16-302* defects. Among these, we found *cwc2* mutations (pos. 37,38) that juxtapose *prp8* mutations (pos. 589-590) in the spliceosome structure (complex C). Since all of these alleles suppress *prp16* defects, we conclude that disruption of Cwc2-Prp8 contacts facilitates the first-to-second step transition, and Prp8 and Cwc2 positions identified in this study participate in stabilizing catalytic interactions.

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666 U2AF is replaced by SPF45 as general splicing factor to recognize weak pyrimidine tract and promotes splicing of human short introns

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There is remarkable pattern in the length distribution of eukaryotic pre-mRNA introns; i.e., a narrow distribution peaking around ~100 nt (short introns) and a broad distribution peaking around several thousand, extending to over a million (long introns). Previously, intron-definition and exon-definition models have been proposed for the splicing mechanism of short and long introns, respectively. Recent global transcriptome analysis demonstrated a multi-step process, such as recursive splicing and nested splicing, for splicing mechanism of huge introns in fly and human genes. However, we do not know much about the differences of the general splicing factors that are selectively involved in short introns and long introns. In fact, established general splicing factors have been identified with model pre-mRNAs with single short introns, which are spliced efficiently in vitro and in cellulo.

To approach this problem, we searched for the general splicing factor that is specifically involved in human short introns. We screened siRNA library targeting 158 kinds of human nuclear proteins based on splicing activity of a model pre-mRNA including 56-nt intron; i.e., intron 7 of HNRNPH1 gene. We identified a known alternative splicing regulator SPF45 (also known as RBM17) as a general splicing factor that is essential for splicing of this HNRNPH1 pre-mRNA with 56-nt intron. Our whole transcriptome analysis in SPF45 deficient cells revealed that SPF45 plays a critical role in the efficient splicing of the vast majority of short introns. Intriguingly, our biochemical experiments in vitro showed that SPF45 replaces U2AF to interact with the U2 snRNP protein SF3b155 for the recognition of short or weak pyrimidine tract.

We conclude that SPF45 is used, instead of U2AF, to splice out a large set of short introns in human pre-mRNAs.

667 RBPMS: A Master Regulator Of The Differentiated Smooth Muscle Cell Alternative Splicing Program

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It has been suggested that a small number of "master" splicing regulators might be responsible for establishing robust cell-type specific transcriptomes and that these master regulators might be identified as RNA binding proteins whose genes are associated with transcriptional superenhancers[1]. Using this approach, we identified RBPMS as a potential vascular smooth muscle cell (VSMC) master regulator. RBPMS is highly expressed in differentiated contractile VSMCs but its levels decrease in dedifferentiated phenotypically modulated cells *in vivo* and *in vitro*. RBPMS is predominantly nuclear in VSMCs and exists in several mRNA isoforms, and two major protein isoforms, RBPMS-A and B, which vary in their extreme C-termini (20 and 43 amino acids respectively). We carried out mRNA-Seq after RBPMS knockdown in differentiated PAC1 cells and after inducible RBPMS-A overexpression in dedifferentiated PAC1 cells. The splicing patterns of many genes important for SMC function were altered and in every case RBPMS promoted the differentiated splicing pattern. Affected exons showed enrichment of optimal CAC binding motifs for RBPMS, with a positional bias depending upon whether RBPMS acts as an activator or repressor. We found that the two major RBPMS isoforms have differential activity. RBPMSA can activate or repress splicing of different target exons, whereas RBPMS-B only activates. Structure-function analysis reveals that RBPMS requires RNA binding, dimerization and the RBPMS-A specific C-terminus for maximal repression, whereas the RBPMSB C-terminus antagonizes repressor activity. Mutagenesis of minigene constructs confirms that CAC motifs are essential to mediate splicing regulation by RBPMS. Two Thr residues of RBPMS adjacent to its RRM domain can be phosphoryated. We find that mutation of these residues to the phosphomimetic glutamate differentially modulates RBPMS activity, inhibiting splicing repression by RBPMS-A and splicing activation by RBPMS-B. Finally, consistent with its designation as a master regulator, we find that RBPMS directly promotes VSMC-specific splicing of the transcription factor Myocardin by activating exon 2a inclusion, which differentiates SMC and cardiac isoforms and promotes the contractile VSMC phenotype. In summary, we have identified RBPMS as a potential master regulator of the differentiated VSMC phenotype via association of its gene with superenhancers.

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668 Determining the Function of Yeast Ecm2 and its Role in Splicing

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While the functions of many spliceosome proteins are known, there still remain some components, like ECM2, whose role in splicing is unclear. Cryo-EM structures of the spliceosome show that ECM2 contacts the U2 and U6 snRNAs, two of the catalytically key molecules of the spliceosome. While all yeast spliceosome structures solved to date which contain ECM2 also show that the N-terminal RNA-binding domain contacts U6, the C-terminal domain's contact with U2 stem IIb appears to be transient and has only been observed in a structure captured just after 5' splice site cleavage. In addition, ECM2 either directly contacts or is close-by a number of other key proteins including Prp8 and components of the Prp19-containing complex (NTC). Since ECM2 is not essential in *S. cerevisiae*, we have investigated ECM2 function by deletion of the protein and assaying for genetic interactions with mutant splicing factors. In addition, we have used ACT1-CUP1 reporter assays to study changes in splicing of consensus and non-consensus introns in ECM2 deletion strains. Our results reveal a dramatic impact of ECM2 on the chemical steps in splicing, particularly with certain introns harboring nonconsensus 5' splice sites. In particular, some ACT1-CUP1 transcripts containing nonconsensus 5' splice sites improve their splicing when ECM2 is deleted when tested by a sensitive copper growth assay. ECM2 also shows strong genetic interactions with U2 stem II mutations that disrupt IIa à IIc transitions, indicating that both ECM2 participates in this process and that IIa must be destabilized for efficient splicing. ECM2 deletion additionally exhibits an array of genetic interactions with mutations in Prp8, Prp16, Prp22, and U6 that are known to perturb either the chemical steps in splicing or transitions between those steps. Together these results reveal a role for ECM2 in helping structure the spliceosome active site for catalysis.

669 Prp19 is an autoinhibited ubiquitin ligase activated by stepwise assembly of three splicing factors

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Human nineteen complex (NTC) acts as a multimeric E3 ubiquitin ligase in pre-mRNA splicing and DNA repair. The transfer of ubiquitin is mediated by Prp19 – a homotetrameric component of NTC, whose elongated coiled coils serve as an assembly axis for other NTC subunits. We found that Prp19 is inactive on its own and becomes active by stepwise assembly of three other NTC components onto the Prp19 tetramer. In complementation with functional assays *in vitro* and *in vivo*, the structure provides mechanistic insight into Prp19's transition from the inactive to the active state, during the assembly of NTC **(De Moura et al., Mol. Cell 2018)**. This work shows that Prp19 can exist in both inactive and active states, and establishes that association with three cofactors constitutes a specific "signature" for the active state. This finding is particularly important for a ubiquitin ligase such as Prp19, present as it is in various complexes and taking part in events associated with them. Moreover, this work unveils a novel mechanism of activation for ubiquitin ligases.

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670 Study of the effect of post-translational modifications on splicing factor Cwc24 in *Saccharomyces cerevisiae*

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The spliceosome is a very dynamic machinery that undergoes extensive conformational changes that are necessary for the catalytic reactions to take place. These conformational changes are promoted mainly by RNA helicases and post-translational modifications of splicing factors, such as reversible phosphorylation, acetylation and ubiquitination. The presence of a RING domain or of its variant, the U-box domain, is commonly related to ubiquitination. The Sacharomyces cerevisiae protein Cwc24 contains a RING domain, and was first identified in a complex with the NTC subunit Cef1, and later was characterized as a general splicing factor. Cwc24 is critical to orchestrate the protein-protein interactions at the catalytic core and to prevent a premature or unspecific 5´SS cleavage, and is released prior to the first transesterification reaction. The molecular changes responsible for binding and release of Cwc24 from the spliceosome may be its post-translational modifications. The aim of this work is to determine whether Cwc24 is a target for ubiquitination and how this post-translational modification regulates the affinity of Cwc24 for other protein factors when joining the spliceosome, or for its release from the complex. Western blot assays revealed that Cwc24 is ubiquitinated in vivo, which was later confirmed by mass spectrometry. With those techniques, we also confirmed previously described Cwc24 interactions with Prp19 and Brr2, and identified new interactions with additional splicing factors, which seem to be ubiquitination-related. In addition, we obtained Cwc24 site-specific mutants to determine the involvement of those residues in ubiquitination. Isolation of splicing sub-complexes by glycerol gradient centrifugation or by size exclusion chromatography revealed different profiles of Cwc24 ubiquitination during splicing. Our results suggest that Cwc24 is ubiquitinated and that this modification is relevant for the Cwc24 interaction with other splicing factors. This event could be the key to determine the joining and release of Cwc24 from the spliceosome.

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671 Single molecule assays resolve ATP dependent and ATP independent binding modes of yeast spliceosomal helicase Prp16

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Pre-mRNA splicing in eukaryotic organisms is catalyzed by the spliceosome, which consists of five small nuclear ribonucleoproteins (snRNPs), and a host of auxiliary proteins. The composition and structure of the spliceosome changes rapidly and drastically with each reversible step in the splicing cycle. This dynamic rearrangement of the pre-mRNA, snRNPs and other protein components plays a pivotal role in accurate detection of and catalysis at the splice sites, with single nucleotide resolution. Eight helicases in the spliceosome promote this structural remodeling by unwinding one RNA secondary structure before it can adopt a different structural conformation. Using single molecule Förster Resonance Energy Transfer (smFRET) microscopy and complementary biochemical assays, we are investigateing the helicase activity of Prp16, a DEAD/DEAH box family helicase essential for the second catalytic step of pre-mRNA splicing. We first explore the binding kinetics of Prp16 to the single stranded 3' overhang of an RNA in the presence of different ATP analogs. Our data show distinct binding preferences at various stages of ATP hydrolysis, supporting previously reported ATP dependent and independent roles of Prp16 during distinct stages of splicing. Furthermore, we show that Prp16 unwinds RNA-RNA and RNA-DNA duplexes in an ATP dependent manner. At relatively low ATP concentration (< 1 mM), Prp16 cannot completely resolve an 18-base pair (bp) duplex. As a result, repetitive unzipping and reannealing is observed with a cycle time of ~5 sec and unfolding rates of about 3-4 bp/sec. In contrast, at higher ATP concentration Prp16 resolves the 18 bp duplex completely. Different binding preferences at distinct stages of ATP hydrolysis are consistent with a previously reported ATP independent role in the first catalytic step, in addition to the known ATP dependent role in the second step of splicing. Furthermore, the dynamic unzipping of the RNA under ATP deficient conditions makes PRP16 unique for proofreading and branchpoint positioning during the second step of splicing. Taken together, our results support a dual function of Prp16 in both catalytic steps of pre-mRNA splicing and provide a detailed mechanistic framework for the binding and unwinding of RNA structures in the activated spliceosome.

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672 Co-transcriptional splicing during erythroid development

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Splicing of pre-mRNAs occurs largely co-transcriptionally. Previously, we have developed single molecule nascent RNA sequencing methods – including single molecule intron tracking (SMIT) and long read sequencing of nascent RNA – to study co-transcriptional splicing in budding and fission yeasts[1]. Using both methods, we were able to get an estimate of the kinetics of single intron removal in both yeasts by relating the 3'end of nascent RNA (position of RNA Polymerase II) to the progress of the splicing reaction. In comparison to yeast, mammalian genes are much more complex – containing on average eight long introns surrounded by short exons. I am adapting SMIT to monitor splicing kinetics in human cells. To initiate studies in mammalian cells, I am using the ß-globin gene as a model gene. The simple gene architecture similar to the previously studied yeast genes and low intron number (two) make ß-globin an ideal candidate. ß-globin mRNA is also abundant during erythroid cell development and can be isolated under endogenous conditions in large amounts for nascent RNA manipulation. I will measure the *in vivo* kinetics of human ß-globin splicing in erythroblasts and in a mouse erythroleukemia (MEL) cell model system transduced with human ß-globin genes. Defects in ß-globin splicing result in ß-thalassemias, allowing us to investigate the effect of thalassemia mutations on kinetics. I will show that erythroid precursor cells are an ideal system to define the kinetics of mammalian co-transcriptional splicing.

1. Oesterreich, F. C. *et al.* Splicing of Nascent RNA Coincides with Intron Exit from RNA Polymerase II. *Cell* **165,** 372–381 (2016).

673 Alternative splicing analysis in human monocytes and macrophages reveals MBNL1 as major regulator

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We report the detailed transcriptomic profiles of human innate myeloid cells using RNA sequencing. Monocytes migrate from blood into infected or wounded tissue to differentiate into macrophages, and control inflammation via phagocytosis or cytokine secretion. We differentiated culture primary monocytes with either GM- or M-CSF to obtain pro- or anti-inflammatory macrophages, and respectively activated them with either LPS/IFNγ or anti-inflammatory cytokines. We also treated the THP-1 monocytic cell line with PMA and similar cytokines to mimic differentiation and activation. We detected thousands of expression and alternative-splicing changes during monocyte-to-macrophage differentiation and activation, and a net increase in exon inclusion. MBNL1 knockdown phenocopies several alternative-splicing changes and strongly impairs PMA differentiation, suggesting functional defects in monocytes from Myotonic Dystrophy patients. This study provides general insights into alternative splicing in the monocyte-macrophage lineage, whose future characterization will elucidate their contribution to immune functions, which are altered in immunodeficiencies, autoimmunity, atherosclerosis and cancer.

674 Self-regulatory network of the core spliceosome

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Pre-mRNA splicing greatly contributes to eukaryotic gene regulation and with its 5 snRNPs and over 200 associated proteins, provides the substrate for its functional regulatory complexity. Strikingly, factors considered to be universally required for intron removal and involved in catalytic activation of the spliceosome can also display distinct regulatory effects. To systematically explore the functions of core splicing factors in alternative splicing, RNA-seq analyses were carried out upon the knockdown of over 300 splicing-related factors. One first insight emerging from this analysis is that expression and/or splicing of 60% of splicing-related factors is affected by the knockdown of least one of the other components of the splicing machinery, with many of the changes leading to assembly of unproductive mRNA or protein isoforms. On average, the knock down of a splicing component affects the expression or processing of 4 other splicing factors. While cross-modulation of splicing regulatory factors was previously reported, our data reveal an extensive regulatory network within the components of the core spliceosome. The U2 snRNP component SF3B1, which is the target of anti-tumor drugs and is frequently mutated in cancer, occupies a central position in this network, its knock down impacting on the expression/splicing of 60% of the other spliceosomal components. Remarkably, analysis of GTEX data indicate that the auto-regulatory mechanisms are partially preserved across tissues, suggesting important roles of core spliceosome self-regulation in determining tissue diversity. Our data imply that understanding the complexity of the splicing self-regulatory network can provide fundamental information to interpret the effects of genetic alterations or other perturbations of the splicing machinery, including those induced by drugs targeting the spliceosome.

675 Intron retention redefines post-transcriptional gene regulation in vertebrate species

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Intron retention (IR) is a unique mechanism of gene regulation. To delineate conserved features of IR in a functionally defined cell type, we analysed white blood cells from five vertebrate species spanning 430 million years of evolution.

In this study we applied an integrative approach utilising next generation sequencing, as well as bioinformatics and systems biology approaches.

We observed an anti-correlation between the number of protein-coding genes in the genome of individual species and the number of genes affected by IR, many of which are functionally related.

An intriguing observation suggests that IR competes with another mechanism of gene regulation suggesting that IR acts to enhance gene regulatory complexity.

Ultimately, we find that IR mediates fine-tuning of gene expression in vertebrates, an imbalance of which can lead to diseases such as cancer.

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676 Functional analysis of RNF113A in human splicing

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Pre-mRNA splicing is the process by which introns are removed and exons are joined to produce a mature mRNA competent for translation. It is catalyzed by the spliceosome, a macromolecular ribonucleoprotein machine, composed of 5 small nuclear ribonucleoprotein particles (snRNPs U1, U2, U4, U5 and U6) and more than 100 proteins. Spliceosome assembly is dynamic and involves RNA-RNA, RNA-protein and protein-protein interactions. Problems in splicing can be associated with tumor development and neurodegenerative diseases. RNF113A is a 39 kDa protein formed by Zinc and RING-finger domains. Previous mass spectrometry experiments reported RNF113A as especially concentrated in B and Bact intermediate spliceosome complexes and also in post-catalytic complexes. However, little is known about its role on spliceosome assembly and catalysis. In this work we analyzed RNF113A interactions within the spliceosome. Interestingly, our immunoprecipitation results show that RNF113A associates to snRNAs U4, U5 and U6 in cell lysates. Mass spectrometry data detected PRP19 as an interacting partner. Taken together, these results indicate RNF113A is important for spliceosome assembly. It is possible that abrogation of these interactions impairs splicing catalysis as well.

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677 Engineered U1 snRNPs redefine a disabled exon from distance

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Spinal muscular atrophy (SMA) is caused by deletions or mutations of *Survival Motor Neuron 1* (*SMN1*) gene. *SMN2*, a nearly identical copy of *SMN1*, cannot compensate for the loss of *SMN1* due to predominant skipping of exon 7. However, correction of *SMN2* exon 7 splicing holds the promise for SMA therapy. Intronic splicing silencer N1 (ISS-N1) located immediately downstream of the 5′ splice site (5′ss) of exon 7 constitutes one of the major regulatory elements responsible for *SMN2* exon 7 skipping and an antisense oligonucleotide (ASO) that targets ISS-N1 is currently the only approved drug for SMA. The stimulatory effect of an ISS-N1-targeting ASO is ascribed to the displacement of the inhibitory factor(s) hnRNP A1/A2 and the structural rearrangement favoring recruitment of the U1 snRNP to the 5′ss of exon 7. Similar stimulatory effects on *SMN2* exon 7 inclusion have been observed employing engineered U1 snRNPs (eU1 snRNPs): the suppressor U1 with improved complementarity to the 5′ss of exon 7 as well as the U1 snRNAs that target downstream intronic sequences. A recently reported individual patient with severe SMA showed complete skipping of *SMN1* exon 7 due to a splice site mutation (G to C substitution at the first position of intron 7, G1C) that destroyed the authentic 5′ss. Here we employed eU1 snRNPs that promoted exon 7 inclusion in the context of G1C mutation by activating a strong cryptic 5′ss (Cr1) located within ISS-N1. While suppressed in the presence of the authentic 5′ss of exon 7, the Cr1 is preferentially activated by U1 snRNPs targeting ISS-N1 as well as other sequences upstream and downstream of ISS-N1 in *SMN1* carrying G1C as well as other splicing mutation associated with different types of SMA. Our findings open up a new therapeutic avenue for patients with pathogenic splicing mutations at the invariant first intronic positions.

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678 Sam68 interaction with U1A modulates mTOR pre-mRNA splicing

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The RNA-binding protein, Src-associated in mitosis (SAM68), affects alternative splicing of transcripts that regulate key cellular processes, such as the mechanistic target of rapamycin (mTor). SAM68 binds to two AU-rich sequences in mTor intron5, to promote splicing at the upstream junction. SAM68 knockout mice had reduced mTOR at the transcriptional and protein levels due to the production of a non-translated transcript that terminates within intron5. Here, we report that SAM68 interacts with U1-small nuclear ribonucleoprotein (U1 snRNP), to promote splicing at the sub-optimal splice site of exon/intron 5 of mechanistic target of rapamycin (*mTor*). We also show for the first time that this interaction of SAM68 and U1snRNP is mediated through the RNA Recognition motif 1 of U1A - the stem loop II binding protein of U1snRNA. SAM68 binds U1A through a conserved region in its C-terminus YY motif, and deletion of this region or mutation in SAM68 binding sites in intron 5 of *mTor,* abrogates U1A recruitment and increases intron 5 retention. To address whether this mechanism may be prevalent in the genome, we performed a genome-wide motif analysis of the intronic regulatory sequences found in mTor and identified putative gene targets of SAM68. These gene sets were enriched in SAM68-related biological processes, suggesting that they may be novel targets of SAM68. Taken together, our results provide the first mechanistic study by which SAM68 modulates alternative splicing decisions at suboptimal 5’ splice sites.

679 Evidence for differential protein-protein interactions as a mechanism underlying cell type-specific regulation of alternative last exon splicing by CDK12

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CDK12 is a transcriptional CDK (cyclin-dependent kinase) that partners with Cyclin K to enhance transcription elongation through the phosphorylation of RNA Polymerase II. It has been classified as a tumor suppressor gene due to recurrent *CDK12* alterations found in several cancer types and a reported role in the regulation of DNA repair genes. Tumor mutations in *CDK12* frequently map to one of its four functional domains: an RS (arginine/serine-rich) domain, a kinase domain, and two proline-rich motifs. While it has been shown that the kinase activity of CDK12 is required to phosphorylate RNA Polymerase II, the presence of other domains suggest additional functions of CDK12 that have yet to be defined. Using mRNA sequencing, we demonstrated that CDK12 regulates a specific subtype of alternative splicing, alternative last exons (ALEs). This regulation was both gene- and cell line-specific, suggesting that CDK12 may associate with other regulatory splicing factors or respond to upstream signaling pathways. To test this hypothesis, we performed affinity purification-mass spectrometry (AP-MS) of CDK12 in multiple cell lines and with different epitope tags. In all cell lines examined, we identified a core CDK12 complex that includes Cyclin K, essential components of constitutive splicing (e.g., the Prp19 complex), and RNA processing factors (e.g., the WTAP complex). Notably, interactions between CDK12 and regulators of alternative splicing (e.g., SRSF proteins) differed depending on the cell line. By performing domain deletion analyses, we found that both the RS and kinase domains of CDK12 were required for its interaction with the Prp19 complex, whereas only the RS domain was necessary for its interaction with alternative splicing and RNA processing factors. The combination of common and differential interaction partners suggest a mechanism for how CDK12 can direct a specific subtype of alternative splicing, but in a gene- and cell line-specific manner. Together, these results provide a mechanistic model for alternative splicing regulation by CDK12.

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680 Near-atomic Structures of the Spliceosomal Complexes: Insights into the Mechanism of Pre-mRNA Splicing

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Splicing of precursor messenger RNA (pre-mRNA) is a accomplished by a dynamic mega-complex known as the spliceosome, which undergoes drastic structural rearrangements during its activation and catalysis. Using single-particle electron cryo-microscopy, we have determined the near-atomic resolution (3.4~4.0 Å) structures of spliceosomal ILS complex from *Schizosaccharomyces pombe* and the spliceosome at 5 key reaction states (B^{act}, C, C^{*}, P, ILS complexes) and a pre-assembled U4/U6.U5 tri-snRNP from *Saccharomyces cerevisiae*. These structures first clearly show the organization of protein and RNA components in the spliceosome at different stages. The spliceosome is proven to be a protein-directed metalloribozyme. The catalytic center of the spliceosome is mainly formed by small nuclear RNAs (snRNAs) whereas spliceosomal protein components play pivotal roles in maintaining active site conformation and promoting the two-step splicing reaction. Besides, at least 20 components remain conformationally unchanged throughout the splicing reaction, forming the rigid core of the spliceosome. The compositional and conformational changes of the spliceosome are crucial for the delivery of critical reaction groups on the RNA molecules into the active site at the right time for the two-step splicing reaction. These 7 structures of key spliceosomal complexes almost cover all working states of the spliceosome during the splicing reaction, revealing the molecular mechanism of pre-mRNA splicing.

681 Systematic Identification and Analysis of pre-mRNA Splicing Regulators in Saccharomyces cerevisiae

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Conventional wisdom has it that splicing in the budding yeast, unlike that in the higher eukaryotes, is constitutive, because nearly all yeast intron-containing genes contain only one intron (therefore, no alternative splicing) and exhibit a ~100% splicing efficiency. Exceptions, however, do exist for meiosis-dependent and environment-responding splicing for particular subsets of transcripts. In this context, there remains, puzzlingly, ~16% of intronic genes that are incompletely spliced even during exponential growth in rich medium, thus raising a hypothesis that meiosis is not the only biological process regulated by splicing. Aiming to test this hypothesis and to search for putative splicing regulators, we developed a high-throughput screening platform against several inefficiently spliced intronic genes. In this modified synthetic genetic array (SGA) scheme, a GFP-marked inefficiently spliced gene (e.g., KIN28) was recombined, by strain crossing, into ~4,500 haploid strains, each containing a specific gene deletion. The resulting haploid strains were then subjected to high-throughput flow cytometry for identifying gene deletions that impact on GFP signal intensity. To control for splicing-specific event, a series of parallel experiments using various GFP-marked strains were performed. These include three completely spliced genes (e.g., RPS17A, RPS17B, BMH2), an intronless gene (ERG11), and a gene containing a non-spliceosomal intron (HAC1). It is anticipated that by statistically analyzing these datasets, gene deletions that alter the intensity of GFP signal through potential splicing regulation can be identified. Preliminary analysis showed that about 40-80 candidates emerged from each set of experiment and candidates unique to inefficiently spliced gene (e.g., KIN28) could be identified. The gene functions of candidates with improved splicing efficiency validated by quantitative RT-PCR enriched in "Transcription regulation" and "Histone modification". Our data suggests a potential gene list that involved in transcription-splicing regulation. We anticipate that this approach may allow us to uncover novel signaling pathways that govern splicing regulation, a largely unexplored territory in the budding yeast.

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682 Cryo-EM shows mechanism of 3' splice site recognition

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Introns are removed from eukaryotic messenger RNA precursors by the spliceosome in two transesterification reactions - branching and exon ligation. Due to the astonishing new power of cryo-EM, there has recently been a revolution in our understanding of pre-mRNA splicing from numerous high-resolution structural snapshots of the spliceosome in action. These structures are producing a detailed picture of how an intron is recognised, how the spliceosome assembles on the intron, and how its RNA-based active site performs chemistry. Despite these new insights, several gaps remain. We have limited understanding of the exon ligation reaction, and the mechanism for recognising the 3'-end of the intron - called the 3' splice site - has been elusive despite decades of genetic and biochemical interrogation. We solved the structure of a spliceosome captured immediately after exon ligation, in which the 3' splice site is still docked in the active site. The structure shows that the universally conserved AG dinucleotide that defines the 3' splice site is recognised by non-Watson-Crick base pairing directly to the branched intron: the A pairs with the branch point adenosine and the G with the G of the 5' splice site. This observation justifies a branching mechanism for splicing, suggests tight coupling between the two chemical steps of splicing, and accounts for the strict conservation of the GU and AG dinucleotide sequences that flank an intron.

683 Conformational Dynamics of Group II Intron Splicing

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Group II introns are self-splicing ribozymes that are evolutionarily related to the eukaryotic spliceosome. Though several crystal structures of group II introns have been determined, there is limited insight into the nature of conformational dynamics during the splicing reaction. To understand the conformational dynamics of splicing, group II introns were probed using selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) to look at the flexibility of individual nucleotides. Comparing the SHAPE profiles of the pre-catalytic, intermediate, and post-catalytic states highlighted regions of the group II intron with significant conformational changes. In particular, the junction regions located between the group II intron domains have large differences relative to nucleotides within the domains. These differences, along with crystal structure data, led us to develop a model where the junction regions undergo specific rearrangements in order for the tertiary contact g-g¢ to engage in the second step of splicing. In addition, I found that the k-k¢ region experiences differences in both the first and second step. Mutagenesis of k followed by *in vitro* splicing assays demonstrated that these differences highlight important dynamics in this region. Cumulatively, these results reveal conformational dynamics that are critical for group II intron catalysis.

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684 Targeting the splice factor kinase SRPK1 in leukaemic cells

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Aberrant splicing patterns are associated with the development of cancer and so there is growing interest in targeting the machinery of splicing. The serine/arginine splice factor kinase SRPK1 phosphorylates splice factors rich in serine/arginine repeats (RS-domain) which are required for pre-mRNA splicing. Studies have previously shown that targeting SRPK1 in prostate cancer cells drastically reduces tumour growth and is a potential target for treatment (Oltean, S. 2012; Mavrou *et al*. 2015). This study was aimed at investigating the effect of inhibiting SRPK1 in leukaemic cells using SPHINX, a specific small molecule inhibitor both alone and in combination with conventional chemotherapy drugs. Chronic myeloid leukaemia (CML) and Acute myeloid leukaemic (Kasumi-1) cell line were used as models.

SPHINX decreases cell viability and growth in Kasumi-1 but not in K562 cells. Both cell lines showed an increase in caspase3/7 activities at higher concentrations of 10µM SPHINX with Kasumi-1 cells exhibiting a dose dependent response. Capase-9 altered its splicing pattern following treatment with SPHINX. When each cell line was treated with a combination of SPHINX and conventional chemotherapeutic drugs (K562s with imatinib mesylate and Kasumi-1 with azacytidine), increased cell death was observed in the case of Kasumi-1 cells treated with SPHINX and azacytidine. These results indicate that there may be potential benefit in treating some leukemic cells with an SRPK1 inhibitor.

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685 Genome-wide CRISPR-Cas9 interrogation of splicing networks reveals a mechanism for recognition of autism-misregulated neuronal microexons

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Alternative splicing has critical roles in diverse cellular, developmental and disease processes. The neural-specific splicing regulator nSR100/SRRM4 activates a large network of neuronal-differential exons that includes a highly conserved program of 3-27 nt microexons with functions in nervous system development[1,2]. We have previously shown that microexons are commonly misregulated in individuals with autism spectrum disorder, with the same subset of patients showing correlated reductions in the expression of SRRM4[2]. Furthermore, mice haploinsufficient for Srrm4 display multiple hallmark ASD-like features[3,4]. However, the molecular pathways and cofactors that converge on Srrm4 to regulate microexons have not been systematically explored.

To address this, we have developed a genome-wide CRISPR-based screening strategy for the comprehensive identification of factors that control the alternative splicing of neuronal microexons. Besides known regulators including Srrm4, Rbfox and Ptbp1, our screens captured ~200 additional genes that regulate microexon splicing. These genes are significantly enriched in RNA processing and chromatin functions, as well as in genetic links to autism. A combination of high-throughput and focused biochemical studies revealed that two screen hits, Srsf11 and Rnps1, preferentially regulate microexons by promoting the recruitment of Srrm4 to target microexons.

Our data culminate in a model for microexon recognition whereby a composite intronic enhancer element comprising C/U-repeat and UGC motif binding sites for Srsf11 and Srrm4, respectively, communicate with Rnps1 bound to exonic sequence. Our study thus presents a generalizable system for the genome-wide definition of splicing regulatory networks, and further reveals interactions required for the recognition and splicing of microexons with critical roles in nervous system development and disorders.

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686 Genetic suppressors of cold-sensitive substitutions that stabilize the U6 internal stem-loop may act on distinct splicing defects

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Pre-mRNA splicing is a prime example of protein-RNA cooperation in catalysis. U6 RNA, a central component of the spliceosome, contains an internal stem-loop (ISL) that positions catalytic magnesium ions. A conserved A-C mismatch at the base of the ISL comprises nucleotides A62 and C85 in *S. cerevisiae*. Substitutions that stabilize this pair result in cold-sensitive (cs) growth, consistent with the need to unwind the ISL for U4/U6 annealing early in spliceosome assembly. The severity of cold-sensitivity, however, does not correlate well with predicted ISL stability: a pyrimidine/purine orientation at U6-62/85 is invariably more cs than the corresponding purine/pyrimidine pair. A C-G pair at U6-62/85 (U6-CG) is lethal, yet U6-GC is less cs than U6-UA. This finding suggests that stabilizing U6-62/85 causes multiple defects. We identified spontaneous genetic suppressors of cs growth for U6-GC and U6-UA. Our study yielded one mutation expected to stabilize U4/U6 (U4-A16G) and many mutations expected to destabilize the free U6 snRNP. Of note were a C-terminal deletion in U6's binding partner Prp24 and null mutations in *LSM7*. *lsm7*Δ deletes one subunit of the Lsm2-8 ring that binds the 3' end of U6. In contrast, deletion of the other non-essential Lsm2-8 subunit, Lsm6, results in very weak or no suppression. These results agree with a reported interaction between Prp24's C-terminus and Lsm2-8 (Rader and Guthrie, 2002), and suggest Lsm7 mediates this interaction. Preliminary analyses of U4/U6 levels indicate that a pyrimidine/purine orientation at U6-62/85 exacerbates a U4/U6 assembly defect, that U4-A16G suppresses by relieving that defect, and that *lsm7*Δ primarily acts by suppressing a later, unidentified defect. These data suggest that suppression of either defect is sufficient to restore cold-resistance to U6-62/85 stabilization mutant strains, and hint that the later defect occurs during spliceosomal activation, when Lsm2-8 dissociates.

687 Caught in Action: Identification of factors sufficient for ATPase activity activation of yeast DEAD-box protein Prp28p

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The DEAD-box protein Prp28p is essential for pre-mRNA splicing since it plays a key role in the formation of an active spliceosome. Prp28p participates in the release of the U1 snRNP from the 5'-splice site during association of the U5.U4/U6 tri-snRNP, which is a crucial step in the transition from a pre-catalytic splicesome to an activated spliceosome. However, it is a long-standing issue why the purified recombinant yeast Prp28p had no detectable RNA helicase activity and poor ATPase activity. It suggests that an additional factor or factors may be required for its activation.

In order to approach this issue, the targets of Prp28p should be identified. To probe how Prp28p contacts its targets in a splicing-dependent manner, we strategically placed a UV cross-linker, benzoyl-phenylalanine (BPA), along the length of Prp28p *in vivo* using a nonsense-suppressor-mediated approach. Prp28p appears to transiently interact with the spliceosome at low ATP concentration, which is known to accumulate A2-1 (or pre-B; mammalian system) complex. Under such a condition, we observed that Prp28p cross-links with a small number of proteins, which is dependent on the presence of UV, ATP, RNA, and, importantly, functional 5'ss and branch site. We then showed that Prp28p cross-links to Prp8p, Brr2p, Snu114p, U1C, and Npl3p. The biological relevance of such interactions is supported by parallel genetic analysis. And ChIP analyses showed that elimination of the BPA site(s) on Prp28p delays the release of U1 snRNP from spliceosome. Moreover, detailed biochemical probing revealed that Prp28p directly contacts pre-mRNA at positions very close to the 5'ss. Surprisingly, we provide the first evidence that the low ATPase activity of purified Prp28p can be activated in both the presence of Npl3p and purified U1 snRNP.

Our data suggest that Prp28p approaches the complex spliceosomal milieu by making prior contact with Npl3p and U1C, thus activates the activity of Prp28p, then contacts with catalytic heart "Prp8p", Snu114p and 5'ss of pre-mRNA to effect U1 snRNP dissociation and then with Brr2p, which may transmit the signal for coupling U1 dissociation with the U4/U6 unwinding, a key step in spliceosome activation.

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688 Coordinated alternative splicing and alternative polyadenylation of *Dscam1* regulated by ELAV is essential for neural development in *Drosophila*

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In *Drosophila*, hundreds of genes express alternative mRNA isoforms that harbor extended 3′ UTRs in the nervous system. However, the functions of these extended 3′ UTR isoforms in neural development remain unclear. The *Dscam1* gene is essential for neural development, and in addition to undergoing extensive alternative splicing, it generates short (*Dscam1-S*) and long (*Dscam1-L*) 3′ UTR isoforms. We found long *Dscam1* isoforms are exclusively expressed in the nervous system while short isoforms are also expressed in non-neuronal cells, including glia and S2 cells. Specific knockdown of Dscam1-L transcripts in neurons caused severely impaired locomotion of flies and death shortly after eclosion. Whole brain development was severely impaired as evidenced by failed bifurcation of mushroom bodies and impaired branching of PDF neurons. We also generated a *Dscam1-L* mutant by CRISPR-introduced deletion of the extended 3′ UTR region, and found that it showed a similar axonal defect that could be rescued by Dscam1 overexpression. Using long-read nanopore sequencing, we found that in adult heads *Dscam1-L* was exclusively associated with the upstream removal of exon 19, which results in a particular protein-coding isoform that is essential for axon terminal outgrowth. The exclusion of exon 19 by alternative splicing was found to be also controlled by ELAV. Thus, ELAV is involved in processing at both the levels of alternative splicing and alternative polyadenylation to generate neuron-specific Dscam1 transcripts that are essential for neural development.

689 Small molecule inhibitors targeting the MALAT1 triple helix exhibit distinct interaction modes

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Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is a long non-coding RNA that is highly expressed in the nucleus and involved in many cancer-related processes including cell proliferation and migration. The 3′ end of this RNA forms a triple helix (TH) which protects the entire transcript from 3′-5′ exonucleolytic degradation pathways and supports persistent oncogenic activity lncRNA. Thus, this TH has been identified as an excellent target for drug discovery. Using a small molecule array screening, our collaborator's lab (S. Le Grice) identified several MALAT1 TH-binding compounds. We investigate the mechanisms of binding for two compounds, M5 and M16, which have been shown to exhibit favorable biological inhibition of MALAT1 in vivo. Using FRET, we show that the two compounds induce opposing structural changes in the RNA. M5 promotes triplex formation while M16 disrupts those interactions. Thermal DS-FRET analysis of M5 and M16 interactions with MALAT1 TH suggest monovalent dependence of M5-binding and a fast off rate of M16. Interestingly, ITC experiments demonstrate micromolar binding affinities for both compounds. However, the enthalpic and entropic contributions are distinct for each compound. Molecular docking studies reveal binding modes for these compounds consistent with FRET and ITC data. M5 binds deep within the major groove of the triplex while M16 preferentially binds on the RNA surface. Taken together, our data uncover divergent interaction modes yet similar affinities for these two MALAT1 triplex inhibitors in vitro. Within the biological context, M5 inhibits MALAT1 more effectively than M16, suggesting that further development of small molecule inhibitors should explore biochemical interactions that promote binding deep within the major groove.

690 Exploring Dumbbell-shaped DNA Minimal Vectors for RNA-Guided Genetic Reversal of Glucose-6-phosphate Dehydrogenase Deficiency

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Recombinant nucleic acid technology has long been used to correct or combat the effects of disease causing mutations. However, gene therapy has been hampered by low clinical efficacy and safety concerns, calling for a better control of off-target effects and an improvement in transgene delivery. Recently, the bacterial CRISPR/Cas system has been explored as highly efficient genome editing tool in mammalian including human cells. However, applications of this RNA-guided genome editing system are limited by safety concerns related to target gene specificity and the delivery vectors used. State-of-the-art delivery vectors mainly comprise viral vectors most of which are associated with safety risks or the rather cost-intensive use of preformed single-guide RNA/Cas9 ribonucleoprotein complexes which are readily degraded in living cells. We explore the use of non-integrating minimalistic dumbbell-shaped DNA vectors for the delivery and expression of the CRISPR/Cas9 system in primary human cord blood stem cells to correct the Glucose-6-phosphate dehydrogenase (G6PD) deficiency-associated *Mahidol* mutation. Unlike plasmid vectors, dumbbell vectors lack untranscribed regions and are not silenced in primary cells. Moreover, their smaller size facilitates cellular delivery and nuclear targeting. We generated an array of dumbbell vectors with different design features and developed a dual fluorescent reporter assay that allows us to easily monitor the editing activity of different vectors. Novel design features comprise (i) computationally selected single-guide RNAs, (ii) a dumbbell DNA nuclear import signal, (iii) a Cas9 mRNA nuclear export signal, and (iv) integrated dsDNA or ssDNA repair templates the latter of which can form one loop of the dumbbell vector. Our preliminary findings indicate that dumbbell vectors successfully deliver and express the CRISPR/Cas9 system and that the dumbbell-CRISPR/Cas9 hybrid technology represents a viable tool to address erythrocytic abnormalities such as glucose-6-phosphate dehydrogenase deficiency.

691 Targeting Translation Termination Machinery with Antisense Oligonucleotides for Diseases Caused by Nonsense Mutations

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One third of the genetic diseases are caused by nonsense or frameshift mutations that generate premature translation termination codons (PTCs). PTCs commonly inactivate gene function due to the production of truncated proteins. Effort to develop treatment for these diseases has focused on identification of small molecular translation read-through drugs. However, to date, no small molecule read-through drug has received final FDA approval, likely due to lack of balance in efficacy and safety. Here we initiated an effort to use antisense oligonucleotides (ASOs) to target translation termination factors to determine if ASO modulation could be a potentially effective and safe therapeutic approach for diseases caused by nonsense mutations.

To our surprise, using in vitro reporter system, we found that the depletion of translation termination factors *ETF1* or *GSPT1* in HeLa cells has minimum effect on promoting translation read-through. However, the depletion of either *ETF1* or *GSPT1* significantly improved the efficacy of small molecule read-through drug Geneticin in HeLa cells.

Safe and efficacious ASOs were developed against mouse translation termination factors *Etf1* and *Gspt1*. We found that the depletion of either *Etf1* or *Gspt1* to ~70% in mouse liver is well-tolerated. Next, we treated hemophilia mice that express a mutant human coagulation factor IX allele containing nonsense mutation R338X, with *Gspt1*-ASO. We found that although *Gspt1*-ASO only elicited a moderate read-through effect on hFIX_R338X mRNA by itself, it worked in synergy with Geneticin, significantly increased production of functional full-length hFIX protein and improved FIX-dependent coagulation activity in these mice. No significant unfolded protein responses were triggered with any of the treatments. Experiments are ongoing to further evaluate the effect of *Gspt1*-ASO on normal translation termination.

Overall our results indicate that modulating translation termination pathway in liver by antisense oligonucleotides may provide a novel approach to improve efficacy of small molecule read-through drugs to treat human genetic diseases caused by nonsense mutations.

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692 Chemically modified siRNAs targeting the RNA structure of the influenza A virus.

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The influenza A virus causes prevalent infections of the human respiratory tract. Due to rapid antigen changes and transmission it poses a serious epidemic and pandemic threat. Moreover, currently applied strategies to combat influenza, which are directed mainly against viral proteins, have remarkable limitations. Therefore, there is an urgent need to develop new approaches to viral inhibition. Recent findings have shown that viral genome consisting of 8 negative-sense RNA molecules, as well as other RNA intermediates of viral replication cycle, are a target carrying great potential. Research concerning secondary structure of viral RNA led to a discovery of many stable structural motifs. Several of them proved to fulfill an important role in viral proliferation, affecting it at distinct stages. In our studies we propose a structure-oriented design of RNA interference technology, mediated by siRNA. We implement a careful selection of target regions in segment 5 influenza virus (+)RNA, based on the secondary structure of viral RNA, predicted in bioinformatics' analyses and experimentally defined by our team. Selected regions are highly structure-conserved among type A influenza strains and especially important from the perspective of their proliferation. Besides considering some general rules concerning effectively interfering molecules in mammalian cells in siRNA design, we also introduced chemical modifications. Incorporation of phosphorothioates and 2'-fluororibonucleotides allows for improving siRNA properties. Duplexes gain increased nuclease resistance and serum stability. The designed siRNAs were tested in cell culture experiments in the MDCK cell line, and infected by influenza virus A/California/04/2009 (H1N1). Viral load was determined through real-time PCR quantitative analysis of viral genomic RNA, in a 24-hour postinfection. Modified variants of siRNA caused a substantial inhibitory effect at 8 nM concentration. The most potent duplex, containing 2'-fluororibonucleotides at specified positions, reduced the viral RNA copy number by c.a. 90%. The obtained results further support the idea that RNA secondary structure and its particular motifs are of vital importance for viral proliferation. Our new approach of target-site preselection, based on structure data may offer interesting possibilities for effective viral inhibition and understanding the impact of structural motifs on the influenza replication cycle.

693 Tumor-targeted delivery of antagomirs by in situ piggybacking on endogenous albumin

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A new class of endogenous small RNAs, microRNAs (miRNAs) play key roles in regulating fundamental cellular processes by targeting multiple genes at the posttranscriptional level. The extensive investigation of miRNAs has revealed that they are often up- or downregulated in a variety of human diseases including cancers. For specific therapeutic purposes, a particular miRNA expression can be silenced or overexpressed using antagomir- or miRNA mimic-based drugs, respectively. Unfortunately, conventional miRNA-based drugs with small molecular size (<5.5 nm) show rapid renal excretion and short blood circulation time (< 5 min). Thus, to harness their therapeutic potential, the miRNA-based drugs require successful delivery technologies. In order to overcome the clinical challenges, herein we suggest to highjack the biological pathways of endogenous albumin for tumor-targeted delivery of miR-21 antagomir. Piggybacking miR-21 antagomir onto endogenous albumin via in situ covalent attachment extended blood circulation half-life (>80 min) via renal albumin reabsorption and neonatal Fc receptor (FcRn)-mediated cellular recycling pathway. As a result, systemic administration of the activatable miR-21 antagomir could effectively silence miRNA 21-dependent gene expression in cancers by improving its tumor accumulation and cellular uptake. Therefore, the in situ antagomir-albumin conjugation strategy has great potential as platform technology for systemic delivery of miRNA antagomirs.

694 AON-induced splice-switching and *DMPK* transcript degradation as therapeutic approaches for Myotonic Dystrophy type 1 (DM1)

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Myotonic Dystrophy type 1 (DM1) is a multi-systemic genetic disease with clinical features primarily related to muscle pathology. DM1 patients carry expanded CTG repeats in the *DMPK* (*dystrophia myotonica* protein kinase) gene, which upon transcription form toxic CUG expansions (CUG^{exp}) sequestering alternative splicing regulators in nuclear foci and perturbing cellular RNA metabolism. To alleviate the burden of RNA toxicity, we designed and tested three potentially therapeutic strategies utilizing antisense oligonucleotides (AONs)-mediated splice-switching or degradation of mutated *DMPK* pre-mRNA. Specific aims involved: (1) skipping of selected constitutive exons to induce frameshift error and decay of toxic mRNAs, (2) exclusion of an alternative exon carrying CUG^{exp}, and (3) elimination of *DMPK* pre-mRNA with intronic gapmers. We investigated whether these approaches could reduce spliceopathy of DM1-related biomarkers, *DMPK* translational defect and CUG^{exp} foci accumulation in distinct DM1 cell models. While constitutive exon skipping failed to stimulate *DMPK* mRNA decay and improve DM1-realed molecular phenotypes, AON-induced exclusion of an alternative exon carrying CUG^{exp} efficiently rescued *DMPK* translational defect. However, accumulation of the CUG^{exp} containing splice by-product prevented spliceopathy correction and foci reduction. In contrast, reduction of early pre-mRNA *DMPK* transcripts by intronic gapmers demonstrated significant therapeutic potential. We also show that chemical modification of AONs backbone might adversely affect crucial splicing regulators leading to induction and aggravation of DM1-like splicing defects. Thus, a combination of the correct AON chemistry and experimental approach should be carefully considered to design a safe and long-lasting AON-based therapeutic strategy for DM1.

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695 Therapeutic potential of small molecules targeting the MALAT1 lncRNA-encoded triple helix

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Human metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also referred to as nuclear-enriched abundant transcript 2 (NEAT2), is a highly conserved lncRNA whose upregulation in non-small cell lung tumors is associated with a high propensity for metastasis. A unique structural feature of MALAT1 is a triple helix, designated the expression and nuclear enhancement element (ENE), located at its 3' terminus and responsible for accumulation of MALAT1 in the nucleus. Utilizing a microarray approach with ~26,000 immobilized small molecules, we identified several chemotypes that recognized the ENE triple helix. Two of these, designated compounds **5** and **16**, were shown to reduce MALAT1-associated branching morphogenesis in a mammary tumor organoid model. While compound **5** was capable of modulating downstream MALAT1 target genes in a dose-dependent manner, preliminary evidence suggests that it failed to reduce expression of multiple endocrine neoplasia b (MENb) lncRNA, which harbors a structurally-related ENE at its' 3' terminus. Using complementary biochemical and biophysical analyses, dissociation constants (K_ds) of 3 μM and 6 μM were determined for binding of compounds **5** and **16**, respectively, to the ENE. Finally, molecular modeling has been implemented in order to identify the binding site of compound **5** within the MALAT1 ENE and determine the basis for selectivity. Our identification of two novel small molecules targeting a MALAT1-encoded triple helix with activity in a breast cancer model will lay the foundation for new classes of anti-cancer therapeutics and molecular probes for treatment and understanding of MALAT1-driven cancers.

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696 Exploring the potential of antisense-oligonucleotide-based *PKM* splice-switching as a targeted therapy for liver cancer

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Macromolecules are the basic building blocks for dividing cells to replicate their cellular content during mitosis. Highly dividing cancer cells increase macromolecule biosynthesis by altering their metabolism to fulfill the requirement for rapid cell-growth. Expression of type II pyruvate kinase M (PKM2) plays a key role in cancer-cell metabolism by diverting glucose metabolites to generate cellular building blocks. PKM2 is an alternative splice isoform of the *PKM* gene that is highly upregulated in many cancer cells. Furthermore, PKM2 is widely recognized as a potential therapeutic target. Previously, we developed antisense oligonucleotides (ASOs) that switch *PKM* splicing from the cancer-specific PKM2 to the normal adult PKM1 isoform. This isoform switch effectively reduces the production of building blocks required for cell proliferation. Cultured glioblastoma cells treated with a *PKM* splice-switching ASO undergo apoptosis in a dose-dependent manner, illustrating the potential of using this ASO for cancer therapy. Systemically delivered ASOs primarily accumulate in the liver, making it a preferred organ for ASO splice-switching therapy. Thus, we are exploring the feasibility of ASO-based *PKM* splice-switching as a targeted therapy for liver cancer. We have identified a lead ASO for *PKM* splice-switching that inhibits the growth of hepatocellular carcinoma (HCC), the most common form of liver cancer, both *in vitro* and in a xenograft mouse model. Currently, we are also testing mouse-specific *Pkm* ASOs in a genetic mouse model of HCC. Together, these models will help establish the potential of ASO-based splice-switching therapy as a novel treatment for liver cancer.

697 Hydrophobicity drives the systemic distribution of lipid-conjugated siRNAs via lipid transport pathways

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Delivery is the fundamental obstacle preventing the widespread clinical use of siRNA-based therapies. Lipid conjugation improves siRNA circulation half-life, tissue penetration, and cellular uptake. However, design of lipid-siRNA conjugates with translatable potential is limited by an incomplete understanding of the impact of structure and lipophilicity on siRNA pharmacokinetics. Here, using a panel of biologically occurring lipids, we show that lipid conjugation directly modulates siRNA hydrophobicity, which in turn governs spontaneous partitioning into defined classes of circulating plasma lipoproteins *in vivo*. Lipoprotein association influences oligonucleotide distribution by delaying glomerular filtration and promoting uptake in lipoprotein receptor-enriched tissues. Lipid-conjugated siRNAs elicit sustained mRNA silencing of up to 65% in liver, 76% in kidney, 35% in ovary, and 37% in adrenal gland after a single, subcutaneous injection, with no increase in systemic toxicological markers. Lipid-siRNA internalization is not dependent on lipoprotein endocytosis, as low density lipoprotein (LDL)-associated siRNAs remain efficiently internalized in LDL receptor deficient animals. We present evidence that LDLR-independent membrane permeation is facilitated by siRNA phosphorothioate modifications. Encapsulation in biomimetic lipoprotein nanoparticles or exosomes has been extensively explored as a strategy for improving siRNA delivery. Our results establish that hydrophobic modifications can be leveraged to target therapeutic oligonucleotides to endogenous lipid transport pathways without the need for synthetic formulation.

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698 A novel heterologous prime/boost vaccine system drives tumor-specific and potent CD8 T cell responses for cancer immunotherapy

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Tumor-specific neoantigens (TSNAs) are present in a majority of tumor types and are key targets for T cells released by immune checkpoint blockade therapy. Given that TSNAs are non-self antigens, they are particularly attractive cancer vaccine targets. We have developed a potent heterologous prime/boost immunization approach to deliver predicted TSNAs to patients, which is comprised of a replication incompetent chimpanzee adenoviral vector (ChAdV) for the prime vaccination and a self-replicating, synthetic RNA vector (srRNA) for repeated boost vaccinations. The ChAdV vector is similar in design to other adenoviral vectors that have demonstrated induction of polyfunctional and durable T-cell responses. The srRNA vector is based on Venezuelan equine encephalitis virus (VEE), where sequences encoding the structural proteins of VEE were deleted and replaced by a TSNA expression cassette. For delivery *in vivo*, synthetic srRNA is formulated with a lipid nanoparticle (LNP), which facilitates efficient cellular uptake of the RNA and enhances antigen expression as well as the resulting immune response. We demonstrate that the srRNA vector effectively replicates *in vitro* and *in vivo* resulting in durable and high levels of antigen expression. To characterize the vectors in pre-clinical animal models, a prototypical expression cassette that encodes multiple mouse antigens for monitoring immune responses in mice and non-human primates (NHPs) was introduced into both vector systems. We demonstrate that immunization of mice with either vector results in strong antigen-specific CD8 T-cell responses against the encoded murine epitopes. The heterologous prime/boost approach provided a statistically significant survival advantage to tumor bearing mice when compared to untreated mice. The potency of the immunization platform was also assessed in Indian rhesus macaques and a quick onset of T-cell responses was observed post ChAdV prime vaccination. The ChAdV primed T-cell responses were effectively boosted by delivery of the LNP formulated srRNA vector. These data demonstrate that our heterologous prime/boost vaccination approach effectively primes and maintains long-term robust T-cell responses to the encoded non-self antigens in NHPs, which is a highly predictive model of vaccine responses in humans. This vaccine platform is targeted for entry into clinical trials in mid 2018.

699 Withdrawn

700 Splice-site changing oligonucleotides targeting the serotonin receptor 2C reduce spasticity after spinal cord injury

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Spinal cord injury (**SCI**) affects approximately 300,000 Americans resulting in devastating neurological and physical limitations. SCI in the chronic phase is complicated by muscle spasms, which are to a large degree caused by hyperactivation of the serotonin receptor 2C (5HT2C) caudal to the injury. Currently, there is no rational treatment available for these spasms. Through a combination of alternative splicing and editing, the 5HT2C pre-mRNA generates at least 25 isoforms with different regulatory properties: one intracellular truncated receptor 5HT2C_tr, one non-edited full-length receptor 5HT2C_Fl_INI and 23 full length edited receptors 5HT2C_FL_ed. The full-length receptors are active in signaling, while the truncated receptor has a dominant negative inactivating function.

We found that SCI removes a 'poison exon' in ADAR2 (adenosine deaminase acting on RNA), resulting in an increase of ADAR2 activity. This is concomitant with an increase of the edited-full-length isoforms and a decrease of the non-edited isoforms. In addition, the 5HT2C_tr isoform generated through alternative splicing is upregulated after SCI. Since the truncated isoform stops 5HT2C activity and the non-edited isoform has reduced activity, these changes could be an adaption to reduce serotonin signaling.

To intervene with the isoform ratios, we developed a series of oligonucleotides that either increase or decrease the 5HT2C_tr /5HT2C_FL_INI ratio, as well as an antiserum that is specific for the 5HT2C_tr protein. One of these oligos (oligo#8) promotes the formation of the truncated isoform when delivered into spinal cords of naïve or injured rats through intrathecal injection. Oligo#8 accumulates in motoneurons after intrathecal delivery and importantly, reduces tail muscle spasms in a rat model of spinal cord injury.

Our data confirm that a deregulation of the 5HT2C pre-mRNA contributes to spasms occurring after spinal cord injury. Furthermore, they show the principle that splicing-changing oligonucleotides could be used to treat spasticity, which is a major co-morbidity of SCI.

701 Maximizing the translational yield of mRNA therapeutics by minimizing 5′-UTRs

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The 5’-untranslated region (5’-UTR) of mRNA contains structural elements, which are recognized by cell-specific RNA binding proteins thereby affecting the translation of the molecule. The activation of an innate immune response upon transfection of mRNA into cells is reduced when the mRNA comprises chemically modified nucleotides, putatively by altering the secondary structure of the molecule. Such alteration in the 5’-UTR in turn may affect the functionality of mRNA. Here we report on the impact of seven synthetic minimalistic 5’-UTR sequences on the translation of luciferase-encoding unmodified and different chemically modified mRNAs upon transfection in cell culture and in vivo. One minimalistic 5’-UTR, consisting of 14 nucleotides combining the T7 promoter with a Kozak consensus sequence, yielded similar or even higher expression than a 37 nucleotides human alpha globin 5’-UTR containing mRNA in HepG2 and A549 cells. Furthermore, also the kind of modified nucleotides used in in vitro transcription, affected mRNA translation when using different translation regulators (Kozak vs. translation initiator of short UTRs). The in vitro data were confirmed by bioluminescence imaging of expression in mouse livers, 6h post-intravenous injection of a lipidoid nanoparticle-formulated RNA in female Balb/c mice. Luciferase measurements from liver and spleen showed that minimal 5’-UTRs (3 and 7), were either equally effective or better than human alpha globin 5’-UTR. These findings were confirmed with a human erythropoietin (hEPO)-encoding mRNA. Significantly, higher levels of hEPO could be quantified in supernatants from A549 cells transfected with minimal 5’-UTR7 containing RNA when compared to commonly used benchmarks 5’-UTRs. Our result demonstrate the superior potential of synthetic minimalistic 5’-UTRs for use in transcript therapies.

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702 RNA-based micelles: a novel platform for paclitaxel loading and delivery

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RNA can serve as powerful building blocks for bottom-up fabrication of nanostructures for biotechnological and biomedical applications. In addition to current self-assembly strategies utilizing base pairing, motif piling and tertiary interactions, we reported for the first time to build RNA based micellar nanoconstruct with a cholesterol molecule conjugated onto one helical end of a branched pRNA three-way junction (3WJ) motif. The resulting amphiphilic RNA micelles consist of a hydrophilic RNA head and a covalently linked hydrophobic lipid tail that can spontaneously assemble in aqueous solution via hydrophobic interaction. Taking advantage of the feature of pRNA 3WJ branched structure, the assembled RNA micelles are capable of escorting multiple functional modules. As a proof of concept for delivery for therapeutics, Paclitaxel was loaded into the RNA micelles with significantly improved water solubility. The successful construction of the drug loaded RNA micelles was confirmed and characterized by agarose gel electrophoresis, atomic force microscopy (AFM), dynamic light scattering (DLS), and fluorescence Nile Red encapsulation assay. The estimate critical micelle formation concentration ranges from 39nM to 78nM. The Paclitaxel loaded RNA micelles can internalize into cancer cells and inhibit their proliferation. Further studies showed that the Paclitaxel loaded RNA micelles induced cancer cell apoptosis in a Caspase-3 dependent manner but RNA micelles alone exhibited low cytotoxicity. Finally, the Paclitaxel loaded RNA micelles targeted to tumor in vivo without accumulation in healthy tissues and organs. There is also no or very low induction of pro-inflammatory response. Therefore, multivalence, cancer cell permeability, combined with controllable assembly, low or nontoxicity nature, and tumor targeting are all promising features that make our pRNA micelles a suitable platform for potential drug delivery.

703 Translation initiation in bacterial polysome through an mRNA stand-by site

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During translation, consecutive ribosomes load on an mRNA to form a polysomal unit. The first ribosome recognizes the start codon and unwinds mRNA structures at the ribosome binding site. The second and the following ribosomes can reach the start codon only when the first ribosome has cleared the initiation site. Here we present the mechanism for the polysome loading on the natural 38 nt-long 5′ untranslated region of *lpp* mRNA from *Escherichia coli*. We find that the second ribosome can load on the mRNA 5′ leader before the leading ribosome has vacated the ribosome binding site and the start codon region. The rapid formation of this stand-by complex with the second ribosome depends on the presence of ribosomal proteins S1/S2 in the leading ribosome. Early recruitment combined to the tight coupling between translation elongation by the first ribosome and the accommodation of the second ribosome can contribute to high translational efficiency of the *lpp* mRNA, suggesting a novel mechanism of translational control in polysomes.

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704 Translational Control by the DEAD-box Helicase Ded1

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Cellular identity, function, and physiology are all determined by the proper regulation of gene expression, and various pathologies can be the result of defects in this process. The messenger RNA is a central component of gene expression, and its structure and the cohort of factors bound to it is constantly changing during the steps of gene expression. Research in the Bolger lab seeks to understand how this remodeling of the mRNA-protein complex is facilitated and controlled during gene expression. We hypothesize that members of the DEAD-box protein (DBP) family, which have the ability to modulate RNA-RNA and RNA-protein interactions, are critical players in this control. As a model for DBP function and regulation, we have focused on Ded1, which has important roles in translation initiation and is frequently mutated in cancer, including the pediatric brain cancer medulloblastoma. Recently, we showed that Gle1, a DBP regulatory factor, acts to limit Ded1 activity during translation, thus positioning Gle1 and Ded1 as gatekeepers for translation initiation. As a follow-up to this study, we have been investigating the control of Ded1's function(s) in translation through its known interactions with Gle1, eIF4G, and homomeric interactions with Ded1 itself. Furthermore, we have expanded the function of Ded1 to a novel role in mediating the translational response to cellular stress. Specifically, in contrast to its role in promoting translation initiation in steady-state conditions, we propose that Ded1 facilitates the disassembly of key translation factors from active complexes in order to down-regulate translation during cellular stress. This work is not only increasing our understanding of the control of translation by RNA helicases, but it also is examining their role in responding to changing cellular conditions.

705 5'UTR control of developmental gene expression dynamics by conserved novel IRES elements

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Gene expression dynamics during organismal development exhibits exquisite spatio-temporal specificity. Despite the importance of post-transcriptional mechanisms in gene regulation, the scope and impact of RNA cis-regulatory elements during development have remained largely elementary. Here we have developed a systems level functional screen of 5'UTR sequences that encompass the most highly conserved elements (HCE) within the genome. Could such extremely conserved sequences encode critical regulatory function in 5'UTRs of mRNAs? In a reporter screen of ~300 long mouse 5'UTRs (250 to 2500bp) that possess HCEs across 60 vertebrate species, we surprisingly identify widespread occurrence of internal ribosome entry (IRES)-like elements, particularly within key developmental genes important for organogenesis and tissue patterning. Polysome profiling of CRISPR-mediated endogenous knockout cells deleted in the conserved elements show that these sequences contribute to translation of developmental genes under physiological conditions, contrary to the dogma that non-canonical translation initiation events are largely limited to stress response transcripts that resist downregulation of cap-dependent translation. Our studies further seek to address whether functional cell-type specific RNA structures may endow cell-type specific IRES activities. To this end, we find that the strength of IRES-mediated translation vary across distinct cell types including embryonic stem cells, mesenchymal cells, neural stem cells, and differentiated neurons, demonstrating the potential for these elements to direct spatiotemporal gene expression. We will describe a novel methodology we have developed to systematically mutagenize and characterize 2D structures of these IRES-like elements in native cellular conditions. In summary, akin to cis-acting transcriptional enhancers that provide combinatorial code in determining body plan and tissue patterning, our findings reveal that unique combinations of 5'UTR RNA elements may guide fundamental aspects of vertebrate development by expanding the variation in expression of the genome at the level of translation.

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706 The landscape of nascent protein synthesis in sensory neurons reveals new insights into pain signaling

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Pro-inflammatory cytokines are harbingers of persistent pain. As a model for probing their effects on nociceptors, we focus on NGF and IL-6. Together, they trigger a rapid induction of cap-dependent translation and promote long-lasting changes in nociceptor plasticity. Accompanying behavioral changes require *de novo* protein synthesis but not transcription. To identify the targets of regulated protein synthesis in cultured dorsal root ganglion (DRGs), we applied the method of translational profiling by ribosome footprinting. We use footprinting to identify the action of NGF and IL-6 on nascent protein synthesis after only 20 minutes. The data provide a comprehensive view of the substantial changes in translation that are induced by these cytokines. From the dataset, we identify motifs which confer preferential translation in response to NGF and IL-6 treatment. We provide evidence for 5'UTRs and lncRNAs as bona fide sites of protein synthesis that undergo differential utilization following cytokine treatment. Regions in the 5'UTR containing many ribosomes, termed upstream open reading frames or uORFs. Importantly, our results on DRG cultures suggest that uORF translation does not appear to require the integrated stress response. Finally, we find that several key players in sensory detection and nociception, including CGRP. Injection of the corresponding peptide results in prolonged mechanical hypersensitivity. We propose that non-canonical translation provides a vast and unanticipated potential source of endogenous signaling peptides that may contribute to nociceptor plasticity in response to inflammatory mediators.

707 Genome-wide analysis reveals that eIF2D coordinates motor neuronal synaptic function and locomotory behavior by modulating translation of specific mRNAs

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Translational control enables rapid, spatially-restricted regulation of gene expression, but its contribution to modulation of synapse composition and function in vivo remains relatively unexplored. Here we reveal an important in vivo role for the non-canonical translation factor eIF2D (a.k.a. Ligatin) in synaptic processes. In vitro studies implicated eIF2D in GTP-independent initiator tRNA delivery and post-termination ribosome recycling, but deleting yeast eIF2D had no discernible impact on growth or translation. Although cultured neuron studies suggested activity regulation of eIF2D mRNA, the biological functions and specific mRNA targets of eIF2D in living cells remain unknown.

To address these questions, we generated flies lacking eIF2D, which appear largely normal, but have locomotion defects. In particular, *eIF2D^{KO}* larvae move slower. Synaptic morphology appeared largely normal in *eIF2D^{KO}* larvae, but electrophysiology data revealed reduced baseline transmission and pre-synaptic homeostasis defects. Rescue experiments reveal that eIF2D is sufficient on either side of the larval neuromuscular junction (NMJ) to promote synaptic signaling for normal locomotion.

To identify mRNAs regulated by eIF2D, we performed polysome profiling from *eIF2D^{KO}* larvae and controls, followed by genome-wide RNA-Seq ("Poly-Seq"). This revealed strong effects on translation profiles of specific mRNAs. Prominent among these were mRNAs coding for proteins implicated in synaptic processes and locomotion, consistent with the observed phenotypes. Moreover, we observe common mRNA characteristics (e.g. 5' UTR cis-elements) within these mRNAs that could mediate coordinate regulation by eIF2D.

Collectively, our results define a new role for eIF2D within the motor system to promote synaptic function via coordinating translation of specific mRNAs.

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708 Biochemical and structural characterization of a novel nucleotide transferase potentially involved in RNA repair

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RNA damage is prevalent in cells and RNA repair systems have been identified in many organisms to counteract this damage. The components of an RNA repair system generally consist of an essential RNA ligase and, if the ends of the damaged RNA are not compatible with the ligase, RNA end-processing enzymes. However, recent bioinformatic analysis of RNA repair systems has revealed that additional proteins may also be involved in RNA repair. One such protein candidate is a family of nucleotide transferases, named NTase14 here, whose encoding genes have been found in many operons also encoding RNA repair systems of both 3'-phosphate and 5'-phosphate RNA ligases. Our *in vitro* biochemical studies of the recombinant NTase14 from *Pseudomonas fluorescens* (*Pfl*NTase14) revealed that: 1) NTase14 is able to utilize all four NTPs to transfer NMP onto RNA, but not onto DNA or protein, isolated from cells. 2) The site of NMP transfer by NTase14 is the OH group at the 3'-end of an RNA molecule. 3) The preferred metal ion for the enzymatic activity of NTase14 is Mn^{+2} instead of Mg^{+2}, which is utilized by most nucleotide transferases. In addition, we also solved the crystal structure of *Pfl*NTase14 at 2.1 Å resolution, which revealed the structure of an N-terminal NTase domain fused to a C-terminal helical domain. Based on the observations described above, we hypothesize that NTase14 is required for repairing RNAs that experience excessive damage at their 3'-ends, and the study to test this hypothesis is currently under way.

709 Translational buffering upon splicing inhibition

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Maintenance of proteome homeostasis is an essential cellular task. Diverse layers of quality control systems monitor protein expression. Pre-mRNA splicing represents a well-known means to control gene expression prior to mRNA export and translation in eukaryotes. Furthermore, improperly spliced pre-mRNAs carrying retained intron sequences are subject to destruction via the nonsense mediated decay pathway (NMD). However, a subset of aberrantly spliced pre-mRNAs can escape NMD, though how cells sense and cope with those mRNAs has been largely unknown. Here, we show that upon inhibition of splicing, the cell activates mTOR-mediated translation initiation control to avoid protein synthesis from unspliced mRNAs. Splicing inhibitors such as spliceostatin A (SSA) have become an established tool for gene regulation studies. We used simultaneous RNA sequencing and ribosome profiling to quantify genome-wide changes in the levels of mRNA and active translation upon SSA treatment. We observed translation of a subset of mRNAs with retained introns. To our surprise, we further observed translation repression for a subset of mRNAs independent of intron retention. Gene ontology analysis implicated downregulation of several signaling systems involved in cell proliferation and translational control, including the mTOR pathway. Furthermore, almost all mRNAs sensitive to the mTOR inhibitor pp242 and those harboring 5′ terminal oligopyrimidine motifs were significantly sensitive to SSA. Accordingly, dephosphorylation of mTOR substrates 4E-BP1 and P70S6K under SSA treatment indicates mTOR dependent translation initiation repression by SSA in different cell lines. RNAi of SF3B, the direct target of SSA recapitulated SSA's effect. In addition, SSA activated the JNK signaling pathway, which senses proteotoxicity and induces mTOR inhibition in cells. Our results suggest that the production of aberrantly truncated proteins from unspliced transcripts is sensed as proteotoxic by the JNK signaling pathway, followed by attenuation through mTOR-dependent inhibition of translation initiation. Further validation experiments should corroborate these findings. For the first time, we are able to observe changes in protein biosynthesis giving a comprehensive picture of gene expression under splicing inhibition. The observed inhibitory effect on cell proliferation pathways, especially mTOR, may explain the selective toxicity towards tumor cells exhibited by splicing inhibitors, such as SSA.

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710 Understanding the regulation of *inl* gene expression in response to inositol in *Neurospora crassa*

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Inositol is important for different cellular processes but its role is highly ponounced in signal transduction pathways in the form of inositol phosphates. Inositol biosynthesis occurs by a well documented pathway which involves *inl* gene encoding inositol-3-phosphate synthase as the first enzyme in the pathway. It catalyzes the rate limiting step of converting D glucose-6-phosphate to 1D myo-inositol-3-phosphate. Interestingly, in 1983 Zsindely *et al.* published a study in *Neurospora crassa* indicating a regulatory repression on this enzyme in presence of increasing concentrations of inositol in the media. This fuelled further research and it was found that the *inl* mRNA contains an upstream open reading frame (uORF) which starts with a non-cognate codon and is translated to form a 26-37 amino acid peptide in different fungal species. uORF containing transcripts have been reported to be under the control of nonsense-mediated mRNA decay (NMD) pathway. Therefore studying the mRNA expression in wild type and NMD deficient cells, both in presence and absence of inositol will help us to understand the regulation of this gene. The preliminary data from RT qPCR experiments show a statistically significant result of about 2 fold change in the levels of *inl* gene compared to a housekeeping control gene in wild type *N. crassa* in presence of inositol. Also initial experiments indicate that the *inl* uORF is translated and shows a potential ribosome associated stall around amino acid position 22. Hence it needs to be validated with the above-mentioned strains and conditions and can also be investigated more deeply using a whole genome transcriptomic approach to fully elucidate the overall effect of inositol. Deep sequencing of ribosome protected RNA fragments or ribosome profiling can be utilized which will further enhance the information and help us understand the molecular regulatory network functional in the cells in response to inositol.

711 EIF3 mediated translation regulation in activated T cells

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Translation capacity of each messenger RNA (mRNA) in human cells depends on a multitude of signals. These signals often converge on translation initiation events, and exploit *cis*-acting elements in the non-coding regions of mRNAs. However, the molecular mechanisms that confer specific regulatory outputs for each mRNA are not well understood. We recently discovered that eukaryotic translation initiation factor 3 (eIF3) serves dual roles in translation. Human eIF3 not only acts as a general scaffold for assembly of translation preinitiation complexes at the start codon, but also has built-in specificity to directly control the translation of specific mRNAs. The mRNAs we identified encode key regulatory proteins involved in, for example, cell cycling, differentiation and apoptosis. We found that eIF3 can either activate or repress the translation of these mRNAs by binding to their 5' untranslated regions (5' UTRs), and can also directly bind the 5'-m^7G cap on certain mRNAs using subunit EIF3D. Building on these results, obtained using a human kidney-derived cell line, we have begun to analyze how eIF3 regulates translation during the early events of T cell activation. Using activated Jurkat cells as a model for T cells, we have obtained preliminary evidence that eIF3 regulates an entirely different network of mRNAs, compared to those identified in HEK-293T cells. Furthermore, eIF3 cross-links to a subset of mRNAs across their entire length, rather than at discrete sites. Using a combination of systems biology, cell biology, and biochemistry we have also obtained direct evidence on how eIF3 mediates translation of these mRNA during the early events of T cell activation. With this study we hope to understand the molecular mechanisms used in human cells to direct eIF3-mediated activation and repression of specific mRNAs important for T cell activation.

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712 Structural cross-talk between transcription and translation

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Bacterial transcription and translation are coupled. This coupling is responsible for optimal gene expression and genome stability. Co-localization and interaction between RNA polymerase (RNAP) and ribosomes has been suggested by previous in vivo and in vitro studies.

We present cryo-EM structures of E. coli RNAP core bound to the small ribosomal 30S subunit. We report high stability of this complex under cell-like conditions. RNAP and the 30S subunit form specific interactions, providing intriguing insights into the mechanism of transcription-translation coupling. The RNA exit tunnel of RNAP aligns with the Shine-Dalgarno binding site of the 30S subunit. Ribosomal protein S1 forms a wall of the tunnel between RNAP and the 30S subunit, consistent with its role in directing mRNAs onto the ribosome. RNAP samples distinct states of clamp opening, suggesting existence of different functional states during transcription-translation coupling. We will discuss new insights into co-localization of the transcriptional and translational systems, and into the mechanism of coupled transcription and translation.

713 Hypoxia-dependent decrease in DDX28 protein levels increases eIF4E2-mediated translation

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Cell survival is dependent upon the ability to respond to changing environmental conditions. A critical component of this process is the synthesis of new proteins, also known as translation. The most common form of translation initiation in eukaryotes occurs in a cap-dependent manner, which requires the recruitment of the eukaryotic translation initiation factor 4F (eIF4F) to the m^7GTP cap structure located at the 5' end of an mRNA. eIF4F is a heterotrimeric protein complex composed of the cap binding factor eIF4E, the RNA helicase eIF4A and the scaffolding protein eIF4G, which together function to initiate cap-dependent translation. However, in response to low oxygen availability (hypoxia), a common feature of several physiological and pathological processes including embryogenesis and cancer, intricate signaling pathways are activated that culminate in the inhibition of eIF4E. Recently, it was discovered that hypoxic cells are able to utilize an alternate 5' cap binding mechanism, whereby cells switch to the use of the eIF4E homologue, eIF4E2, in order to maintain selective cap-dependent translation of critical hypoxia-response mRNAs. While there is currently some understanding of how this non-canonical hypoxic translation initiation complex, named $eIF4F^H$, is functioning, there is still little known about its composition or its regulation. We hypothesize that DDX28, a DEAD-box RNA helicase family member, acts as a negative regulator of eIF4E2-mediated translation. Our data from proliferation assays, polysome profile analysis, co-immunoprecipitation, and cap-affinity assays suggest that a decrease in DDX28 protein expression under hypoxia may serve to enhance the cap-binding affinity and overall activity of eIF4E2, while overexpression of DDX28 appears to have an antithetical effect. Given the novelty of this hypoxic cap-dependent translation mechanism, identification and characterization of $eIF4F^H$ regulatory mechanisms will not only aid in understanding the plasticity of translation, but could also ultimately lead to the development of cancer-therapeutics that selectively target the hypoxic protein synthesis machinery.

714 Most circular RNAs are potentially translated

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Most circular RNAs (circRNAs) are known to be produced from back-splicing, however their general function is still an open question. Recently we and other groups reported that a subset of circRNAs are translated in vivo via different Internal Ribosome Entry Sites (IRES), however the scope of circRNA translation is unclear. To systematically identify new sequences that drive circRNA translation, we developed a cell-based reporter system to screen a random 10-nt library for short elements that drive circRNA translation. Through a near-saturate screen followed by high-throughput sequencing, we found that a large number random 10-mer sequences have IRES-like activity to initiate cap-independent translation of circRNA. We further identified enriched motifs from these 10-mers, resulting in ~100 enriched hexamers (mostly AU-rich) that can drive circRNA translation. These IRES-like hexamers were clustered into 11 classes based on their sequence similarity, and their activities were further confirmed by experiments. Since these IRES-like hexamers account for ~1/40 of all hexamers, any circRNAs longer than 100-nt should contain roughly two of such elements by chance to initiate translation. Consistently, we tested two circRNAs containing only the coding sequences but without stop codon, and found in both cases that the circRNAs are translated in a rolling cycle fashion, presumably from some internal IRES-like short elements in the coding region. More importantly, the IRES-like hexamers are significantly enriched in circRNAs compared to all RefSeq RNAs, implying that these elements are positively selected in circRNAs and thus most circRNAs in human cells may indeed be translated. We further identified trans-acting factors that regulate circRNA translation. Taking together, our data suggest an extensive translation of circRNA driven by short IRES-like elements, which may be a general function of circRNAs in cytoplasm. Since many circRNAs contain the natural start codon of host gene, our study raises an important open question regarding the biological function of the circRNA-coded proteins or new isoforms.

715 Controlling tissue patterning by translational regulation of signaling transcripts through the core translation factor EIF3C

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Gene expression is tightly regulated during mammalian embryonic development in time and space to give rise to diverse cell type and patterned tissues. Over the last decades, numerous studies have demonstrated multiple layers of regulation at transcription and epigenetic levels, while little is known about the regulation at the level of translation. To understand the impact and prevalence of translational regulation during tissue patterning, we performed state-of-the-art ribosome profiling genome-wide in mid-gestation mouse embryos as cells become specified and organize into tissues. We identified differential translational regulation between neural tube and limb for hundreds of mRNAs guiding critical tissue-specific functions as well as developmental signaling cascades, demonstrating that translation regulation acts to diversify gene expression across tissues. Unexpectedly translation of the core development signaling circuitry including Shh, Wnt, Hippo, PI3K and MAPK pathways are widely repressed, revealing pervasive regulation at the level of translation for signaling transcripts. To further extend molecular understanding into this layer of control to gene expression, we identified and functionally characterized a complex landscape of upstream open reading frames (uORFs) across 5′-untranslated regions (UTRs) of key signaling components. Using CRISPR-mediated mutagenesis of ES cells, coupled with *in vitro* differentiation into neurons, we demonstrate the significance of uORF-mediated translational repression within the major SHH receptor, PTCH1, in control of cell signaling and neuronal differentiation. Finally, phenotypic analysis revealed that the core component of eukaryote translation initiation factor 3 subunit c (EIF3C) is specifically required for Shh-mediated tissue patterning. The eIF3 complex has been thought to be required for general translation initiation. However, *Eif3c* heterozygous embryos show a selective reduction of *Ptch1* mRNA translation in the developing neural tube and EIF3C has a specific requirement in control of limb and neural tube patterning. Altogether, this work unravels the designing principles of mammalian embryogenesis at the level of translational control and reveals surprising specialization of housekeeping translation initiation machinery in regulating fundamental aspects of the developmental signaling circuitry.

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716 Bacterial ribosome conformations probed by ribosome profiling

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Ribosome decodes codon and incorporate each new amino acid during translation elongation. Although this step is driven by massive rearrangement of ribosome conformation, ribosome forms two distinct conformations based on the tRNA positions: classical state and hybrid state. In the classical state, ribosome possesses peptidyl-tRNA stays in P-site and aminoacyl tRNA recruited to A-site. Along with peptidyl transfer reaction, ribosome rotates between large and small subunits and then dynamic tRNA rearrangement is induced. In this hybrid state of ribosome, tRNAs occupies E/P, and P/A states, in which P- and A-site tRNA heads lean to E or P-site, respectively. Finally, along with translocation prompted by EF-G, ribosome returns back to classical state for the next round of elongation cycle. The conformation change between classical and hybrid states is the basis of translation elongation reaction. Although single-molecule and biochemical assays have revealed the detail, the conformational changes occurred *in vivo* has been still largely unknown. Here we probed *in vivo* conformations of bacterial ribosomes in genome-wide manner by ribosome profiling, a deep-sequencing of ribosome-protected RNA fragment followed by RNase digestion. Combined the ribosome profiling with well known translation inhibitors that stabilize *E.coli* ribosomes in classical or hybrid state, we revealed that ribosome has different footprint length correspond to each conformation: classical state; ~27 nt and hybrid state; ~36 nt. In contrast to a previous study showing that hybridization to SD motifs insensitizes the footprint to nuclease treatment, we observed that long footprints induced by hybrid state ribosomes are independent of SD motifs. Along with the similar observations of two distinct footprint lengths in yeast, our analysis paves a way to analyze the conformational changes of ribosome *in vivo* genome-widely.

717 Measuring the Mechanical Forces During Ribosome Translocation via EF-G Crosslinking

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The ribosome is the complex molecular machine found in all living cells that is responsible for the synthesis of protein. The ribosome is associated with various proteins, including the GTPase Elongation Factor G (EF-G). EF-G is responsible for catalyzing tRNA and mRNA translocation on the ribosome, however, the mechanism of this translocation remains elusive. A recent crystallographic study has implied large conformational changes of EF-G during translocation. Previous studies observed only the elongated, post-conformational state; however, a compact, pre-translocation state has recently been observed. The question regarding the biological relevance of these conformational changes remains. To answer this, we have generated double-cysteine EF-G that was then internally crosslinked with various lengths of crosslinkers. If the large conformational change does occur in solution, then translocation will be affected by the crosslinking. To determine if crosslinking was successful, crosslinked samples were run on SDS-PAGE gels until band separation was observed. A purification protocol for large scale preparation of crosslinked EF-G was developed. To purify crosslinked EF-G, we performed electro-elution with a 5% SDS-PAGE gel to collect crosslinked and non-crosslinked fractions. We have done preliminary biophysical measurements on the crosslinked EF-G that implies large conformational change in EF-G may indeed occur.

718 Ribosome heterogeneity and specialization during cellular differentiation

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Protein synthesis is under extensive regulation to ensure that genes are expressed in the proper times, places, and quantities within the cell, but many of the mechanisms underlying translational control remain unclear. Recently, the ribosome itself has been suggested as a novel source of translational regulation: previously believed to be identical across all cell types, the ribosome has now been shown to have heterogeneous compositions which alter its capacity to translate specific mRNAs. To determine whether ribosomes with distinct compositions and specialized translation functions are required for cell fate specification, we quantitatively measured the abundance of the core ribosomal proteins (RPs) on actively translating ribosomes as stem cells differentiated down a lineage. We identified multiple RPs that change dynamically during cell differentiation, several of which are known to regulate the translation of specific mRNAs, suggesting that these alterations in ribosome composition may specialize cells for the production of certain proteins. Furthermore, mice lacking these heterogeneous RPs have tissue-specific defects that correlate with the cell types where the RPs are enriched, indicating that these increases in ribosomal abundance occur in the cells where the RPs perform specialized functions necessary for proper organismal development. The ribosome accordingly may be a crucial regulator of gene expression during cellular differentiation, and these findings may lead to the discovery of new gene networks, demarcated by novel RNA regulons, undergoing translational regulation, as well as improving our understanding of how cells make fate decisions and form complex tissues.

719 5′-UTR RNA structure modulates protein translation of SERPINA1 in alpha-1 antitrypsin deficiency

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Alpha-1 antitrypsin (A1AT) deficiency is one of the most common monogenic diseases in North America that contributes to emphysema and severe liver disease for patients. Hundreds of disease-associated mutations are known that result in pathological aggregation in the liver, insufficient levels of A1AT in the lungs, and subsequent excessive lung remodeling from excess local protease activity. The α-1-antitrypsin gene, SERPINA1, expresses over ten known mRNA isoforms, all generated by alternative splicing in the 5′-untranslated region (5′-UTR). Although all SERPINA1 mRNAs encode exactly the same protein, expression levels of the individual mRNAs vary substantially in different human tissues. Distinct 5'-UTR sequences result in alternative RNA structures and can either enhance or disrupt translation of a reporter gene. However, complete structural models of these motifs and the extent to which these structures regulate protein translation in living cells is relatively unexplored. Thus, A1AT represents a unique opportunity to define RNA-mediated regulation of translation, liver and lung disease and, in the long term, new classes of RNA-targeted therapeutics.

We have identified and characterized higher-order RNA structures in the SERPINA1 gene at single-nucleotide resolution and across multiple cell types using modern quantitative chemical probing methods and massively parallel sequencing. The functional importance of newly discovered mRNA structures were examined through screening tiling mutations that together cover the entirety of the SERPINA1 5'-UTR. Several distinct RNA structural components are shown to either promote or disrupt protein translation. We anticipate these structural motifs will define new translational regulatory elements in 5'-UTRs and provide preliminary targets for future RNA-targeted ligand discovery efforts in alpha-1 antitrypsin deficiency.

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720 Rbfox1 Regulation of Translation in Neurons

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The neuron-enriched Rbfox family of RNA-binding proteins has been implicated in neurological diseases including epilepsy, schizophrenia, and autism spectrum disorders. Each of the three members of this protein family can be alternatively spliced to form nuclear and cytoplasmic isoforms. While the nuclear isoforms have been extensively studied as splicing regulators, the functions of cytoplasmic Rbfox are less well understood. We previously found that cytoplasmic Rbfox1 (Rbfox1-C) stabilized mRNA levels by binding to 3'UTRs. We then identified VAMP1, a vSNARE protein, as a major target of Rbfox1-C, containing seven Rbfox binding motifs in the 3'UTR. We showed that 3'UTR binding by Rbfox1-C increases Vamp1 transcript and protein levels mainly by antagonizing miR-9 binding at a nearby site, but may also directly affect translation. We also showed that Vamp1 is specifically expressed in inhibitory neurons, indicating that the programs of Rbfox1 regulation differ between inhibitory and excitatory cells. We aim to further investigate mechanisms of Rbfox1-C mRNA regulation in neuronal subtypes and the effect of loss of Rbfox1-C on expression level of its target transcripts. To do so, we are using ribosome footprinting to compare the ribosomal profiles of inhibitory vs excitatory neurons in Rbfox1-cKO mouse brains. Using this technique, we hope to measure Rbfox1-dependent changes in ribosome loading and elongation to gain better insight into the mechanisms of Rbfox1 function in the cytoplasm. Furthermore, we will also identify additional transcripts whose expression is dependent on Rbfox1-C and which might determine the neurological phenotypes of Rbfox1 loss. Ultimately, these studies will help clarify the specific roles of Rbfox in neuronal function and neuropsychiatric diseases.

721 How 2′-O-methylation in mRNA disrupts tRNA decoding during translation elongation

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Chemical modifications of messenger RNA (mRNA) may regulate many aspects of mRNA processing and protein synthesis. Recently, 2'-O-methylation of nucleotides has been identified as a frequent modification in human mRNA, but little is known about its effect on translation. We used single-molecule, bulk kinetics and structural methods to determine how 2′-O-methylation affects key steps of protein synthesis in a bacterial model system. The modification caused excessive rejection of cognate aminoacyl-tRNAs by inhibiting GTP hydrolysis on elongation factor Tu and aminoacyl-tRNA accommodation in the A site. Our results suggest that 2′-O-methylation may sterically perturb the interactions between ribosomal monitoring bases (G530, A1492 and A1493) and cognate codon anticodon helices, leading to significant inhibition of proteins synthesis.

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722 Lethal effect of denatured RRF on *E.coli* during the lag phase—Formation of 40S particles and upregulation of ArfA.

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Ribosome recycling factor (RRF) is an essential protein factor responsible for disassembly of the post-termination complex(1). Inactivation of tsRRF (temperature sensitive RRF) at non-permissive temperature (43°C) is bactericidal during the lag phase in *E.coli.* In contrast, it is bacteriostatic at log and stationary phase (2). Significant amount of protein synthesis remains under the lethal condition due to inactivation of RRF. Addition of the protein synthesis inhibitor (tetracycline) prevents the bactericidal effect of tsRRF loss. Under the condition, new particles with 40S sedimentation coefficient containing 16S rRNA were formed. Antibiotics which prevents the bactericidal effect also prevented the emergence of the 40S particles. The proteomics and EM studies of the 40S particles revealed the heterogeneous nature of the particles having majority of proteins from 30S ribosomal subunits. The 40S particles is disintegrated in 1 mM Mg^{2+}. Restoring the Mg^{2+} back to 10 mM reformed the 40S particles again. We found upregulation of ArfA, RbfA, RimM and RimJ only at the non-permissive temperature at the lag phase by proteomics analysis. On the other hand, under this condition, protein DbpA, responsible for 50S ribosome subunits assembly, was not expressed. In addition, other protein factors involved in 50S subunits assembly, such as CsdA, SrmB and EngA were significantly reduced. On the basis of these findings we postulate that, under the lethal conditions, unusual proteins are formed because of unscheduled translation (re-initiation). These toxic proteins influence, among other things, the assembly of ribosome subunits resulting in the formation of 40S particles. To cope with the lethal condition where ribosome is bound to the mRNA abnormally, a factor such as ArfA is increased to retrieve mRNA-stuck ribosomes. Further studies are in progress to characterize the 40S particles formed and possible role of this particles in the bactericidal effect of denatured RRF.

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723 Translational readthrough in *Drosophila melanogaster*

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The fidelity with which a translating ribosome can recognize in-frame stop codons and mediate termination is largely dictated by the identity of the stop codon and the local mRNA context. A fraction of ribosomes, upon encountering a leaky stop codon context can lead to the production of C-terminally extended protein isoforms in a process known as programmed readthrough (RT). In this study, we analyze a set of genes from *Drosophila melanogaster* that have been phylogenetically predicted to undergo programmed RT. Using a dual luciferase reporter assay in Schneider 2 cell lines, we have determined that the RT efficiency for some genes exceeds 10%; notably, poor stop codon context alone did not explain the high RT of some genes, suggesting that additional information on the mRNA sequence provides a cumulative effect in enhancing programmed RT. In order to understand the biological significance and phenotypic outcome of gene specific RT, we employ CRISPR/Cas9-based genome editing to create transgenic flies with abolished or constitutive RT. Such an approach allows us to study the significance of the signals appended by RT as well as monitor the direct effects of RT at a cellular level.

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724 The Non-Canonical RNA-Binding Protein, Pyruvate Kinase, Regulates the Translation of Protein Synthesis Machinery

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We purified and characterized the proteome of polysomes and mRNPs isolated from the cytosol and ER of human cells. Interestingly, we discovered that several glycolytic enzymes, including pyruvate kinase (PKM), were enriched in the cytosolic polysome/mRNP pools. To further explore the relationship between metabolism and polysome-content, we isolated mRNPs from glycolytically-inhibited cells and discovered that this treatment further promotes PKM-association. We also found that the tethering of PKM to a reporter mRNA represses its translation. Using eCLIP we identified the transcripts associated with PKM and found that this enzyme binds to the open-reading frames (ORF) of mRNAs encoding protein translation machinery: ribosomal protein subunits, initiation factors and elongation factors. In light of PKM's propensity to bind the ORF, we found that it associates with ribosomes in vivo and in vitro. Overall, these results indicate a model where PKM senses glycolytic perturbation and responds by repressing the translation of mRNAs encoding translational machinery thus resulting in the overall suppression of protein synthesis capacity.

725 Evolutional conservation of genome-wide eIF4F dependency in protein synthesis

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Translation initiation has been widely accepted as a rate-limiting step in protein synthesis *in vivo*. Therefore, its control allows rapid and reversible control of gene expression. The eukaryotic translation pre-initiation complex (PIC) is recruited to the capped 5′-ends of mRNAs, which are facilitated by eukaryotic initiation factor (eIF) 4F—a multi-protein complex consisting of cap-binding protein 4E, scaffold protein eIF4G, and DEAD-box protein eIF4A. eIF4F complex has been thought to be uniformly required for translation of almost all mRNAs in cells. However, recent genome-wide analyses, including ribosome profiling, indicated differential eIF4A- or eIF4E-dependencies among mRNAs in mammals and yeast. The widely conserved mechanism of translation initiation among eukaryotes raised a question of how the eIF4A- and eIF4E-dependencies of cellular mRNAs are preserved during evolution.

Here, we performed ribosome profiling in the presence of eIF4A and eIF4E inhibitors in human HEK293 and *Drosophila* S2 cells and measured the genome-wide changes in translation efficiency. As a result, we found that diverse sensitivities by eIF4E and eIF4A inhibitions among different mRNAs were overlapped both in humans and flies. Intriguingly, the distribution of eIF4F-dependencies in the transcriptome showed striking similarity between the two species. Our data imply an ancient and common mechanism of translational initiation regulation conserved for ~700 million years since the two lineages were separated.

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726 Alternative splicing of cytoplasmically recapped mRNAs

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Until recently, removal of the 5’ cap was thought to be an irreversible step leading to the degradation of the decapped mRNA. Our lab has discovered and characterized a cytoplasmic capping complex that promotes the stability and translation of specific mRNAs by restoring the 5’ cap onto their decapped forms [1]. Our earlier microarray work detected a pool of uncapped mRNA transcripts both in normal cells and in cells expressing a dominant negative cytoplasmic capping enzyme (K294A) [2]. These uncapped transcripts retain poly(A) tails and the locations of capped analysis of gene expression tags correlate with uncapped 5’ ends on several well-characterized cytoplasmic capping target mRNAs [3, 4]. Using a dual-adaptor RACE approach, we detect uncapped 5’ RNA ends, including three non-coding RNAs and several mRNAs identified by our earlier microarray study in both normal and K294A-expressing cells. Surprisingly, 11 of the 46 mRNAs detected contained sequences unique to an individual splice isoform. We examined this intriguing finding with 5’RACE experiments targeting three known cytoplasmic capping targets. We didn’t detect isoform-specific products for ITGB1 or SARS; however, one uncapped 5’ end of ZNF207 was restricted to a single alternatively-spliced isoform. Collectively, these data both support our earlier works and also suggest that sequences included or excluded by alternative splicing may play a role in targeting transcripts for- and/or determining the position of- cytoplasmic recapping.

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727 Multiple ways to frameshifting in human immunodeficiency virus type 1

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Many viruses use programmed ribosome frameshifting (FS) to increase genome-coding capacity and to regulate stoichiometric ratio between viral proteins. Human immunodeficiency virus type 1 (HIV-1) uses –1 frameshifting to produce the Gag-Pol fusion polyprotein which is then processed into mature viral enzymes. Significant changes in the ratio between the 0-frame and –1-frame products is detrimental for the replication, particle formation, and infectivity of the virus. In the cell, frameshifting can proceed via two distinct pathways resulting in a signature peptide FFR (about 25%) or FLR (about 75%). Here we show that the virus can switch between the two different frameshifting regimes while maintaining the robust overall frameshifting efficiency. The main modulator of frameshifting regime is the UAA-specific Leu-tRNA^{Leu5} reading the second codon of the slippery site, which is rare in human cells. Under Leu-tRNA^{Leu5} limitation FFR pathway is dominant while at saturating translation conditions FLR route becomes prevalent. The lack of specific aminoacyl-tRNAs can also lead to –2 and +1 frameshifting events. In addition to the canonical slippery site, HIV-1 contains the second slippery site, which contribution to frameshifting is normally marginal. However, when this site is mutated in response to antiviral drug therapy, it could support rather efficient –1 and –2 slippages. Together, these different frameshifting regimes allow the virus to maintain a constant –1 frameshifting efficiency regardless of the changes in the host environment and the effects of antiviral therapy.

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728 Mutually Exclusive amino acid Residues Of L13a Regulate its Role in Ribosomal Incorporation and Transcript-Specific Translational Silencing.

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Ribosomal protein L13a is essential for transcript-specific translational silencing of mRNAs encoding several inflammatory proteins e.g. chemokines and chemokine receptors. Series of studies from our laboratory showed that phosphorylation-dependent release of L13a from 60S ribosomal subunit and its assembly into the IFN-gamma-activated inhibitor of translation (GAIT) complex, which binds to the GAIT element located in the 3' untranslated region (UTR) of target mRNAs, is essential for translational silencing. Moreover, our studies with macrophage-specific knockout mice showed the essential role of this mechanism as a physiological defense against uncontrolled inflammation. However, the amino acid residue(s) of L13a essential for translation silencing of GAIT element bearing target mRNAs and the residue(s) important for ribosomal incorporation are still not known. Previous studies in our laboratory showed that arginine at position 68 is essential for the ribosomal incorporation of L13a. Structural homology modeling using crystal structure of prokaryotic L13 as a model showed that eukaryotic L13a possesses an extra helix of about 55 amino acids long at the C-terminal end. Interestingly, we observed that deletion of this helix impairs the ability of L13a to play its extra-ribosomal function i.e. translational silencing of inflammatory genes. This truncated L13a protein also fails to incorporate into the ribosomes. We have identified the amino acids within this helix at position 159(K) and 161(K) that are required for nucleolar import of L13a and incorporation into the ribosome. Also, the amino acids at position 185(V), 189(I) and 196(L) of L13a are involved in an interaction with RPL14 and mutating these residues abrogates the nucleolar import and ribosomal incorporation of L13a. In addition, amino acids at positions 169(R), 170(K) and 171(K) are required for translational silencing activity of L13a. Altering these amino acids fails to silence the translation of GAIT element-bearing mRNA transcripts in an in vitro translational silencing assay. However, these changes do not block ribosomal incorporation. Thus showing the presence of mutually exclusive ribosome incorporation and translational silencing domain. Together, these studies provide a comprehensive analysis of the critical amino acid residues essential for ribosome incorporation and translational silencing activity of L13a, a physiological attenuator of inflammation.

729 A CRISPRi screen in eIF4E heterozygous cells reveals a new genetic interaction landscape for the major cap binding protein

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Control of gene expression at the step of translation is one of the most tightly regulated processes in the cell, yet the regulatory mechanisms that confer translational specificity at the organismal level remain poorly understood. We found that a 50% reduction in eIF4E expression is surprisingly compatible with normal development and global protein synthesis but compromises the cellular response to stress and oncogene-induced transformation. While these results present new paradigms for the requirement of eIF4E in translation initiation, they raise the outstanding question of the nature of eIF4E genetic interactions that support the fitness landscape of $eIF4E^{+/-}$ cells in both normal and transformed cellular states. By knocking down each gene within the mammalian genome in the background of eIF4E haploinsufficiency, we aimed to identify not only specific and unknown genetic interaction partners of eIF4E, but also unique proteins which can be potential targets for cancer therapy. To this end, we established a genome-wide synthetic lethal screen employing $eIF4E^{+/-}$ primary and transformed cells achieved by CRISPR interference (CRISPRi), whereby dCas9 is fused to the Krüppel associated box (KRAB), a transcriptional repressor domain. We surprisingly uncovered several genetic interactions between eIF4E and distinct cellular pathways that were not previously described including the translation machinery, mRNA export, splicing as well as transcription. These synthetic lethal clusters define the potential points of cross talks that link eIF4E activity to important molecular and cellular processes. For example, $eIF4E^{+/-}$ cellular fitness is drastically reduced when the expression of specific components of the splicing machinery or apoptotic pathways are downregulated. We are currently delineating the mechanisms that connect eIF4E with a new landscape of genetic interactions and functionally characterizing their implications for protein synthesis control in normal and diseased states.

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730 Genome-encoded heterogeneous rRNAs regulate gene expression by preferentially selecting a subset of mRNAs

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Each organism is assumed to have evolved to possess a unique ribosomal RNA (rRNA) species that is optimal for its physiological needs; this assumption is the basis for the wide use of rRNA sequences in quantifying evolutionary relationships among organisms. However, some organisms express genome-encoded heterogeneous rRNAs, whose functional roles remain unknown. Here, we show that ribosomes containing the most variant rRNAs encoded by the *rrn*I operon (herein designated I-ribosome) contribute to differential protein synthesis in *Vibrio vulnificus* CMCP6. Ribosome profiling and luciferase fusion reporter analyses identified a subset of mRNAs whose ribosome binding site is selectively protected and is thus preferentially recognized by I-ribosomes. In *rrn*I-deleted cells, inefficient translation of I-ribosome target mRNAs resulted in reduced ability of *V. vulnificus* cells to adapt to environmental changes, such as temperature and nutrient shifts; these phenotypic changes were restored when the *rrn*I operon was exogenously expressed in these cells. In addition, genetic and functional analyses of I-ribosomes and target mRNAs suggest that both I-ribosomal subunits are required for the preferential translation of specific mRNAs, in a manner that does not depend on the interaction between the Shine-Dalgarno (SD) and anti-SD sequences. This study identifies genome-encoded heterogeneous rRNAs for the first time as regulators of gene expression, converting the protein synthesis machinery into a regulatory hub that modulates the cellular proteome in response to environmental changes.

731 Cellular response to small molecules that selectively stall protein synthesis by the ribosome

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A small molecule capable of blocking translation of PCSK9 transcript by targeting its nascent peptide in the 80S exit tunnel has recently been described (Petersen et al., 2016). Using ribosome profiling, our laboratory showed highly selective drug-induced stalling of translation consistently on the N-terminal sequences of PCSK9 and a small percentage of off-target proteins (Lintner et al. 2016). The high transcript selectivity and preponderance of early stall sites raises the possibility that the selectivity basis for the inhibitor extends beyond the environment of the nascent chain and ribosome peptidyl transferase center. To explore this, we are using CRISPRi genomic screens with high levels of compound to uncover possible cellular pathways contributing to the drug's selectivity and the regulatory pathways induced to resolve stalling. Among the proteins affecting the cell's sensitivity to the drug, we found a significant enrichment of mitochondrial and nuclear proteins suggesting a stress response. The main proteins currently associated with translation stall detection having a significant impact on cell fitness were PELO, HBS1L, NEMF and LTN1, suggesting an involvement of the No-Go Decay pathway upon stalling. We are now exploring further the role of these proteins and others more recently associated with ribosome quality control pathways in the drug's mechanism of action.

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732 Single-molecule FRET study of prolyl peptide bond formation

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The ribosome assembles the activated amino acids into proteins at the peptidyl transfer center. The translation rate in vivo is fast, except the prolyl tRNA. In a complete translation system, the overall rate of protein synthesis is ~10 s^{-1}, and peptide bond formation follows instantaneously. Because accommodation precedes peptide bond formation, it limits the rate of product formation as long as it is slower than peptidyl transfer. Thus, studying the catalytic mechanism of peptide bond formation is possible only when peptidyl transfer is uncoupled from accommodation. Recent research also showed that the slowest step in incorporation of N-alkylamino acids is accommodation or peptidyl transfer step.

To detect the kinetics of the prolyl peptide bond formation, single molecule-FRET system is an appropriate way to study the mechanism, because changes of interaction of tRNAs could be shown by the most sensitive change of FRET. In this way, it can help us to track the pathway of interaction of A-site and P-site tRNAs. L27 is the nearest ribosomal protein that approaches within only 8-10 Å of the reaction center. L27 could be labeled with FRET-relevant dyes to show the kinetcs of tRNAs in peptidyl transfer reaction. The kinetics of prolyl tRNA incorporation and stalling will be discussed.

733 Ribosome binding by yeast eIF4B drives stress-induced changes in translation

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The eIF4 group of eukaryotic translation initiation factors promotes mRNA binding to the ribosome. Our previous work indicated that ribosome binding by the N-terminal domain of yeast eIF4B is critical for robust initiation *in vitro* and *in vivo*. In contrast, RNA-binding activities by the RRM domain of eIF4B were dispensable. Our work left open the possibility that the RRM of eIF4B could allow for enhanced translation under stress conditions. Here we have compared the effects of disrupting RNA and ribosome binding of yeast eIF4B under ~1400 different growth conditions. The RRM was dispensable for vegetative growth in all conditions tested, but we found that ribosome binding promotes growth in response to a number of stressors through changes in translation. In particular, the NTD confers a strong growth advantage in the presence of the denaturing reagent, Urea and a number of osmolytes. Ribosome profiling of cells with and without the ribosome-binding NTD of eIF4B reveals gross changes in translation of mRNAs containing structured 5-prime untranslated regions in response to Urea stress. These changes are dependent on the ribosome-binding activity of eIF4B. This analysis indicates the cellular response to urea in yeast includes a strong translational component, driven by increased translation of membrane associated proteins. The results of these studies highlight the importance of eIF4B in adapting to external conditions, and allow us to tie mechanical functions of a translation factor to specific phenotypes.

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734 Inhibition of Poly(A)-Binding Protein with an RNA mimic reduces pain sensitization in mice

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Post-transcriptional controls permeate neuronal plasticity. Pain sensing neurons, termed nociceptors, rely on cap-dependent translation to rapidly induce protein synthesis in response to pro-inflammatory signals. Comparatively little is known regarding the role of the 3' end of mRNA in nociceptor plasticity. Poly(A) binding protein (PABP) stimulates translation initiation by bridging the poly(A) tail to the eIF4F complex associated with the mRNA cap. PABPs are extremely well studied. Yet, their biological functions in mammals remain poorly understood in part owing to their conserved requirement during development. An unbiased assessment of PABP binding specificity was used to generate a chemically stabilized RNA-based competitive inhibitor. The resulting RNA mimic is an order of magnitude more stable than unmodified RNA and binds PABP with high affinity and selectivity *in vitro* and in cells. PABP is required for nascent protein synthesis in primary afferent neurons and their axons. Intriguingly, behavioral responses caused by induction of nociceptive plasticity require PABP. Collectively, these results suggest that PABP is integral for nociceptive plasticity. The general strategy described here provides a broad new source of mechanism-based inhibitors for RNA-binding proteins and is applicable for *in vivo* studies.

735 Caloric Restriction Regulates Circadian Rhythms in Protein Translation.

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Calorie restriction (CR) is a dietary intervention which is known to delay various age-related pathologies and extend the lifespan. There are numerous studied mechanisms by which CR bring about its beneficial effects on the lifespan such as 1) Reduce IGF signaling, 2) Reduce mTOR signaling, 3)Reduce oxidative stress, 4)Synchronizing circadian clocks, and 5) regulation of gene expression. Our research is focused on understanding the effects of CR on the post-transcriptional regulation of gene expression in the mouse liver. CR was employed in young mice and the animals were kept on alternate 12hrs light and dark cycles and fed, 30% fewer calories than their usual daily food consumption, 2hrs after the lights were turned off. Adlibitum (AL, Control group) animals had access to the food all the time. To get an insight, we employed ribosomal profiling and high thorough output RNA Sequencing on the poly ribosomal fractions collected from the liver tissue. Tissues were collected across 24hr cycle at 6 different time point in a day at the interval of every 4hrs. The results demonstrate that CR affects the circadian rhythms in the protein translation with the peak activity at 4 hours after the feeding. RNA sequencing results further identified 1307 transcripts whose abundance in the actively translating poly ribosomal fractions expressed circadian rhythmicity, whereas the abundance of 946 transcripts had circadian rhythmicity before CR. Further analysis showed that differential abundance of actively translating transcripts was not only diet dependent but also time of the day dependent. Most of the differentially abundant actively translating transcripts were involved in various metabolic pathways as discovered by KEGG pathway analysis.

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736 Human tRNA-ThrUGU contains ncm^{5}U34 and can decode all four synonymous ACN codons in human cells; studies using stable and unstable mRNAs

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tRNAs are post-transcriptionally modified, enhancing their translational activity. U residues at wobble positions of tRNAs (U34) are modified by complex activities in a tRNA-specific manner to accommodate wobble decoding. We previously reported that tRNAThrUGU is the least abundant tRNA in human (HEK293) cells and that its modest over-expression increases the translation and steady state levels (~5-fold) of cotransfected eGFP, whose mRNA accumulates to high levels and contains only 1 ACT and 15 ACC Thr codons, which remarkably suggests efficient U:C wobble basepair decoding by tRNAThrUGU. We show for the first time that in a higher eukaryote, tRNAThrUGU contains ncm^{5}U34, as had been reported for *S. cerevisiae* tRNAThrUGU. We developed four nanoluciferase (nLuc) reporters that differ only in their eleven Thr codons, which are all a single identity in each construct, ACT, ACC, ACA or ACG. We cotransfected each nLuc reporter with a plasmid expressing one of each of the three tRNAsThr in HEK293 cells, tRNAThrUGU, tRNAThrCGU and tRNAThrAGU (the latter undergoes A34 to I34 modification and can decode ACT and ACC codons), and confirmed that each accumulated to comparable levels. For each of the four nLuc construct mRNAs, the tRNAThrUGU led to significantly higher nLuciferase activity than the other two tRNAs. These data provide evidence that tRNAThrUGU can wobble to all four Thr ACN codons as an efficient decoder of each codon in human cells. Although ncm^{5}U34 is found on yeast tRNAProUGG which has been shown to decode all four CCN codons, tRNAThrUGU had not previously been shown to decode all four of its codons. Results of experiments that test effects on this system of knocking down the elongator U34 modification enzyme will be reported.

Differential use of synonymous codons and tRNA levels can affect mRNA stability and translation, to which our nLuc system lends itself. To examine effects of codons and tRNA levels as a function of mRNA abundance, we next inserted a 3' UTR instability element into the nLuc constructs. Analysis of effects of codons and tRNA levels using high vs. modest levels of mRNA accumulation, and with varying levels of tRNAsThr will also be reported and discussed.

737 Examining the function of yeast eIF4E using the auxin-inducible degron system.

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While the cap binding protein eIF4E is regarded as essential for eukaryotic translation, certain mRNAs are over and under-represented in eIF4E RIP-seq experiments (Costello *et al.* 2015), and eIF4E dysfunction can result in specific non-lethal phenotypes (e.g. Danaie *et al.* 1999). These findings indicate that initiation's requirement for eIF4E might be less straightforward than is currently thought. A closer examination of eIF4E's roles, however, is obscured by the lethality of eIF4E knockout in yeast, and the secondary effects likely possessed by dysfunctional alleles. We are using the auxin-inducible degron (AID) system to examine the translational response of yeast to the rapid degradation of eIF4E. The AID system is a conditional protein degradation system based on plant auxin signalling (Nishimura *et al.* 2009). A protein of interest is genetically fused to a tag based on the *Arabidopsis* protein IAA17 (the mAID tag); in the presence of the cognate F-box protein TIR1, the addition of auxin causes ubiquitination of the mAID tag by SCF^{TIR1}, and thus the rapid proteasomal degradation of the protein of interest. The rapidity of degradation is a significant advantage - it allows for the earlier assaying of effects, which reduces both the impact of secondary effects and the toxicity resulting from the removal of essential proteins. We have tagged endogenous eIF4E with the mAID tag and can achieve >80% reduction in eIF4E levels within 60 minutes of the beginning of auxin treatment. Interestingly, while this significantly slows yeast growth, it does not appear to be lethal. Moreover, degradation lasting >2 hours results in defects in cell-cycle distribution and cell size. We have performed ribosome profiling on yeast that have had eIF4E degraded for 60 minutes: there is a wide variety in the amplitude of changes in ribosome density resulting from this treatment, indicating that transcripts are differentially sensitive to the absence of eIF4E. We will present our current findings regarding the mechanistic basis for this observation.

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738 Measuring Nonsense Suppression Promoted Readthrough in a Simplified Pure Translational System

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Nonsense mutation, resulting in premature termination codons (PTCs) giving rise to truncated proteins, is the root cause for many diseases. Nonsense suppressor drugs (NonSups) which stimulate near-cognate tRNA binding to PTCs, thus promoting readthrough of the PTC, can mitigate consequences of nonsense mutation. Here we report a novel simplified cell-free pure translational readthrough assay which can determine readthrough efficiency and EC_{50}s of NonSups and provide a platform for determining the mechanisms of action of NonSups. Here we report results indicating two or more classes of NonSups. Aminoglycosides such as G418, gentamicin B1, and commercial gentamicin mixture promote readthrough via apparent binding at a single high affinity site, with EC_{50}s of 0.1 – 5 μM. In contrast, ataluren and other similar compounds (RTC13 and GJ072) induce readthrough at higher EC_{50}s (100 – 300 μM) via apparent multi-site binding. Doxorubicin and negamycin may belong to separate classes. Mechanistic studies are underway with the goal of improving NonSup therapeutic properties by rational drug design.

739 How tRNA modifications and nucleotide insertions impact decoding and mRNA frame maintenance

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The ribosome is a complex and highly conserved molecular machine that is responsible for cellular protein synthesis (translation) in all three domains of life. During translation, the ribosome decodes the mRNA sequence three nucleotides at a time (i.e. in a universal triplet reading code) with extreme precision to ensure accurate protein expression critical for cellular growth and function. However, it remains unclear how the ribosome maintains, or deliberately deviates from this three-nucleotide mRNA reading frame. Since the mRNA frame directly corresponds to protein sequence, the molecular mechanism of both frame maintenance and programmed frameshifting is an important question of the Central Dogma. While many frameshifting events have been attributed to multiple factors, tRNAs alone can influence the ribosome to allow for a shift into noncanonical mRNA reading frames. We previously demonstrated that certain tRNA modifications and insertions alter how the ribosome interprets the mRNA sequence during decoding using structural biology approaches. Methylation at position 37 of the anticodon loop of $tRNA^{Pro}$ has the same impact on disrupting the structural integrity of the anticodon loop as an inserted anticodon nucleotide as $tRNA^{SufA6}$ ($tRNA^{SufA6}$ is a variant of $tRNA^{Pro}$ with a guanosine insertion between positions 37 and 38). Both situations cause a +1 shift in the mRNA frame. Furthermore, interactions between the 32-38 pair in the anticodon loop are prevented, a universally conserved pair in all tRNAs known to affect the ability of the ribosome to discern correct from incorrect tRNAs. Here, I determined how the $tRNA^{Pro}$ modification and insertion influences decoding and selection. I determined that the m^1G37 methylation of $tRNA^{Pro}$ is essential for binding to the cognate codon, corroborating the increased levels of frameshifting observed in cells lacking the TrmD methyltransferase. Modulation of the 32-38 pair in $tRNA^{SufA6}$ shows a restoration of tight binding and thus selection by the ribosome. These studies provide insights into mRNA frame maintenance by the ribosome and further suggest rational approaches to engineer tRNAs for recoding of the genetic code with non-natural amino acids.

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740 Shep is a conserved translational regulator that controls dendrite morphogenesis in sensory neurons

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RNA binding proteins (RBPs) mediate post-transcriptional gene regulatory events throughout development. During neurogenesis, many RBPs are required for proper dendrite morphogenesis within *Drosophila* sensory neurons. Despite their fundamental role in neuronal morphogenesis, little is known about the molecular mechanisms in which most RBPs participate during neurogenesis. In *Drosophila*, *alan shepard (shep)* encodes a highly conserved RBP that regulates dendrite morphogenesis in sensory neurons. Moreover, the *C. elegans* ortholog *sup-26* has also been implicated in sensory neuron dendrite morphogenesis. Nonetheless, the molecular mechanism by which Shep/SUP-26 regulate dendrite development is not understood. Here we show that Shep interacts with the translational regulators Trailer Hitch (Tral), Ypsilon schachtel (Yps), Belle (Bel), and Poly(A)-Binding Protein (PABP), to direct dendrite morphogenesis in *Drosophila* sensory neurons. Moreover, we identify a conserved set of Shep/SUP-26 target RNAs that are enriched for regulators of cell signaling and post-transcriptional gene regulation.

741 Mechanistic Analysis of Glorund-Mediated *nanos* Translational Repression during *Drosophila* Late Oogenesis.

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Translational control, coupled with other post-transcriptional mechanisms such as mRNA localization and mRNA degradation, plays a key role in the spatial and temporal regulation of maternal transcripts. The selective translation of maternal *nanos* (*nos*) mRNA at the posterior pole of the *Drosophila* oocyte and embryo is required for proper development of the anterior-posterior body axis. Spatial control of *nos* is accomplished by localization of a small pool of maternal *nos* mRNA to the posterior of the oocyte, translational activation of this localized *nos*, and translational repression of the remaining *nos* throughout the bulk cytoplasm. Translational repression of *nos* is largely mediated by a 90-nucleotide *cis*-acting element, the *nos* translational control element (TCE), present in the *nos* 3'UTR. During late oogenesis, *nos* translation is repressed by a largely unknown mechanism conferred by the *Drosophila* hnRNP F/H homolog, Glorund (Glo), whose function is later replaced by Smaug during embryogenesis to inhibit translational initiation of *nos*. Previous biochemical studies have suggested that the translational repression of *nos* during late oogenesis involves inhibition at both initiation and post-initiation. In this study, we investigate the detailed mechanism by which Glo represses *nos* translation through the identification of Glo interacting proteins and genetic analysis of Glo mutant proteins. In addition, we use a high-throughput sequencing technique, ribosome footprinting, to investigate the post-initiation block, to identify other *Drosophila* maternal transcript whose translation is regulated post-initiation like *nos*, and to explore how translational control of *Drosophila* maternal transcripts is developmentally regulated. A footprint peak-finding algorithm has been developed to identify potential ribosome stalling point transcriptome-wide.

742 Orthogonal regulation of ferritin light chain translation by eIF3 and the iron response proteins

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While iron is an essential micronutrient of life, excess iron has detrimental consequences for cellular viability. In order to prevent this iron toxicity, intracellular iron homeostasis is in part regulated by an evolutionarily conserved protein complex called ferritin. This complex is composed of a dynamic mixture of two subunits: the ferritin light chain (FTL) and the ferritin heavy chain (FTH). FTL and FTH levels are post-transcriptionally regulated by the iron response proteins (IRPs) which bind iron responsive element (IRE) RNA structures in the 5' untranslated region (5' UTR) in both the *FTL* and *FTH* mRNAs. This IRP/IRE interaction was believed to be the sole form of post-transcriptional regulation of the ferritin complex. However, we find that *FTL* mRNA translation is additionally regulated in an IRP-independent manner by eukaryotic initiation factor 3 (eIF3). Ordinarily, eIF3 serves as a scaffold, stimulating the organization of other translation initiation factors and the 40S subunit of the ribosome onto the mRNA target and promoting the subsequent scanning for the start codon. Here, we show that eIF3 also acts as a distinct repressor of FTL translation by interacting with a region of the 5′ UTR immediately downstream of the IRE. Understanding this orthogonal form of regulation reveals not only a distinct regulatory function of eIF3, but also illuminates a novel mechanisms by which cells control iron supplies.

743 Cas9-induced DNA double-strand breaks trigger ribosome remodeling and translational shutdown

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DNA damage activates a robust transcriptional stress response, but much less is known about its impact on translation. The advent of genome editing via Cas9-induced DNA double-strand breaks has intensified the importance of understanding the cellular response to DNA damage. Here we demonstrate that Cas9-induced DNA double-strand breaks lead to a reduction of select core ribosomal proteins and to the shutdown of translation through phosphorylation of eukaryotic initiation factor 2 alpha. Depletion of these core ribosomal proteins is caused by double-strand DNA damage but not other kinds of genomic lesions. The reduction of these ribosomal proteins is post-transcriptional and p53-independent. Multiple mechanisms are responsible for the loss of these ribosomal proteins, including proteasomal degradation and translational inhibition. Strikingly, translation can continue after depletion of these core ribosomal proteins, suggesting cells may create specialized ribosomes in response to DNA double-strand breaks.

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744 Evidence against RNA structure-based translation initiation in bacteria

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Canonical eukaryotic translation initiation relies on the presence of the mRNA 5' cap, which is recognized by initiation factors that recruit the ribosome to initiate translation of the encoded peptide. Internal ribosome entry sites (IRESs) are RNA elements capable of recruiting ribosomes and initiating translation on an internal portion of a mRNA. While all IRESs function independently of the 5' cap, the intergenic region (IGR) IRES of the Dicistroviridae virus family is unique as it does not require any initiation factors [1]. This non-canonical activity relies on a triple pseudoknot tertiary structure, which recruits the ribosomal subunits and mimics a canonical tRNA-mRNA duplex [2, 3]. Recently the IGR IRES from the *Plautia stali intestine virus* has been reported to drive translation in *E. coli* lysate, bridging billions of years evolutionary divergence [4]. Interestingly, while the mechanism of translation initiation appears to be different between taxonomic domains, RNA tertiary structure appeared to be important in both systems [1, 4]. Here we present a thorough analysis of translational activity of several IGR IRESs *in vivo* as well as a biophysical assessment of IRES interactions with prokaryotic ribosomes *in vitro*. Our data suggest that the prokaryotic translational activity of the IGR IRESs is not based on their tertiary structure and is instead likely mediated by the canonical Shine-Dalgarno mechanism.

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745 Thermodynamic study of macrolides using ITC competition experiments

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Bacterial infections responsible for nosocomial diseases has become one of the most important public health problem worldwide. Considering the emergence of resistance against existing drugs and the drop in the development of new ones, the fight against those multi-resistant pathogens has been classified as a top priority by the WHO.

Half of clinically used antibiotics target the bacterial ribosome. Consequently, the translation machinery is extensively studied in order to better understand the mechanism of action of antibiotics and their interactions with the ribosome, mainly through biochemical and structural works. However, thermodynamic data, which are yet essential for a fully understanding of the mechanism of inhibition of drugs, are still sparse.

ITC (Isothermal Titration Calorimetry) Microcalorimetry is the method of choice to obtain a complete thermodynamic profile, including affinity parameter of drugs binding. In this study, we investigate the interaction of some macrolides (erythromycin, azithromycine and telithromycin), a historical class targeting the 50S subunit of the ribosome of E. coli and more precisely the peptide exit tunnel. The high affinity of macrolides for ribosome (less than 10 nM) cannot be measured directly with ITC. To overcome this problem, we used an incremental ITC approach (Bec et al., JACS 2013) together with competitive experiments. Proline-rich antimicrobial peptides (PrAMPs), which recently appeared as interesting protein synthesis inhibitors, were here used as competitors since they share a similar binding region in the ribosome peptide exit tunnel, however with a lower affinity than macrolides. After precise determination of the binding parameters of PrAMPs competitors, like Bac 7 or pyrrhocoricin, the thermodynamic profile and affinity of the studied macrolides can be obtained.

In the end, this original thermodynamic approach using ITC competition experiments will provide new data on well-known and more recent compounds in order to contribute to the development of new efficient antibiotics against multidrug-resistant bacteria.

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746 The IGF2 mRNA binding protein 1 (IGF2BP1) impairs the formation of adherens junctions in cancer cells by promoting SRC activity

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The IGF2 mRNA protein family comprises three canonical RNA-binding proteins controlling cytoplasmic mRNA fate by modulating mRNA turnover or translation. Two members of the protein family, IGF2BP1 and 3, show an oncofetal pattern of expression characterized by their abundance during embryogenesis and de novo synthesis or severe upregulation in various solid cancers. Elevated expression of IGF2BP1 is associated with enforced mesenchymal tumor cell properties including single cell migration and reduced cell-cell adhesion. Adherens junctions (AJ) are essential cell-cell contact sites interconnecting intercellular adhesion via β-catenin (CTNNB1) and the trans-membrane cadherin proteins like E-cadherin (CDH1) or N-cadherin (CDH2). At their cytoplasmic side, AJs are associated with the actin cytoskeleton involving catenins, in particular α-catenin (CTNNA1). In "aggressive" carcinomas, intercellular adhesion is frequently diminished by epithelial-to-mesenchymal transition (EMT). In our study we investigated the role of IGF2BP1 in impairing adherens junctions. Our findings demonstrate that IGF2BP1 interferes with the integrity of these contacts and promotes the decay of the AJ-proteins CDH2, CTNNA1 and CTNNB1. This regulation essentially relied on the activation of the SRC kinase, a potent driver of EMT. Surprisingly, IGF2BP1 induces SRC activity whereas SRC expression remains largely unaffected by IGF2BP1. This suggests that IGF2BP1 mediates SRC activation by a RNA independent ligand-binding induced mechanism. In summary, our studies indicate that IGF2BP1 promotes a mesenchymal, aggressive tumor cell phenotype not only by controlling cytoplasmic mRNA fate but also by promoting the activation of the SRC kinase.

747 Identification of Yeast Translation Initiation Regulating Factors using a Fluorescence-Based Reporter Assay

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Eukaryotic translation initiation is a critical node at which protein expression can be regulated in response to environmental factors. We have developed a versatile method of evaluating bulk translation initiation in eukaryotes that allows for identification of potential genetic and environmental contributors. This method uses fluorescence-activated cell sorting (FACS) of yeast cells containing a reporter in which expression of GFP is driven by cap-dependent translation and expression of RFP is driven by cap-independent translation to identify conditions under which canonical factor-dependent translation initiation is impaired. This dual-fluorescence reporter allows us to screen different gene knockout yeast strains for defects in translation initiation and to screen different environmental conditions that may influence translation initiation. Studies of translation regulation have primarily focused on specific stress response pathways that influence translation of individual genes; our reporter enables us to screen for genes associated with translation under a variety of conditions on a large scale, providing a more complete picture of the different factors that work together to allow for accurate and efficient translation.

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748 Selective translation mechanism through two paralogs of eukaryotic initiation factor 4A

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Translational control is crucial for various biological processes and diseases. Although multiple steps of the protein synthesis are regulated in cells, translation initiation, where the ribosome small subunit is recruited to the 5'-teminal cap and scans the 5' untranslated region (UTR) for a start codon, typifies the target of the regulation. A complex called eukaryotic initiation factor (eIF) 4F plays a pivotal role in the translation initiation and its control. The eIF4F complex is mainly composed of the cap-binding protein eIF4E, the scaffold protein eIF4G, and DEAD-box protein eIF4A. eIF4A has been supposed to act as an RNA helicase and unwind secondary structures in 5' UTR that inhibit smooth scanning of ribosome, however recent genome-wide studies proposed that eIF4A has an alternative function in translation other than melting RNA.

Mammalian cells have two eIF4A paralogs, eIF4A1 and eIF4A2, that share >90% identity in amino acid level. Both eIF4A1 and eIF4A2 can be assembled into eIF4F complex, suggesting that these two factors are exchangeable. However, a series of studies have supposed that eIF4A1 and eIF4A2 are not functionally redundant: the expression pattern of eIF4A1 and eIF4A2 varies among tissues and the insufficient complement of eIF4A1 depletion by eIF4A2 overexpression. These findings imply that eIF4A1 and eIF4A2 have distinct roles in translation, however, the functional difference between the paralogs has been poorly understood.

To understand this in genome-wide manner, we generated eIF4A1- and eIF4A2-knockout HEK293T cell lines using the CRISPR/Cas9 system. Ribosome profiling revealed that translation efficiency (TE) changes in both eIF4A1- and eIF4A2-knockout cells were diverse across transcriptome and distinct group of mRNAs were regulated by each paralog. Moreover, RNA-immunoprecipitation sequencing (RIP-seq) via each paralog showed biased interaction of mRNA to eIF4A paralogs. From these results, we suggest that eIF4A1 and eIF4A2 preferably bind to specific mRNAs and facilitate their translation selectively.

749 The RNA-binding protein Bfr1p transnationally regulates an ergosterol biosynthesis protein Erg4p in *Saccharomyces cerevisiae*.

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The Brefeldin A resistance protein (Bfr1p) is a non-essential RNA-binding protein conserved in *ascomycetes*. Deletion of *BFR1* in *S. cerevisiae* results in multiple defects, including chromosomal mis-segregation, as well as altered cell shape and size(1). Bfr1p is important for delayed entry of certain mRNAs into P-bodies(2) and prevents P-bodies formation under normal growth conditions(3). Although Bfr1p does not contain canonical-RNA binding domains, UV-crosslinking and high resolution mass-spectrometry revealed six RNA-binding sites(4). Analysis of mRNAs bound to Bfr1p revealed an enrichment of mRNAs translated at the ER(5). However, Bfr1p's molecular function remains unclear.

Here, we show that the N-terminus of Bfr1p ($Bfr1_{(1-397)}$), which contains all identified RNA binding sites is sufficient to maintain correct chromosomal ploidy and localizes to the ER like the full-length protein. However, a single mutation (F239A) at a highly conserved, RNA crosslinking phenylalanine abolishes ER localization of Bfr1p even in the context of the full-length protein. This suggests that mRNA interaction is required for Bfr1p to localize to the ER. We also demonstrate that Bfr1p binds to *ERG4* mRNA, encoding an enzyme that catalyzes the final step of ergosterol synthesis. Mutations in RNA binding sites of Bfr1p diminish this interaction. Deletion of *BFR1* leads to down-regulation of Erg4p and abolishes its ER targeting although *ERG4* mRNA is still found at the ER. However, ribosomal occupancy on *ERG4* mRNA is reduced, suggesting that Bfr1p is required for translation of Erg4p. We propose that Bfr1p regulates efficient translation of specific membrane protein encoding mRNAs (e.g. *ERG4*) at the ER.

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750 Sign epistasis as a signature of collision-stimulated quality control during translation

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Ribosome stalling reduces protein expression in eukaryotes by activating co-translational quality control mechanisms. However the kinetic events leading to recruitment of quality control factors to stalled ribosomes have not been clearly delineated. Here we use an inverse approach combining protein expression measurements with mathematical modeling to study the kinetic events that occur upon ribosome stalling in the budding yeast, *S. cerevisiae*. Our measurements reveal that protein expression increases from stall-containing reporters when the initiation rate or the elongation rate in the 5′ region of the mRNA is decreased. This counterintuitive sign epistasis is recapitulated by a kinetic model in which collision with trailing ribosomes stimulates abortive termination of leading stalled ribosomes. By contrast, canonical models of quality control without a stimulatory role for ribosome collisions do not exhibit sign epistasis. Modeling shows that sign epistasis requires multiple successive stalls and a collision-stimulated abortive termination rate that exceeds the forward elongation rate. Quantifying the sign epistasis signature in deletion mutants enables identification of quality control factors whose activities are stimulated by ribosome collisions. Our results illustrate the utility of mathematical modeling for dissecting the *in vivo* kinetics of co-translational quality control in eukaryotes. We further expect that the open-source modeling framework developed here will facilitate the specification, simulation, and sharing of kinetic models for RNA metabolism.

751 Investigating function and evolution of mammalian expansion segments (ES)

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While ribosomes across all kingdoms of life share a conserved core region, the complexity of an organism appears to correlate with its ribosome size. Although the core of the eukaryotic 80S ribosome is almost identical to the bacterial 70S ribosome, it contains additional layers of ribosomal protein and rRNA covering its conserved core. This observation hints at the requirement for further levels of translational regulation in more complex organisms

This trend is observed within eukaryotes, with ribosome size increasing from simple eukaryotes such as yeast (~3.3 MDa), to complex metazoans such as humans (~4.3 MDa). Most of this size increase can be attributed to the expansion of rRNA in localized regions, called expansion segments. Recent research suggests that there are intra- and interspecies-specific variants in these highly variable domains, with potential tissue-specific expression.

The role of these expansion segments in mammals, if any, is largely unknown. Functional studies in mammals are largely hindered by the difficulties in genetically manipulating the numerous rDNA repeats. As such, we propose alternative approaches to investigate the specific role of these expansion segments in mammalian translational regulation, mainly by using mammalian cells which lack certain expansion segments as a natural knock-out model. In particular, we hypothesize that these expansion segments may serve as sites of interaction for ribosome-associated proteins (RAPs) that our lab has identified.

To identify candidate ribosome-associated proteins that interact specifically with individual expansion segments, we use techniques such as modified sulfolink ribosome purification and domain-specific chromatin isolation by RNA purification (dChIRP). These interactions may shed light on the possible role of particular expansion segments and their corresponding RAPs in the translational regulation of complex organisms.

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752 Conformational control of translation termination on the 70S ribosome

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Translation termination ensures proper lengths of cellular proteins. During termination, release factor (RF) recognizes a stop codon and catalyzes peptide release. Conformational changes in RF are thought to underlie accurate translation termination. However, structural studies of ribosome termination complexes have only captured RFs in a conformation that is consistent with the catalytically active state. Here, we employ a hyper-accurate RF1 variant to obtain crystal structures of 70S termination complexes that suggest a structural pathway for RF1 activation. We trapped RF1 conformations with the catalytic domain outside of the peptidyl-transferase center, while the codon-recognition domain binds the stop codon. Our structures reveal that stop-codon recognition induces 30S decoding-center rearrangements that precede accommodation of the catalytic domain. The observed separation of codon recognition from the opening of the catalytic domain suggests how rearrangements in RF1 and in the ribosomal decoding center coordinate stop-codon recognition with peptide release, ensuring accurate translation termination.

753 Dynamic post-transcriptional regulation by the yeast RNA-binding protein Mrn1p

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Post-transcriptional regulation of mRNA translation and decay plays a key role in dynamic cellular responses to changing environments. Transcript-specific RNA-binding proteins (RBPs) underlie many of these regulatory responses. One such RBP is Mrn1p, a protein with 4 RNA recognition motifs (RRMs) that is reported to bind over 300 mRNA targets in yeast, with a preference for those encoding proteins localized to the cell wall, endoplasmic reticulum, and plasma membrane. MRN1 also associates with many mRNAs that require unusual modes of translation regulation, including those containing upstream open reading frames and proposed internal ribosome entry sites. Finally, MRN1 has many overlapping targets with Pub1, an RBP required for the stability of many mRNAs. Despite these compelling data that MRN1 is a post-transcriptional regulator with many mRNA targets, little is known about its physiological function. Here we show that the N-terminal domain of MRN1 drives post-transcriptional repression of target mRNAs. In addition, the loss of MRN1 alters the cell's ability to shift to respiratory growth during glucose deprivation. We hypothesize that MRN1 represses the expression of certain mRNAs during fermentative growth, and this repression is relieved when the cell switches its mode of metabolism from glycolysis to the utilization of ethanol. These data may suggest that MRN1 functions in this and other aspects of cellular homeostasis by repressing target mRNA expression until the mRNA is required by the cell.

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754 An alternative translated isoform of MK2 determines functional diversity

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Shaping of the proteome by alternative translation events is an important mechanism of post-transcriptional gene regulation. Multiple isoforms originating from the same mRNA can execute distinct functions and alter cellular phenotypes. Here we show that the $p38^{MAPK}$-activated kinase MK2, a key regulator of stress-induced transcription, death signaling and post-transcriptional gene regulation, can be translated from an alternative CUG translation initiation start site located in the 5'UTR of its mRNA. GC-rich sequences and putative G-quadruplex structures influence the usage of the CUG as a translation initiation start site. Importantly, we can recapitulate the usage of this codon, determine the molecular properties of a short and a long MK2 protein isoform derived from the same transcript and provide evidence that activity of the RNA helicase eIF4A1 is needed to assure accurate translation. Furthermore, we show that depending on the expression of the MK2 isoforms, distinct immediately early gene (IEG) expression patterns are detected upon stress. Currently, interaction profiling of MK2 isoforms by mass-spectrometry is performed aiming to detect differentially interacting proteins that could explain the phenotypical differences in MK2-deficient cells exclusively expressing only one of the isoforms. Overall, our results demonstrate an example for an alternative translation process, that results in kinase isoform expression which in turn impacts on important physiologically relevant functions.

755 Control of mammalian limb patterning by the ribosome

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During development, precise control of gene expression is crucial for proper patterning of the mammalian body plan. For example, in the developing limb, a combination of signaling centers that modulate developmental pathways along orthogonal axes are important for patterning the limb. Although progress has been made in understanding how gene expression at the level of transcription can regulate embryonic patterning, less is known about how post-transcriptional processes, such as translation, can impact patterning programs. Such post- transcriptional regulation of patterning does occur as evident by human mutations in the ribosome that lead to limb patterning defects. However, the mechanisms for how perturbations in the ribosome could lead to such defects is not well understood. To better understand these mechanisms, we conditionally inactivated one allele of a ribosomal protein (RP) in the developing mouse limb. The mice manifest with limb defects, most notably either absent or underdeveloped radii relative to the ulna. Given that the developmental programs that differentially pattern the radius and ulna are not well understood, we sought to use this mouse as a model to understand these programs in addition to understand how perturbations in the ribosome lead to such a phenotype. Currently, we are assaying changes in expression of limb patterning genes as well as perturbing potential modulators of the phenotype via genetic crosses to understand the pathways both at an organismal and cellular level that may contribute to the phenotype. In addition, we are also assaying translational changes in the limbs of these mice to understand how such programs may be perturbed as well. In summary, our work will set out to understand how RP reduction can impact patterning and may also contribute to a greater understanding of how skeletal elements in the limb are differentially specified.

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756 Cancer-associated mRNAs regulated by the HLH motif of human eIF3A

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The initiation of translation is a vital check-point of protein synthesis in the cell and is regulated by a multitude of initiation factors. Eukaryotic translation initiation factor 3 (eIF3) plays a major role in the assembly of the 40S translation initiation complex by bringing together mRNA and the 40S ribosomal subunit. eIF3 is composed of thirteen subunits and the dysregulation of their expression has been linked to a number of cancers. Overexpression of the eIF3A subunit has been associated with increased translation of mRNAs involved in cell proliferation, suggesting that regulation of eIF3A binding can confer specificity for a subset of mRNAs. Mutations in the N-terminal Helix-Loop-Helix (HLH) motif of the eIF3A subunit have been shown to interfere with Hepatitis C virus IRES-mediated translation initiation *in vitro.* To assess how the HLH motif contributes to the translation regulation of cellular mRNAs *in vivo*, we introduced triple mutant eIF3A (HLH*) into HEK293T cells and knocked down endogenous eIF3A. Ribosome profiling and RNA-seq analysis of the mutant cell line showed that the eIF3A HLH motif regulates the translation of a discrete subset of the transcripts, which is enriched for oncogenic mRNAs involved in regulation of proliferative pathways. Among those most affected are the mRNAs encoding PRL3 and MYC. These two oncogenic genes are mis-regulated and overexpressed in a number of cancers and thus are high profile chemotherapy targets. Through a series of *in vivo* and *in vitro* assays, we show that the HLH motif of eIF3A acts directly and specifically on the 5'UTR of the target mRNA by modulating the recruitment of other initiation factors and provides a counterbalance to eIF4A1 in particular to help guide the mRNA through the scanning translation initiation complex.

757 Phosphorylation of eIF4A drives dynamic changes in gene expression in response to diverse environmental stressors

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Recruitment of mRNA to the ribosomal translation preinitiation complex (PIC) serves as a key point of regulation of gene expression in eukaryotic cells. The current model for ribosomal mRNA recruitment hinges on a strong role for the eIF4 group of factors, which are proposed to bind to the 5'end of an mRNA and activate it for translation by unwinding secondary structures. The unwinding activity of eIF4F is proposed to generate a single-stranded (ss) ribosomal landing pad, and eIF4B was thought to stimulate this activity by binding to ssRNA. Our recent data support a model in which yeast eIF4B binds directly to the small ribosomal subunit in a manner that promotes both eIF4A function and a receptive state of the mRNA binding channel, rather than by acting as a ssRNA-binding protein. To further dissect the molecular functions of eIF4F and eIF4B, we are probing the role of known phosphorylation sites of these factors that are predicted to affect interaction with the ribosome and mRNA. We have mutated a number of these sites to phosphodeficient and phosphomimetic forms, and analyzed the effects on growth and translation in vitro and in vivo. Our data indicate that the phosphorylated form of eIF4A is required for resistance to membrane and cell wall stresses, while the dephosphorylated form promotes resistance to LiCl, an inhibitor of glycolytic pathways. We are currently analyzing specific in vitro and in vivo changes in response to phosphorylation of eIF4A, to better understand how it modulates translation under normal conditions and in response to stress. These studies illustrate dynamic changes conferred by modification of a general translation initiation factor in response to diverse environmental conditions.

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758 Translational Frameshifting Induced by Mutually Jostling Between Ribosomes

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The ribosome is a ubiquitous protein manufactory for all living creatures. There are usually copious ribosomes co-translating on one mRNA, forming a complex called polyribosome. Generally, a translating ribosome has a chance to shift its reading frame on a slippery sequence, usually with a nucleotide pattern of XXXYYYZ, followed by an mRNA secondary structure like a hairpin or pseudoknot as a stimulator to produce different protein products from the same nucleotide sequence. We find that frameshifting can occur in polyribosomes without mRNA secondary structures via the interaction between two neighboring ribosomes. In that case, a downstream ribosome mimics the role of an mRNA structure to promote the following ribosome to shift its reading frame backward on the slippery sequence. Conversely, an upstream ribosome can press the preceding ribosome as well to cause it to slide forward on the slippery sequence. Our research demonstrates that the interaction between ribosomes can affect their behavior and lead to different translation outcome. In addition, the mRNA secondary structure following a slippery sequence may act as an obstacle to provide a force to push ribosomes to slip on mRNA.

759 Multi-protein Bridging Factor 1(Mbf1), Rps3 and Asc1 prevent stalled ribosomes from frameshifting.

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To ensure the fidelity of translation, ribosomes accurately maintain the reading frame throughout the coding region, although in some cases, ribosomes execute programmed frameshifts to regulate gene expression. In bacteria, ribosomes that are slowed by some suboptimal codons are sometimes rescued by frameshifting. In the yeast Saccharomyces cerevisiae, ribosomes translate 12 inhibitory codon pairs, including CGA-CGA, slowly and inefficiently, but do not frameshift at these pairs in wild-type yeast. However, ribosomes lacking a small ribosomal protein Asc1, homolog of human RACK1, efficiently frameshift at CGA-CGA codon pairs. It is unclear how Asc1 prevents frameshifting and by what mechanism, wild-type yeast cells maintain the reading frame during slow translation.

In a selection for mutants that frameshift at CGA codon repeats in yeast, I identified mutations in the Multi-protein Bridging Factor 1 gene (MBF1) and in a specific region of the universally conserved small ribosomal protein gene RPS3. I will present evidence that these two proteins function similarly in reading frame maintenance. By contrast, Asc1, which mediates both read-through and frameshifting at CGA-CGA pairs, appears to play a related, but distinct role from Mbf1, based on two observations. First, deletions of both ASC1 and MBF1 exhibit much more frameshifting than either single mutant, indicative of mutants induce efficient frameshifting at the sameΔdistinct roles. Second, mbf1 codon pairs at which Asc1 mediates read-through, indicative of related roles. In the double mutant, frameshifting occurs at the seven most slowly translated codon pairs in yeast, evidence of the link between slow translation and frameshifting.

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760 Studies of an Altered-Substrate, β-Amino Acid Translating Ribosome, P7A7.

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The ribosome the most efficient tool for generation of sequence-defined polymers, but remains limited to catalysis on α-amino acids. While the ribosome easily translates a wide variety of α-amino acids, many with exotic side-chains, it struggles to handle any backbone-modified substrates. Due to their differences in size, flexibility, and reactivity, monomers including peptoids, N-methyl amino acids, and β-amino acids are barely tolerated as single insertions, let alone for consecutive polymerizations. These issues are some of the primary roadblocks for the potential of the ribosome as a platform for more general sequence-defined polymer synthesis. As first steps to overcome these challenges, screens have identified an *E. coli* ribosome, P7A7, with a mutant 23S rRNA that can incorporate a single β-amino acid monomer with greatly improved efficiency over wild type. Here we present structural, *in vitro*, and *in vivo* studies of P7A7 as steps to understand its unusual catalytic abilities and as a guide for more general ribosome engineering towards exotic, non-α-amino acid polymerization.

761 RNA Pseudoknots: Folding and Interaction with the Ribosome

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Ribosomal frameshifting is stimulated by the mRNA that forms specific structures in the coding region. The common structures are hairpins or more complex pseudoknots, in which tertiary interactions, such as base triples, are present to stabilize the conformation. How RNA structures stimulate ribosomal frameshifting has been extensively studied, but the underlying molecular mechanism is still not clear. Here we used single-molecule approaches, including optical tweezers and smFRET, to study the folding of a model RNA pseudoknot (DU177) and its interaction with the ribosome to characterize the important RNA structural features. Our results show that the stem-loop S1-L1 of DU177 was the first secondary structure to fold, followed by base-pairing of stem S2 to form the pseudoknot. The second step was slow and frequently resulted in misfolded pseudoknots. Masking the last two or four nucleotides of stem S2 by complementary DNA strands greatly rescued the pseudoknot from misfolding. On the other hand, masking the first two nucleotides of stem S1 made this stem less stable and thus favored the structural folding through the stem-loop S2-L2 instead, followed by instant base-pairing of stem S1. This folding pathway yielded in structures, all of which were native pseudoknots. Moreover, the two masked nucleotides were recovered from the complementary DNA strand to form the full-length stem S1 during the folding. The result suggests that the well-folded pseudoknot, which contains several base triples among the interior stems and loops, would strengthen the integrity of the upstream stem S1. The stem S1 is the first target of the ribosome when it encounters the pseudoknot during translation. This would provide a mechanical explanation for ribosomal frameshifting. In fact, we further observed that the native pseudoknot was resistant to unfolding by the ribosome, which was programmed to translocate stepwise through the pseudoknot, whereas a mutant RNA that lacked the core base triples to maintain the structural strength failed to resist ribosomal translocation. Thus, our data provide great insights into RNA pseudoknot folding and the structural features that may affect ribosomal frameshifting.

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762 The Ribo-Interactome Reveals Novel Post-Translational Modifications of Ribosomal Proteins

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Ribosome immuno-precipitation enables isolation of hundreds of previously unidentified ribosome-associated proteins (RAPs). Among these RAPs is Ufl1, the E3 ligase for a ubiquitin-like post-translational modification called Ufm1. Substrate proteins modified by Ufm1 have been implicated in the ER stress response and in estrogen receptor signaling. Here we report that Ufm1 is conjugated to the ribosome and that ufmylated substrates are detectable in 60S and 80S polysome fractions. Currently, we are investigating the potential roles of ufmylation in ribosome biogenesis, mRNA selectivity, ER-associated translation, and other means of translational regulation.

763 Global analysis of translation processivity in *E. coli*

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During translation, the ribosome sometimes fails to complete synthesis of full-length protein product. Based on previous reporter gene studies, mutations that slow down elongation reduce translation processivity. It was envisioned that longer decoding dwell time causes a higher frequency of peptidyl-tRNA drop-off in these mutant ribosomes. Here, we used ribosome profiling to globally measure the processivity errors in wild-type and various mutant strains. In contrast to the earlier predictions, we have found that translation processivity is generally independent of decoding rate. Our findings suggest that processivity errors mainly occur during the translocation step of elongation.

764 Translational regulation in development and regeneration

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Regulation of gene expression is of critical importance during embryonic development as it guides the proliferation, differentiation and migration of cells and the formation of discrete organs and tissues. While the role of transcription in this process has been widely studied, regulation at the level of mRNA translation has historically received less attention and little is known about the role, or indeed, the extent of translational regulation during vertebrate development. To address this critical question, we used state-of-the-art ribosomal profiling to track mRNA translation at a genome-wide-level in the developing mouse embryo at mid-gestation. We demonstrate that the mesoderm, one of the three embryonic germ layers, which gives rise to a variety of cell types ranging from blood to bone, is subject to extensive translational regulation. Most intriguing, is our finding that more than 300 genes encoding core components of the developmental signaling toolkit, including multiple components of the MAPK, PI3K, FGF, WNT, Hippo and SHH pathways are under robust translational repression arises from cis-acting elements in the 5' untranslated regions (5'UTRs) of regulated mRNAs. Using CRISPR-mediated mutagenesis of ES cells, coupled with in vitro differentiation into neurons, we definitively demonstrated that translational repression is encoded in a complex landscape of upstream start sites peppered across 5'UTRs of translationally repressed genes. Comparing translation efficiency between tissues, neural tube and limb, we demonstrate that translation regulation of core developmental signaling cascades acts to diversify gene expression across tissues and we explore the role of the EIF3 translation initiation complex in tuning this tissue-specific signaling. Lastly, we explore the role of rapid translational remodeling as a means of enacting rapid wound closure and limb regeneration in the axolotl, a species of aquatic salamander. Together, these studies push the boundaries of our current understanding of the scope and the functional significance of translational regulation in the life cycle of complex vertebrates.

765 Variants of the 5'-terminal region of p53 mRNA influence the ribosomal scanning and translation efficiency

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The p53 protein is one of the major cell cycle regulators. The protein is expressed as at least twelve protein isoforms resulting from the use of alternative promoters, alternative splicing or downstream initiation codons. Importantly, there is growing evidence that translation initiation of p53 mRNA may be regulated by the structure and length of the naturally occurring variants of the 5′-terminal region of p53 mRNA transcripts. Here, several mRNA constructs were synthesized with variable length of the p53 5′-terminal regions and encoding luciferase reporter protein, and their translation was monitored continuously *in situ* in a rabbit reticulocyte lysate system. Moreover, four additional mRNA constructs were prepared. In two constructs, the structural context of AUG1 initiation codon was altered while in the other two constructs, characteristic hairpin motifs present in the p53 5′-terminal region were changed. Translation of the last two constructs was also performed in the presence of the cap analogue to test the function of the 5′-terminal region in cap-independent translation initiation. Superposition of several structural factors connected with the length of the 5′-terminal region, stable elements of the secondary structure, structural environment of the initiation codon and IRES elements greatly influenced the ribosomal scanning and translation efficiency. This work was supported by the National Centre of Science grant No. 2013/09/B/NZ1/01884. This publication was also supported by the Polish Ministry of Science and Higher Education under the KNOW program.

766 Potential involvement of La protein in the nuclear pre-tRNA splicing pathway

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La is an abundant RNA-binding protein found throughout eukarya. La facilitates and orders the complex process of pre-tRNA maturation; by high-affinity sequence-specific binding to the universal pre-tRNA trailer, 3' UUU-OH, it facilitates 3' endonucleolytic processing by protecting pre-tRNAs from 3' exonucleases, and also exhibits RNA chaperone activity. Its chaperone function can 'rescue' structurally challenged pre-tRNAs from nuclear surveillance-mediated decay and assist them in adopting conformations that facilitate productive processing. In all eukaryotes examined, with the exception of fungi, La proteins contain an additional C-terminal RNA recognition motif of unknown function. La is dispensable in yeasts but essential for mouse and other eukaryote viability; it's tempting to speculate that this RRM2a mediates an essential activity that was lost in fungi.

Pre-tRNA splicing is essential in all eukaryotes and defects are associated with heritable disorders in humans. While tRNA biogenesis is otherwise highly conserved there is a striking divergence in the subcellular localization of pre-tRNA splicing; in animals it occurs in the nucleus whereas in yeast it occurs in the cytoplasm. In yeast, nuclear intron-containing pre-tRNAs are 5' and 3' processed before export to the cytoplasm. Thus, loss of the 3' UUU-OH trailer before splicing leaves no role for La in pre-tRNA splicing in yeast. However, in mammals the abundant La has ample opportunity to interact with the nuclear pre-tRNA splicing pathway. In fact, deletion of La in mouse cortex leads to specific loss of leader- and trailer-containing spliced pre-tRNA intermediates, but not the corresponding intron-containing nascent transcripts. RNA IP of La from mouse cortex revealed that La is physically associated with unspliced as well as these spliced pre-tRNAs. This activity appears conserved, as human La is also associated with these pre-tRNAs before and after splicing has occurred. Here we characterize a potentially novel activity for mammalian La that interacts with the tRNA biogenesis pathway, which may be responsible for the essential function of La in metazoans. Data on the role of human La phosphorylation at serine 366 by protein kinase CK2, relevant to its activity for the different pre-tRNA intermediates will also be presented.

767 Identification of Nuclear-encoded Mitochondrial tRNAs Using Isotype-Specific Models and tRNAscan-SE 2.0

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Transfer of mitochondrial DNA to the nucleus has been an ongoing evolutionary process in many eukaryotes. With the improvement of sequencing and genome assembly techniques, researchers have discovered many more nuclear sequences of mitochondrial origin, named as NUMTs. Recently, over 700 segments of NUMTs were found scattered across the human nuclear genome. Multiple copies of mitochondrial genes with different levels of conservation exist in NUMTs, which indicate the relative age of DNA transfer. Previously, tRNAscan-SE 1.3 identified 27 human tRNA genes that are located in NUMTs. Thus, we undertook a comprehensive analysis of nuclear-encoded mt-tRNAs in the genome.

Using the latest version of the Infernal covariance model search software, we developed a new multi-model searching strategy for tRNAscan-SE 2.0, where input sequences can be assessed with the full set of isotype-specific covariance models. To detect the mt-tRNAs in NUMTs, we scanned the human genome with the mammalian mt-tRNA covariance models integrated in tRNAscan-SE. We identified 748 likely mt-tRNAs in the nuclear chromosomes, 15% of which are located outside of the previously noted NUMTs regions. This suggests that an alternate method may be needed to better identify NUMTs. Notably, these nuclear-encoded mt-tRNAs (nmt-tRNAs) are more numerous than the high confidence set of 417 cytosolic human tRNA genes. With the aid of small RNA-seq data and epigenetic chromatin modification data, we re-examined these predictions and found that most do not show significant abundance and appear to be epigenetically silent. Although a small number of human diseases have been shown to be caused by NUMT integration, the function and possible effect of the nmt-tRNAs in the nuclear genome is still not known. Comparison and analysis of nmt-tRNAs across multiple species may help better understand the biological importance mitochondrial tDNA transfer and their unique patterns of conservation.

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768 Elucidating the biological significance of multiple, parallel primary tRNA nuclear exporters in yeast cells

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Eukaryotic tRNAs are transcribed in the nucleus and are exported to the cytoplasm. Some tRNA genes also encode introns that must be removed from the pre-tRNAs. In budding yeast, end-processed and modified intron-containing as well as intronless primary tRNAs are exported to the cytoplasm primarily by the canonical tRNA nuclear exporter Los1/Exportin-t and splicing occurs post nuclear export in the cytoplasm. Our recent genetic, cytological and biochemical data revealed the mRNA exporter Mex67-Mtr2 heterodimer co-functions with Los1 in tRNA nuclear export. We are currently assessing whether the nuclear protein exporter Crm1, implicated by genetic studies, also directly functions in tRNA nuclear export. The question thus arises: why do cells have multiple tRNA nuclear exporters? Nuclear tRNA export by Los1 provides an initial tRNA quality control step to generate a pool of functional tRNAs as Los1 preferentially binds to end-processed, appropriately structured tRNAs. However, proofreading of pre-tRNAs by Los1 is not error-free, as low levels of aberrant tRNAs are detected in Los1 over-expressed yeast cells. Thus, we sought to assess the fidelity of Los1-independent tRNA nuclear export pathways in yeast. Consistent with previous studies, we show that Los1-mediated export pathway proofreads pre-tRNA 5' and 3' ends, as enhanced levels of 5',3'-end extended, spliced tRNAs are detected in *los1Δ* cells. Compared to Los1, Mex67-Mtr2 exhibits error-prone tRNA nuclear export, as 5'-end unprocessed, but spliced tRNAs are detected in elevated levels in yeast cells when Mex67-Mtr2 is over-expressed. Interestingly, we also observe that individual tRNA exporters have differential tRNA substrate specificities, raising the possibility that the cellular proteome can be controlled at the level of tRNA nuclear export. Thus, these multiple, parallel, nuclear tRNA export pathways may confer selective advantage to the yeast cells under specific conditions.

769 In vitro-based workflow for exogenous and endogenous expression of tissue- and disease-specific tRNAs

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Transfer RNAs are the largest, most complex non-coding RNA family, universal to all living systems. Often disregarded as passive players in gene expression, renewed focus on eukaryotic tRNA biology has revealed their involvement in regulatory pathways outside their fundamental function in mRNA translation. Recent evidence suggests tRNAs undergo tissue-specific expression, processing, and modifications, resulting in a constellation of tRNAs and tdRNAs (tRNA-derived small RNAs) with the potential to regulate myriad pathways of gene expression in response to changes in the cellular and extra-cellular environments. Furthermore, dysregulation of expression of both tRNA and tdRNAs have been shown in a variety of diseases. Unfortunately, eukaryotic tRNA/tdRNA expression and modification data is almost entirely lacking due to difficulty in measuring both in a high-throughput manner. Moreover, certain tRNAs and tdRNAs appear to be expressed in specific tissues, if at all, making in vitro study of their expression and effects on global gene expression cumbersome. Here, we present data establishing a workflow for HEK293-based tRNA expression using both plasmid overexpression and CRISPR/Cas9 activation of specific tRNA genes. Focusing on tRNAs/tdRNAs with known or putative tissue- and disease-specific expression and processing, we are able to show their specific overexpression using Northern Blot analysis. Using these tools to modulate in vitro expression of specific tRNA and tdRNAs will allow for deeper biochemical analyses of those atypical tRNA and tdRNAs discovered using high-throughput tRNA-centric sequencing.

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770 Distinct substrate specificities of human tRNA methyltransferases TRMT10A and TRMT10B

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Substantial biological resources are invested into producing functional tRNA molecules, including an extensive system of post-transcriptional nucleotide modifications. In this universal process, chemical functional groups are changed, rearranged, and added to individual nucleotides in a specific pattern for each target tRNA. One such modification is the addition of a methyl group to the N-1 atom of ninth-position purines (m1N9), which occurs in archaea and eukarya and is catalyzed by the Trm10 family of enzymes. We sought to explain the unusual presence of two predicted cytosolic Trm10 homologs (TRMT10A and TRMT10B) in humans and other metazoa. A yeast genetic system established in our lab suggested that TRMT10A and TRMT10B are not functionally redundant, leading to the use of in vitro approaches to investigate the substrate specificities of each enzyme. Using a combination of kinetic and binding assays, we demonstrate that human TRMT10A and TRMT10B exhibit distinct tRNA substrate specificities that are consistent with known modification patterns in human tRNA. Ongoing experiments suggest a new model for control of Trm10 substrate recognition based on interactions between Trm10 and other tRNA modification enzymes, which had not been previously observed for this enzyme family and has important implications for the behavior of Trm10 enzymes in diverse biological systems.

771 Comprehensive sequence analysis of tRNAs in the three domains of life

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Transfer RNAs (tRNAs) are short non-coding RNAs, and typical cytoplasmic tRNAs are approximately 70–90 ribonucleotides long. The secondary structure of almost all tRNA molecules is a cloverleaf structure fold with four arms (the acceptor arm, D-arm, anticodon arm, and TΨ-arm) and is essentially conserved in the three domains of life, the Bacteria, the Archaea, and the Eukarya. tRNAs adopt an L-shaped three-dimensional structure, with the anticodon region at one end and the CCA acceptor sequence on the other.

In recent years, Next-Generation Sequencing technology has yielded a huge amount of tRNA sequence data that contribute toward a more accurate understanding of the molecular evolution of tRNAs. Therefore, a comprehensive sequence analysis of the canonical tRNAs was conducted in 83 bacterial, 182 archaeal, and 150 eukaryotic species. Our results showed that two main classes of nucleotide regions are broadly conserved; (1) the three tRNA regions (the anticodon loop and the CCA terminal region, and the D-stem) that interact with aminoacyl-tRNA synthetase, and (2) the two additional loop regions (D-loop and TΨC-loop) known to be important to form the tRNA L-shaped structure. We also found sequence conservation near the tRNA discriminator in the Bacteria and Archaea, and an enormous number of non-canonical tRNAs in the Eukaryotes; over 59% of the eukaryotic tRNAs did not conform to the conventional tRNA numbering rules. This is the first global view of tRNA nucleotide conservation and/or evolution based on the unprecedented number of sequence data.

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772 Structural and functional characterization of the mammalian tRNA ligase complex

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Eukaryotic tRNA ligases play critical roles in tRNA splicing, unfolded protein response and RNA repair. In metazoa, tRNA ligase activity is provided by the unconventional GTP-dependent ligase HSPC117 (RtcB) which is part of the pentameric tRNA ligase complex together with a DEAD-box helicase DDX1 and subunits FAM98B, CGI-99 and Ashwin of unknown function. Interestingly, the activity of the complex is not limited to RNA ligation but has been implicated in axon regeneration, RNA transport along microtubules and transcription modulation. Our aim is to obtain structural insights into the molecular mechanisms of the tRNA ligase complex to shed light on its roles in cellular RNA metabolism.

To date, we have used cross-linking-coupled mass spectrometry and biochemical assays to define the basic architecture of the human tRNA ligase and determined the crystal structure of the catalytic subunit HSPC117. We have also determined the crystal structure of the N-terminal domain of the CGI-99 subunit, revealing that it adopts a calponin homology fold and suggesting that it might mediate actin or microtubule binding. We are currently investigating this putative activity along with continuing our characterization of the assembly of the entire tRNA ligase complex.

773 Monitoring the 5-methoxycarbonylmethyl-2-thiouridine (mcm5s2U) modification in eukaryotic tRNAs via the gamma-toxin endonuclease

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Uridines located at the wobble position of eukaryotic tRNAs often undergo the addition of multiple methyl groups and thiolation. The 5-methoxycarbonylmethyl-2-thiouridine (mcm5s2U) modification has been linked to the modulation of translation, specifically of transcripts necessary for protein stress response pathways. Until now, the current detection methods for mcm5s2U modifications require instrumentation and technical expertise beyond the capabilities of many laboratories. Here, we explore the utilization of the gamma-endonuclease as a detection method of mcm5s2U modification in a diverse array of eukaryotic species. We show that gamma-toxin endonuclease from the yeast *Kluyveromyces lactis* can be used as a probe for assaying mcm5s2U status in the tRNA of diverse organisms ranging from protozoans to mammalian cells, highlighting the evolutionary conservation of the modification in eukaryotes. Our technique involves a brief incubation of purified gamma-toxin with total RNA followed by either Northern blotting or quantitative RT-PCR to detect tRNA cleavage. Additionally, this assay is highly specific for the detection of the mcm5s2U modification as tRNAs possessing mcm5U or mchm5U failed to undergo cleavage by the endonuclease. The ease and cost efficiency of this assay renders itself useful for a wide range of biological questions involving the role of the mcm5s2U modification *in vivo*. We have also used this assay to characterize predicted *Caenorhabditis elegans* homologs of Trm9 and Trm112. Our findings demonstrate the use of purified gamma-toxin to monitor the mcm5s2U modification in a wide range of eukaryotic organisms with implications to better understand the dynamic nature and biological role of this modification, as well as for the discovery of novel proteins involved in mcm5s2U formation.

774 Evidence supporting the role for Mtr10 in tRNA nuclear import

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In eukaryotes, tRNAs are transcribed in the nucleus and subsequently exported to the cytoplasm where they serve as essential adaptor molecules in protein synthesis. Once in the cytoplasm, however, tRNA can be trafficked back into the nucleus in an evolutionarily conserved process termed "retrograde tRNA nuclear import". Several functions of this process have been identified, including maturation of select tRNA species, tRNA quality control, translation regulation and cell cycle control, as well as import of viral reverse transcription complexes, such as HIV. However, the mechanism of retrograde tRNA nuclear import, particularly under non-stress conditions remains unclear. One protein believed to mediate this process in *S. cerevisiae* is the karyopherin, Mtr10. Here, we provide genetic, molecular and biochemical evidence supporting a role for Mtr10 as a retrograde tRNA nuclear importer. First, by assessing in situ tRNA subcellular localization, our genetic data indicates that Mtr10 functions upstream of two proteins involved in tRNA nuclear re-export, Los1 and Msn5. Although *los1Δ* and *msn5Δ* cells exhibit nuclear accumulation of tRNA upon amino acid deprivation, *los1Δmtr10Δ* and *msn5Δmtr10Δ* double mutants do not, similar to *mtr10Δ* mutants. Secondly, we developed an RT-PCR assay that takes advantage of the reverse transcription-blocking methylation of G_{37} on certain tRNAs. This modification is catalyzed by the nuclear-localized modification enzyme, Trm5. Since Trm5 recognizes spliced tRNAs, but not intron-containing pre-tRNAs, and intron splicing in yeast occurs in the cytoplasm, any spliced tRNA with the m^1G_{37} modification is evidence of cytoplasmic to nuclear retrograde trafficking. Cells lacking Mtr10 show an increase in m^1G_{37}-hypomodified tRNA as compared to wild-type cells, indicating an inability of these cytoplasmic tRNAs to access the nucleus. We have expanded this assay to be performed on a genome-wide level in yeast to screen for additional proteins involved in tRNA nuclear import. Thirdly, we are testing whether Mtr10 functions directly in tRNA nucleocytoplasmic dynamics using co-immunoprecipitation assays followed by RT-PCR. Our current data supports an interaction between Mtr10 and multiple tRNA species. Together these findings support a role for Mtr10 as a tRNA nuclear importer and highlight the importance of this protein in modulating numerous key aspects of cellular physiology.

775 Large-scale phylogenetic analysis and diversity of Clp1 polynucleotide kinase family protein

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In many eukaryotes and archaea, transfer RNA (tRNA) genes often possess an intron in their anticodon loop region, and therefore exact pre-tRNA splicing is required to produce mature and functional tRNAs. It has been reported that polynucleotide kinase (Clp1) phosphorylates the 5'-end of the tRNA 3'-exon half during tRNA splicing. However, the research of the molecular evolution of Clp1 is still in its early stages. In the present study, we conducted the large-scale molecular evolutionary analysis of Clp1 to find out their structural diversification and phylogenetic distribution. The BLASTp search against the UniProt Knowledgebase (99,261,416 entries as of November 20, 2017) identified 2,890 Clp1 homologous proteins in the three domains of life. Among the representative species, Clp1 homologous proteins were found in most of eukaryotes (18/18; 100%) and archaea (11/18; 61%), but in the limited number of bacteria (3/36; 8.3%). The phylogenetic distribution of Clp1-related proteins involved in pre-rRNA processing such as Nol9 and Grc3 was also examined. We found approximately 100 novel proteins having both Clp1-like domain and other functional domains, and their length in amino acids (1,000-2,700 aa), are longer than those for most of the Clp1 homologous proteins (up to 1,000 aa). The domain structure and the motif essential for phosphorylation were conserved in the Clp1 proteins of bacteria and archaea, suggesting that bacterial Clp1 may have been acquired through horizontal gene transfer. Finally, in order to investigate the polynucleotide kinase activity of the predicted bacterial Clp1 proteins, we produced the recombinant bacterial Clp1 proteins in *Escherichia coli* and examined their activities. Possible evolutionary scenarios of Clp1 family proteins and their profiles of the polynucleotide kinase activities will be discussed.

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776 Characterization of tRNA intronic circular (tric)RNA biogenesis

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Mature tRNAs are generated by multiple post-transcriptional processing steps, which can include removal of introns. Recently, our lab discovered a new class of metazoan circular RNAs, formed by intramolecular ligation of excised tRNA introns. We term these molecules tRNA intronic circular (tric)RNAs. We have found tricRNAs to be stable, highly abundant, and evolutionarily conserved.

To investigate the mechanism of tricRNA biogenesis, we generated reporters that replace the majority of the endogenous introns of two *Drosophila* tRNA genes with the Broccoli fluorescent RNA aptamer. Using these reporters, we investigated the *cis*-acting elements required for proper tricRNA splicing in human and animal cells. We observed that disrupting the anticodon-intron base pair, which is a conserved feature of eukaryotic pre-tRNAs, results in dramatically reduced tricRNA production. Although the integrity of this base pair is necessary for tricRNA splicing, it is not sufficient. Furthermore, we found that strengthening the weak base pairs that are proximal to the anticodon-intron base pair also leads to a reduction in tricRNA formation. The presence of a weakly pairing stem likely facilitates proper splicing.

In addition to these *cis* element studies, we have also used the Broccoli reporter to identify tricRNA processing factors. We found that several known tRNA processing factors, such as the RtcB ligase and components of the TSEN endonuclease complex, are involved in tricRNA biogenesis, as depletion of these factors reduces proper tricRNA splicing. Interestingly, we observe that depletion of cbc, a kinase whose human homolog (Clp1) has been shown to phosphorylate tRNA 3' exons *in vitro*, results in increased tricRNA levels, both endogenous and reporter. These findings support a model wherein Clp1 is an *in vivo* negative regulator of tRNA biogenesis. Furthermore, we identified Dis3 as a potential tricRNA endonuclease, as its depletion increases tricRNA levels, whereas its overexpression decreases them. In summary, we have characterized the major players in the tricRNA biogenesis pathway, and provide evidence for a negative regulator of tRNA and tricRNA biogenesis.

777 Detecting novel genetic code reassignments

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Almost all of life uses the same genetic code for translation, suggesting two possibilities: either the genetic code has reached an optimal state, or that changes to the genetic code are so deleterious that it is unable to evolve further. The existence of alternate genetic codes demonstrates that the genetic code can evolve to some degree; however, the evolutionary trajectories by which this happens are still poorly understood. To better study how the genetic code evolves, we have developed a computational tool that can systematically search for new alternate genetic codes among sequenced organisms and characterize their phylogenetic distribution. This tool uses conserved protein domains encoded in nucleotide sequences to quickly and accurately infer the amino acid meaning of each codon. Using this method, we have identified several yeast species that use an ambiguous genetic code and encode two different isotype tRNAs with the same anticodon, which may resemble an intermediate stage in genetic code evolution.

778 Using DNA sequence variation to infer tRNA activity

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Transfer RNAs (tRNAs) are fundamental to protein synthesis, and are among the most highly conserved and frequently transcribed genes in all living things. However, the evolutionary forces on genomes at tRNA loci are not well characterized. Comparative genomics analyses of humans and a variety of model eukaryotes provide strong evidence that transcription-associated mutagenesis and strong purifying selection drive patterns of sequence variation within and surrounding tRNA genes. In the human and mouse genomes, we find highly significant positive correlations between tRNA conservation, flanking region divergence and tRNA transcription profiles. The strength of these correlations enables broad inference of tRNA activity across almost any mammalian or vertebrate genome. By applying machine learning techniques to deep cross-species alignments, we identify tRNA loci likely to be constitutively expressed versus those likely to be inactive, as well as outliers producing tRNA-like transcripts that may be associated with alternative RNA processing pathways.

779 A gammaherpesvirus modulates host RNAPIII transcription at specific repetitive loci

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Increased RNA polymerase III (RNAPIII) activity is a phenotype observed in many types of cancerous and transformed cells, including those generated by infection with DNA tumor viruses. RNAPIII is responsible for transcribing several classes of noncoding RNAs (5S RNA, 7SL, tRNAs, etc.) that are vital for cell growth and metabolism. Additionally, a highly abundant class of repetitive mobile elements called short interspersed nuclear elements (SINEs) are transcribed by RNAPIII. Though the ~1 million SINE loci in our genomes are normally silenced, viral infection can trigger an epigenetic switch to stimulate expression of noncoding RNA from specific SINE loci. Previously we showed that cytoplasmic B2 SINE RNAs produced during murine gammaherpesvirus 68 (MHV68) infection activate the NF-kB pathway, a manipulation that results in enhanced viral gene expression. Prior to this work, SINEs were the only RNAPIII gene class known to be induced during MHV68 infection. Here, we present evidence that a subset of tRNA genes are also transcriptionally upregulated. We see increased abundance of pre-tRNAs belonging to several different isotypes in infected fibroblasts. Interestingly, infection-induced pre-tRNAs are exported to the cytoplasm following infection, indicating that the fate of these pre-tRNAs does not follow the canonical maturation pathway. We are currently performing Dm-tRNA-Seq to identify upregulated tRNA loci and investigating the fate of infection-induced pre-tRNAs. Additionally, future work investigating the mechanism of increased RNAPIII activity at specific SINE and tRNA loci during infection should reveal how gammaherpesviruses alter the epigenetic landscape of the cell. As increased expression of both SINEs and tRNAs have been reported in a variety of cancers, gammaherpesviruses may hijack RNAPIII activity to help establish an oncogenic growth environment.

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780 From powerhouse to warehouse: novel and conserved role of mitochondrial outer membrane proteins in tRNA splicing

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tRNAs function to deliver amino acids to ribosomes during protein synthesis. In all studied eukaryotes, splicing of intron-containing tRNAs is essential for protein synthesis because at least one tRNA species is exclusively intron-containing. Intron removal is catalyzed by the heterotetrameric tRNA splicing endonuclease (SEN) complex. In yeast *S. cerevisiae*, the SEN complex is located on the cytoplasmic surface of mitochondria. However, whether the mitochondrial localization of SEN complex is conserved has been controversial. Moreover, how and why SEN subunits (Sen2, Sen15, Sen34, and Sen54) assemble on the surface of mitochondria are unknown. A genome-wide screen from our lab discovered that deletion of genes encoding mitochondrial outer membrane proteins *TOM70* or *SAM37* causes pre-tRNA splicing defects (Wu et al., 2015). Here we report that end-matured, intron-containing tRNAs accumulate in the cytoplasm in *tom70D* and *sam37D* strains. The pre-tRNA splicing defect is not due to the loss of respiratory metabolism, but rather to altered association of SEN subunits to the mitochondrial surface. Employing molecular, cellular, and biochemical biology approaches of strains with each SEN subunit tagged with GFP at its endogenous locus in wild-type*, tom70Δ* and *sam37Δ* backgrounds, we documented that the quantity of SEN subunits on mitochondria was significantly reduced in the mutant cells compared to wild-type cells. Thus, our data indicate that via direct or indirect interactions, Tom70 and Sam37 are required for the proper localization, assembly, and catalysis of the SEN subunits on mitochondria. We also report that in *S. pombe*, endogenous Sen2, Sen34 and Sen54 localize to mitochondria, and that Tom70 is required for their mitochondrial localization, documenting a mechanism that has been conserved for >500 million years. Several other RNA processing steps, including tRNA modifications, and piRNA 5' and 3' maturation in metazoans, occur on the mitochondrial surface and thus, the mitochondrial surface seems to function as a "warehouse" for RNA processing, in addition to the canonical mitochondrial role as the cellular "powerhouse". Our studies provide insights into how and why mitochondrial outer membrane proteins function in tRNA processing in two evolutionarily divergent model organisms and this may be relevant to other RNA processing steps in metazoans.

781 RNA structure in Influenza A virus ribonucleoproteins

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The Influenza A virus (IAV) genome consist of eight (-) sense viral RNA (vRNA) segments of different length, each of them coding for at least one essential viral protein. A heterotrimeric polymerase complex is bound at the extremities of each vRNA, while internal parts of the vRNAs are associated with multiple copies of the viral nucleoprotein (NP) in order to form viral ribonucleoproteins (vRNP). NP forms a double helix on which vRNA binds. However, while the structure of the NP scaffold has been partially elucidated by cryo-EM, the structure of the vRNA within vRNPs remains elusive.

To address this question, we performed chemical probing of in vitro transcribed vRNAs 7, and 8 of the A/WSN/1933 (H1N1) and A/Puerto Rico/8/34 (PR8) strains using SHAPE (selective 2'-hydroxyl acylation analyzed by primer extension).

The secondary structure of naked vRNAs appears highly structured, although most helices (70-85% depending on the considered vRNA) contain less than 6 base-pairs. We next compared the SHAPE reactivity of "naked" vRNAs 7 and 8 with those of vRNA/NP complexes formed in vitro. The vRNAs were either modified by SHAPE reagent in complex with NP or after removal of the protein, allowing us to discriminate the NP binding sites from its chaperone activity, respectively.

These experiments address the following questions: 1) does NP recognize specific sequence and/or structural motifs? 2) to what extent does NP destabilize vRNA secondary structures? 3) does NP permanently modify vRNA secondary structures? (i.e. does NP possess an RNA chaperone activity?). We observed many protected sites in single stranded but also double-stranded regions that appear to become single-stranded with NP binding. Moreover, in some vRNA regions, the NP seems to have a chaperone activity, suggesting that once the vRNA/NP complex is formed the RNA conformation is changed and subsists even after the removal of the protein.

Taken together, our results suggest the NP destabilizes RNA helices adjacent to its binding sites. Another possibility could be that NP not only binds to single-stranded region but also to helices, in an asymmetric manner.

This work was supported by grant ANR-15-CE11-0014-01 'PSiFlu' from the Agence Nationale de Recherche (ANR) to RM.

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782 An RNA-Centric Dissection of Host Complexes Controlling Flavivirus Infection

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Flaviviruses including dengue virus (DENV) and Zika virus (ZIKV) cause significant human disease. Co-opting cellular factors for viral translation and viral genome replication at the endoplasmic reticulum (ER) is a shared replication strategy, despite different clinical outcomes. While the protein products of these viruses have been studied in depth, how the RNA genomes operate inside human cells is poorly understood. Using comprehensive identification of RNA binding proteins by mass spectrometry (ChIRP-MS), we took an RNA-centric viewpoint of flaviviral infection and identified several hundred proteins associated with both DENV and ZIKV genomic RNA in human cells. Genome-scale knockout screens assigned putative functional relevance to the RNA-protein interactions observed by ChIRP-MS. ER RNA binding proteins such vigilin and RRBP1 directly bound viral RNA and each acted at distinct stages in the life cycle of flaviviruses. Thus, this versatile strategy can elucidate features of human biology that control pathogenesis of clinically relevant viruses.

783 CBP80/20-dependent translation initiation factor (CTIF) inhibits HIV-1 Gag synthesis by targeting the activity of the viral protein Rev

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The Human Immunodeficiency Virus type-1 (HIV-1) Gag polyprotein is synthetized from the unspliced mRNA in a process involving a non-canonical nuclear export pathway as well as cap-dependent and cap-independent mechanisms of translation initiation. The viral protein Rev is critical in ensuring proper Gag synthesis during viral replication by acting as the master post-transcriptional regulator of the unspliced mRNA. Previous data have suggested that translation of the unspliced mRNA occurs by a cap-dependent, CBC-driven mechanism, which is active during a virally-induced inhibition of eIF4E activity. In addition, it was show that Rev interacts with the CBC subunit CBP80, suggesting that Rev is involved in CBC-mediated translation. However, the role of other components of the CBC-dependent translation initiation complex is unknown. Here, we show that the CBP80/20-dependent translation initiation factor (CTIF) acts as a strong inhibitor of Gag synthesis by interfering with the activity of the viral protein Rev. We also report that the N-terminal, CBP80-binding domain of CTIF, contains all the determinants required for this inhibitory activity. As such, both full-length CTIF and the CBP80-binding domain interacts with Rev inducing the retention of the viral protein in the cytoplasm. These data reveal an unexpected role of CTIF as a cellular restriction factor for HIV-1 gene expression.

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784 Structure-Activity Relationship of Raloxifene Analogs as Potential Hepatitis B Virus Therapeutics, Through Targeting of Encapsidation Signal, Epsilon, of Viral Pregenomic RNA

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Despite the advent of an effective vaccine against HBV, 300 million individuals are living infected with chronic Hepatitis B virus (HBV) which results in cirrhosis and hepatocellular carcinoma. Current nucleos(t)ide analog treatments target viral reverse transcriptase, are successful at reducing viral load but do not clear the virus. HBV encapsidation signal, epsilon (HBVε), a cis-acting regulatory RNA element of HBV pregenomic RNA (pgRNA), is involved in pgRNA packaging, as well as signaling for viral polymerase presents a novel method for treatment. Potential disruption of these events by small molecule interaction with HBVε presents an attractive drug target. To start, a library of 26,000 immobilized small molecules were screened against a fluorescently labeled 61nt HBVε, identifying an FDA-approved drug, raloxifene, a selective estrogen receptor modulator (SERM), as warranting further investigation. Biophysical, biochemical, and in silico testing revealed raloxifene interacts with the flexible 6-nt bulge region of HBVε with an IC50 of 69 uM. Herein we present the chemical synthesis of a focused library of analogs, their biochemical evaluation using a dye displacement assay and NMR study to provide insight into structure-activity relationship of this class of HBVε small molecule binders. Substitutions at positions 2 and 3 of the benzothiophene core of raloxifene were explored. Analogs with various linker composition at the 3-position were tolerated and improved binding to HBVε was achieved through further manipulation of this side. Changes to the 4-hydroxy group of benzene ring at the 2-position however were less tolerated and is being further investigated. Furthermore, chemical shift perturbation studies along with molecular docking of this class of compounds provided molecular detail of interaction with HBVε, In summary, a class of raloxifene-based small molecules have been developed and fully characterized to target a novel motif of HBVε.

785 N^6-methyladenosine modification and the YTHDF2 reader protein play cell type specific roles in lytic viral gene expression during Kaposi's sarcoma-associated herpesvirus infection

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Methylation at the N^6 position of adenosine (m^6A) is a highly prevalent and reversible modification within eukaryotic mRNAs that has been linked to many stages of RNA processing and fate. Recent studies suggest that m^6A deposition and proteins involved in the m^6A pathway play a diverse set of roles in either restricting or modulating the lifecycles of select viruses. Here, we report that m^6A levels are significantly increased in cells infected with the oncogenic human DNA virus Kaposi's sarcoma-associated herpesvirus (KSHV). Transcriptome-wide m^6A-sequencing of the KSHV-positive renal carcinoma cell line iSLK.219 during lytic reactivation revealed the presence of m^6A across multiple kinetic classes of viral transcripts, and a concomitant decrease in m^6A levels across much of the host transcriptome. However, we found that depletion of the m^6A machinery had differential pro- and anti-viral impacts on viral gene expression depending on the cell-type analyzed. In iSLK.219 and iSLK.BAC16 cells the pathway functioned in a pro-viral manner, as depletion of the m^6A writer METTL3 and the reader YTHDF2 significantly impaired virion production. In iSLK.219 cells the defect was linked to their roles in the post-transcriptional accumulation of the major viral lytic transactivator ORF50, which is m^6A modified. In contrast, although the ORF50 mRNA was also m^6A modified in KSHV infected B cells, ORF50 protein expression was instead increased upon depletion of METTL3, or, to a lesser extent, YTHDF2. These results highlight that the m^6A pathway is centrally involved in regulating KSHV gene expression, and underscore how the outcome of this dynamically regulated modification can vary significantly between cell types.

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786 Revisiting the RNA Genome Architecture of Influenza Virus

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Influenza A virus (IAV) genomes are composed of eight single-stranded RNA segments that are coated by viral nucleoprotein (NP) molecules, reminiscent of the nucleosomal 'beads-on-a-string' conformation. Using HITS-CLIP, we identified the NP binding profiles for two H1N1 IAV strains in virions. Contrary to the prevailing model for viral RNA packaging, NP does not bind uniformly along the entire length of segments, but instead exhibits an irregular pattern of both enriched and depleted regions of NP association. Even though NP has been shown to bind RNA in a sequence-independent manner, the NP binding profiles of highly conserved H1N1 strains are markedly distinct. By introducing small changes in nucleotide sequence, we observe that secondary structures facilitate NP binding, as evidenced by the induction of ectopic NP peaks. Intriguingly, these small changes affect the NP binding profile *in trans* at other regions of the genome as well. By conducting the RNA equivalent of 3C, we identify an intricate three-dimensional organization of the viral genome, which underlies the global NP binding profile. Taken together, our genome-wide study of NP interaction with viral RNA indicates that the textbook model of genome packaging is oversimplified and thus has implications for our understanding of the influenza viral life cycle.

787 Biophysical characterization of non-coding regions of Japanese Encephalitis Virus

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The Japanese encephalitis virus belongs to the Flaviviridae family of RNA viruses that also include other pathogenic viruses such as the dengue, West Nile, and Zika. These viruses have a conserved 3′ terminal region that interact with host proteins. In order to ascertain to which conserved RNA structures the host proteins interacts with, specifically, the proteins belonging to the DEAD-box family of helicases, we have designed multiple constructs of the terminal regions. We are studying the secondary structure of terminal regions using selective 2'-hydroxy acylation analyzed by primer extension method and the low-resolution 3-dimensional structures using small-angle X-ray scattering technique. We are combining the secondary structure information with the low-resolution structures and computational methods to obtain the high-resolution structure models. We will continue applying this approach to obtain solution structures of Japanese Encephalitis Viral terminal regions of host proteins. Our work on the interaction between the viral terminal regions and the host proteins will ultimately help us not only specify the interacting domains of host proteins and of the terminal region but also to determine if the conserved terminal regions between different Flaviviridae viruses also interact with the host proteins.

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788 Kaposi's sarcoma-associated herpesvirus ORF57 protein protects viral transcripts from two host-mediated RNA decay pathways

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In eukaryotes, nuclear RNAs are subject to quality control systems that ensure the integrity of a transcript. Kaposi's sarcoma-associated herpesvirus (KSHV) expresses its genes in the nucleus using the host machinery. Consequently, KSHV evolved mechanisms that allow its transcripts to evade host nuclear RNA decay pathways. The multifunctional KSHV ORF57 (Mta) protein increases the nuclear stability of viral transcripts by protecting them from cellular RNA quality control pathways. However, neither the RNA decay pathway(s) inhibited by ORF57 nor the mechanism by which ORF57 protects viral RNAs from degradation is known. We used RNAi to target host nuclear decay pathways and monitored their contributions to the degradation of an unstable form of the nuclear noncoding KSHV PAN RNA. Depletion of cellular factors involved in two different nuclear RNA decay pathways, PABPN1 and PAPα/γ-mediated RNA decay (PPD) and CBC-ARS2-mediated RNA decay, stabilized the transcript. However, ORF57 primarily protected from CBC-ARS2 mediated decay in transcription pulse-chase assays. In the context of a viral infection in cultured cells, inactivation of both decay pathways restored the expression of a subset of ORF57-dependent viral genes produced from an ORF57 null bacmid. Thus ORF57-mediated protection of viral RNAs from these nuclear decay pathways is important for viral replication. Mechanistically, we show that ORF57 inhibits recruitment of the exosome co-factor MTR4 to viral RNAs in cells and in whole cell lysate. In addition, our data suggest that ORF57 promotes the recruitment of ALYREF to some viral RNAs to increase the stability of the viral transcript by impeding the interaction between ARS2 and MTR4. These studies describe complex host-virus interactions that control KSHV RNA stability in the nucleus. They show that KSHV RNAs are subject to nuclear degradation by two host pathways, PPD and CBC-ARS2-mediated decay. Our results further support the conclusion that ORF57 protects viral transcripts from decay by inhibiting MTR4 recruitment.

789 Screening for novel anti-HCV lead compounds via attenuation of the 40S ribosomal subunit

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Chronic hepatitis C virus (HCV) infection is an epidemic viral disease that threatens ~3% of the world population and is a major cause of hepatic cirrhosis, liver failure, and hepatocellular carcinoma. Current therapy by orally administrated direct-acting antiviral agents (DAAs) is effective but highly expensive. In addition, the development of novel anti-HCV vaccines and therapies has been seriously hampered by HCV's fast antigen shift and rapid emergence of resistance-associated mutations, which often cripple the efficacy of the antiviral agents targeting at specific HCV proteins. An alternative strategy that explores the genetic stability of host factors indispensable for HCV replication would thus be desirable. We have recently reported that specific attenuation 40S ribosomal subunit can effectively repress HCV without negatively impacting on the host vitality. A highly plausible model to explain this finding is that reducing 40S ribosomal subunit level below a threshold places HCV under severe disadvantage in competing for 40S subunit for its protein synthesis. This is because translation of HCV's mRNA depends on the internal ribosomal entry site (IRES) to recruit the translation machinery, which is less effective than the host 5'-cap-dependent recruitment system. Our results thus suggest a novel approach to achieve selective suppression of HCV by means of manipulating the level of 40S ribosomal subunit. To this end, we have established a Huh-7.5puro reporter system for screening HCV IRES-specific inhibitors. By taking advantage of our institute's ultra-high throughput facility and the extensive compound collection, we have screened more than 125,000 small molecule compounds and obtained 58 effective compound leads. These compound candidates were further grouped into five categories based on their key structure. The most effective lead will be chemically derivatized to improve its IC50 and other important features.

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790 Human RNase, MCPIP1, Degrades Specific Human and Viral Pre-miRNAs and Inhibits Herpesvirus Infection

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Kaposi's sarcoma-associated herpesvirus (KSHV) is the responsible agent for Kaposi's sarcoma (KS), primary effusion lymphoma and multicentric Castleman's disease. KSHV expresses 25 miRNAs that modulate human gene expression. In patient-derived cell lines, certain KSHV miRNAs are expressed at higher levels than cellular miRNAs. This raised the question of how the virus can manipulate the miRNA biogenesis pathway to promote expression of viral miRNAs. MCP-1-induced protein-1 (MCPIP1), a critical regulator of immune homeostasis, has been shown to suppress miRNA biosynthesis via cleavage of human precursor miRNAs (pre-miRNAs). Since MCPIP1 in strongly up-regulated by inflammatory cytokines, including IL-1, we hypothesized that MCPIP1 could be used as an antiviral strategy to inhibit biogenesis of viral miRNAs at the time of infection. We demonstrated that MCPIP1 can directly cleave KSHV and EBV precursor miRNAs. MCPIP1 expression is repressed following de novo KSHV infection, while Dicer and TRBP were up-regulated. MCPIP1 degraded the majority of KSHV pre-miRNA, but a specific KSHV pre-miRNA, miR-K6, which was resistant to MCPIP1 degradation, generated a mature miRNA that directly targeted the MCPIP1 transcript and decreased MCPIP1 expression. Our pre-miRNA cleavage assays revealed that MCPIP1 degraded human and viral pre-miRNAs with different efficiencies, but MCPIP1 binding strength was not the factor determining MCPIP1 cleavage specificity. We analyzed the loop structures of pre-miRNAs and made point mutations within our predicted motif associated with MCPIP1-mediated degradation. We found that these mutations in key locations inhibited MCPIP1-mediated degradation of both KSHV and human pre-miRNAs. We propose that KSHV infection inhibits a negative regulator of miRNA biogenesis (MCPIP1) and up-regulates critical miRNA processing components to evade host mechanisms that inhibit biogenesis of viral miRNAs. KSHV-mediated alterations in miRNA biogenesis factors represent a novel mechanism by which KSHV interacts with its host and a new mechanism for the regulation of viral miRNA expression.

791 Unraveling the function and subcellular localization of DDX6 in Human cells

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DDX6 is a conserved DEAD-box protein (DBP) that plays central roles in cytoplasmic RNA regulation, including processing body (P-body) assembly, mRNA decapping, and translational repression. Several reports have shown that the orthologs of DDX6 in various organisms localize to nuclei, while the function of DDX6 in nucleus is still unknown. In addition, it is unclear if DDX6 is generally present in human cell nuclei and the molecular mechanism underlying DDX6 subcellular distribution remains elusive. Here we showed that DDX6 is commonly present in the nuclei of human-derived cells. Our structure- and molecular-based analyses deviate from the current model that the shuttling of DDX6 is directly mediated by the canonical nuclear localization signal (NLS) and nuclear export signal (NES), which are recognized and transported by Importin-α/β and CRM1, respectively. Instead, we show that DDX6 can be transported by 4E-T in a piggyback manner. Furthermore, we also provide evidence for a novel nuclear targeting mechanism where DDX6 enters the newly formed nuclei by "hitchhiking" on mitotic chromosomes with its C-terminal domain during M phase progression. Together, our results indicate the nucleocytoplasmic localization of DDX6 is regulated by these dual mechanisms.

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792 Defining the targeting mechanism of the Kaposi's sarcoma-associated herpesvirus RNA endonuclease

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Kaposi's Sarcoma (KS) presents as neoplastic lesions on patients during instances of severe immune suppression. While aggressive antiviral therapy has aided to reduce the rate of death in patients with KS in the west, KS is still at endemic levels in many areas of Africa. The etiologic agent of KS is KS-associated herpesvirus (KSHV). KSHV has a biphasic life cycle: a relatively quiescent latent phase, and a lytic replication stage, where the virus expresses the full set of viral genes and multiplies to generate progeny virions. During the lytic cycle, the virus dramatically alters the gene expression landscape of a cell to evade the immune response and create an environment favorable to viral replication. One of the major drivers of host gene expression changes during lytic KSHV infection is a virally encoded, messenger RNA (mRNA)-specific endonuclease termed SOX. While we have gained great insight into how SOX changes the gene expression landscape during infection, the mechanism underlying SOX mRNA targeting remains poorly understood. I have recently established an *in vitro* assay that preserves the site specificity of SOX targeting, which I will leverage to identify cellular factors involved in guiding SOX to its mRNA targets using comparative pull-down and mass spectrometry approaches.

793 Structure of Frequency-interacting RNA Helicase from Neurospora crassa reveals high flexibility in a domain critical for circadian rhythm and RNA surveillance

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The FRH (Frequency-interacting RNA helicase) protein is the *Neurospora crassa* homolog of yeast Mtr4, an essential RNA helicase that plays a central role in RNA metabolism as an activator of the nuclear RNA exosome. FRH is also a required component of the circadian clock, mediating protein interactions that result in the rhythmic repression of gene expression. Here we show that FRH unwinds RNA substrates *in vitro* with a kinetic profile similar to Mtr4, indicating that while FRH has acquired additional functionality, its core helicase function remains intact. In contrast with the earlier FRH structures, a new crystal form of FRH results in an ATP binding site that is undisturbed by crystal contacts and adopts a conformation consistent with nucleotide binding and hydrolysis. Strikingly, this new FRH structure adopts an arch domain conformation that is dramatically altered from previous structures. Comparison of the existing FRH structures reveals conserved hinge points that appear to facilitate arch motion. Regions in the arch have been previously shown to mediate a variety of protein-protein interactions critical for RNA surveillance and circadian clock functions. The conformational changes highlighted in the FRH structures provide a platform for investigating the relationship between arch dynamics and Mtr4/FRH function.

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794 Mechanistic Insights into the Regulation of let-7 Biogenesis by hnRNP A1

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Understanding the mechanisms by which an RNA-binding protein (RBP) recognizes its target is essential in deciphering its function. hnRNP A1, one of the most prevalent RBPs in cell, plays important roles in many RNA-mediated pathways and the development of various diseases such as Alzheimer's disease and Cancer. One particular function of hnRNP A1 is regulating miRNA biogenesis by binding to the terminal loop of primary miRNA (pri-miRNA) and thereby modulating the activity of the Microprocessor complex (Drosha in complex with its cofactor Dgcr8). Interestingly, the association of hnRNP A1 and the terminal loops inhibits the processing of all the let-7 family members. How hnRNP A1 specifically recognizes the terminal loops and inhibits their functions is a long-standing mystery in the field of RNA biology research.

Based on four crystal structures of Protein-RNA complexes composed of hnRNP A1 and various let-7 terminal loop sequences, and a comprehensive set of biochemical results, we revealed several modes of RNA recognition by hnRNP A1 via intermolecular cooperation. We found that the terminal loop of pri-let-7 has two hnRNP A1-binding sites, one near the 5' end and the other near the 3' end. The protein binds to these two sites and inhibits the formation of the Microprocessor:pri-miRNA complex due to space confrontment with the Rhed domain of Dgcr8. When we eliminate the pri-let-7's two binding sites by site-directed mutations, the effect of hnRNP A1 is negligible. These results provide important mechanistic insights into the recognition of RNA and the regulation of miRNA biogenesis by hnRNP A1, suggesting a gene manipulation strategy that enables the recovery of tumor suppressor gene let-7 for the management of cancer types that feature up-regulated hnRNP A1 and down-regulated let-7.

795 Single-molecule visualization of human RNA polymerase II transcription complexes assembly

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Transcription initiation of protein-encoding genes in human cells starts with the assembly of the RNA polymerase II (Pol II) preinitiation complexes (PICs) on genes' promoters. A functional PIC is comprised of Pol II itself, five essential General Transcription Factors (GTFs), sequence-specific activators and co-activators. Based on classical *in vitro* experiments with purified factors, the PIC is believed to be a biochemically stable (lifetimes >1 min) and structurally defined entity. Over the last decade, the notion of the stable PIC has been challenged by *in vivo* experiments, e.g. fluorescence recovery after photobleaching and single-molecule tracking, in which fleeting factor-DNA interactions (lifetimes ~1 sec) were observed. To resolve this contradiction, we have established a single-molecule system to visualize the assembly of human PICs on immobilized promoter DNA templates in real-time. The system employs super-resolution Total Internal Reflection Fluorescence (TIRF) microscopy and single-molecule detection by co-localization. To visualize single GTF molecules, we fused every essential GTF (IIB, IID, IIF, IIE, and IIH) with the Halogenase tag (Halo), followed by covalent fluorescent labeling. Specifically, endogenous IID-Halo and IIH-Halo were obtained by tagging at their essential subunits (TAF4 and XPB, respectively) using CRISPR/Cas9 knock-in gene editing in human cells with homozygous integration. Recombinant IIB-Halo, IIF-Halo and IIE-Halo were expressed and purified from bacterial cells. The *in vitro* transcription activities of all purified Halo-tagged factors have been verified using classical radioactivity-based assays. We are currently focused on single-molecule analysis of the PIC assembly under the TIRF microscope. In these experiments, single-molecule protein-DNA interactions are detected as co-localization between respective diffraction-limited spots, and correlated with subsequent RNA synthesis from the DNA molecule of origin. As a reporter of single-molecule RNA synthesis, we employ the tandem phage MS2 coat protein (tdMCP-Halo).

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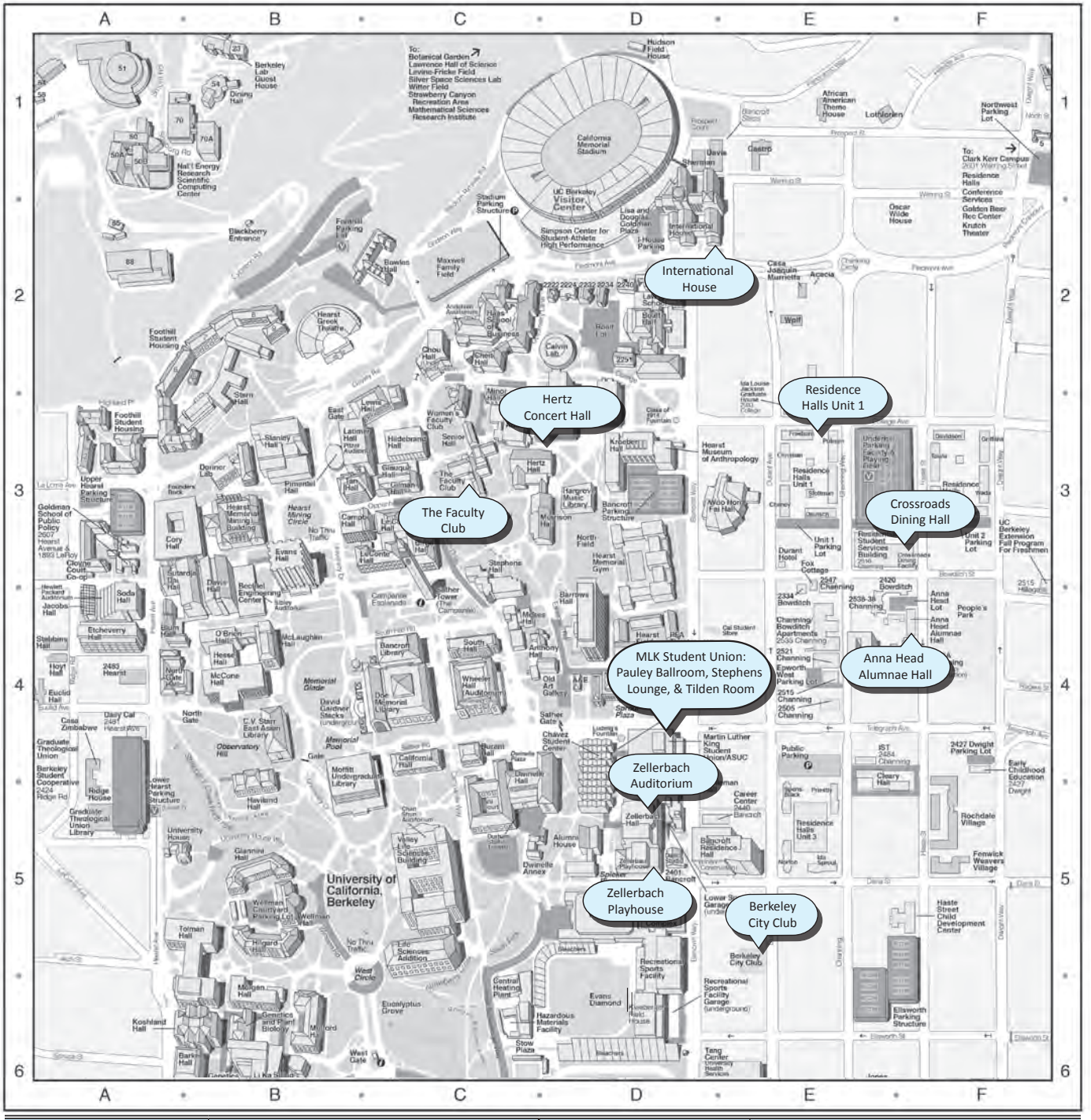
BERKELEY CAMPUS MAP

(see key on next page)



Meeting Facility	Event	Meeting Facility	Event
Zellerbach Auditorium	Plenary Session 1, 2, 3, 4, & 5, Keynotes, Concurrent Session 2, 3, 5, & 7, Awards and Workshop 1 & 5	Anna Head Alumnae Hall	Meetings Committee Dinner/Meeting Board of Directors Dinner/Meeting Mentoring Lunch
Zellerbach Playhouse	Concurrent Session 1, 4, 6, & 8 and Workshop 3 & 6	International House	Mentoring Lunch
Hertz Concert Hall	Workshop 2 & 4 & Industry Session	Crossroads Dining Hall	Meals
Pauley Ballroom & Patio	Welcome Reception & Poster Sessions	Tilden Room & Patio	Junior Scientists Social
Berkeley City Club	Sponsored Seminars	The Faculty Club	Reception/Dinner/Dance
		Residence Halls Unit 1	Housing

UC BERKELEY CAMPUS MAP KEY

Alumni House, D-5
Andersen Auditorium (Haas School of Business), C-2
Anthony Hall, C/D-4
Architects and Engineers (A&E), D-4
Bancroft Library, C-4
Banway Bldg., D-7
Barker Hall, A/B-6
Barrow Lane, D-4
Barrows Hall, D-4
BART Station, C-7
Bechtel Engineering Center, B-3/4
Berkeley Art Museum, C-6/7
Birge Hall, C-3
Blum Hall, A/B-4
Boalt Hall, D-2
Botanical Garden, C-1
Brain Imaging Center, B-5
C.V. Starr East Asian Library, B-4
California Hall, C-4
California Memorial Stadium, D-1
Calvin Laboratory, D-2
Campanile (Sather Tower), C-3
Campbell Hall, B/C-3/4
Career Center, D/E-5
Chan Shun Auditorium
(Valley Life Sciences Bldg.), C-5
Chávez Student Center, D-4
Cheit Hall, C-2
Clark Kerr Campus, F-1
Class of 1914 Fountain, D-3
CNMAT, A-5/6
Cory Hall, A/B-3
Cyclotron Rd., B-2
Davis Hall, B-3/4
Doe Memorial Library, C-4
Donner Lab, B-3
Durant Hall, C-4
Durham Studio Theatre (Dwinelle Hall), C-5
Dwinelle Annex, C/D-5
Dwinelle Hall, C-4/5
East Gate, B-3
Edwards Stadium, D-6
Energy Biosciences Building A/B-6/7
Eshleman Hall, D-4/5
Etcheverry Hall, A-4
Evans Diamond, D-6
Evans Hall, B-3
Eye Center (Minor Hall Addition), C/D-3
Eye Center (Tang Center), D/E-6
Faculty Club, C-3
Faculty Glade, C-3
Founders' Rock, A/B-3
Fox Cottage, E-3
Frank Schlessinger Way, C-6
Gayley Rd., B/C-2
Genetics and Plant Biology Bldg., B-6
Giannini Hall, B-5
Giauque Hall, C-3
Gilman Hall, C-3
Golden Bear Recreation Center, F-2
Goldman Field, D-6
Goldman Plaza D-1/2
Goldman School of Public Policy, A-3
Greenhouse, A-7
Grinnell Natural Area, C-6
Haas Pavilion, D-5
Haas School of Business, C-2
Hargrove Music Library, D-3
Haste Street Child Development Center, F-5
Haviland Hall, B-4/5
Hazardous Materials Facility, C/D-6
Hearst Field Annex, D-4
Hearst Greek Theatre, B-2
Hearst Memorial Gymnasium, D-3
Hearst Memorial Mining Bldg., B-3
Hearst Mining Circle, B-3
Hearst Museum of Anthropology, D-3
Heating Plant, Central, C-6
Hellman Tennis Complex, C-6
Hertz Hall, C/D-3
Hesse Hall, B-4
Hewlett-Packard Auditorium (Soda Hall), A-3/4
Hildebrand Hall, C-3
Hilgard Hall, B-5
Insectary, A-7
International House, D-2
Ishi Court, C-5
Jacobs Hall, A-4
Jones Child Study Center, E-6
Kleeberger Field House, D-6
Koshland Hall, A/B-6
Kroeber H[illegible]
Krutch The
Latimer H
Lawrence Berkeley National Laboratory, B-2
Lawrence Hall of Science, C-1
LeConte Hall, C-3
Legens Aquatic Center, E-6
Levine-Fricke Field, C-1
Lewis Hall, C-2/3
Life Sciences Addition, C-5
Lower Sproul Plaza, D-4/5
Martin Luther King Jr. Student Union, D/E-4
Mathematical Sciences Research Institute, C-1
Maxwell Family Field, C-2
McCone Hall, B-4
McEnerney Hall, A-5/6
McLaughlin Hall, B-4
Memorial Glade and Pool, B-4
Minor Hall, C-2/3
Minor Hall Addition, C-3
Moffitt Undergraduate Library, B/C-4
Morgan Hall, B-5/6
Morrison Hall, C/D-3
Moses Hall, C-4
Mulford Hall, B-6
Natural Resources Laboratory, A-6
North Field, D-3
North Gate, A/B-4
North Gate Hall, A-4
Northwest Animal Facility, A/B-6
O'Brien Hall, B-4
Observatory Hill, B-4
Old Art Gallery, C/D-4
Optometry Clinic (Eye Center,
Minor Hall Addition), C-3
Optometry Clinic (Eye Center, Tang Center), D/E-6
Parking Lots/Structures, A-3, A-4/5, A-6, C-7,
D-3, D-5, D-7, E-4, E/F-3, E/F-5/6
Pimentel Hall, B-3
Pitzer Auditorium (Latimer Hall), C-2/3
Police, UC (Sproul Hall), D-4
Recreational Sports Facility, D-5/6
Residence Halls
Bowles Hall, C-2
Clark Kerr Campus, F-1
Cleary Hall, E/F-4/5
Foothill Residence Halls, A/B-2/3
Ida Louise Jackson Graduate House, E-2/3
Martinez Commons E/F-4
Stern Hall, B-2/3
Unit 1, E-3
Unit 2, F-3
Unit 3, E-5
Residential and Student Services Bldg., E-3
Sather Gate, D-4
Sather Rd., C-4
Sather Tower (Campanile), C-3/4
Senior Hall, C-3
Sibley Auditorium (Bechtel Engineering
Center), B-4
Silver Space Sciences Laboratory, C-1
Simon Hall, D-2
Simpson Center, C/D-1/2
Soda Hall, A-3/4
South Hall, C-4
Spieker Aquatics Complex, D-5
Spieker Plaza, D-5
Springer Gateway, C-6
Sproul Hall, D-4
Sproul Plaza, D-4
Stadium Rim Way, C-1/2
Stanley Hall, B-3
Stephens Hall, C-3/4
Strawberry Canyon Recreation Area, C-1
Sutardja Dai Hall, A/B-3/4
Tan Hall, B/C-3
Tang Center, E-6
Tolman Hall, A/B-5
UC Berkeley Extension, B-7
Underhill Playing Field, E-3
University Dr., B-5
University Hall, B-6
University Health Services, D/E-6
University House, A/B-5
Valley Life Sciences Bldg., C-5
Visitor Center (Memorial Stadium), D-1/2
Warren Hall, A-6/7
Wellman Hall, B-5
West Circle, B/C-5/6
West Gate, B/C-6
Wheeler Hall, C-4
Wickson Natural Area, B-5
Witter Field, C-1
Women's Faculty Club, C-3
Woo Hon Fai Hall, D/E-3
Wurster Hall, D-2/3
Zellerbach Hall, D-5
Zellerbach Playhouse, D-5

ABBREVIATED SCHEDULE

TUESDAY MAY 29

14:00 – 20:00	Registration	Zellerbach Lobby
16:30 – 19:00	Welcome reception Featuring music by Harry Noller and the RiboBand	Pauley Ballroom & Patio
19:00 – 21:30	Opening session: Award talks (1-5)	Zellerbach Auditorium

WEDNESDAY MAY 30

08:00 – 08:45	Sponsored Seminar (page 25) [Sponsor: Oxford Nanopore Technologies]	Berkeley City Club
09:00 – 11:45	Plenary session 1: Splicing (6-14) [Patricia Coltri]	Zellerbach Auditorium
11:45 – 12:30	Keynote 1 (15) [Mikiko Siomi]	Zellerbach Auditorium
12:30 – 14:00	Lunch	Crossroads Dining Hall
14:00 – 16:45	Concurrent session 1: RNA Turnover (16-24) [Katherine Berry]	Zellerbach Playhouse
	Concurrent session 2: Regulatory RNAs (25-34) [Ayelet Lamm]	Zellerbach Auditorium
17:00 – 18:30	Workshop 1: Capturing Dynamic RNPs (35-41) [Julian König]	Zellerbach Auditorium
	Workshop 2: Single-cell RNAseq (44, 42, 43, 45, 46) [Jeremie Breda]	Hertz Concert Hall
	Workshop 3: Probing RNA Structure (49, 47, 50, 48, 51) [Sharon Aviran]	Zellerbach Playhouse
18:30 – 20:00	Dinner	Crossroads Dining Hall
18:30 – 20:00	Meetings Committee dinner/meeting	Anna Head Alumnae Hall
19:00 – 20:30	Junior Scientists Social	Tilden Room & Patio
20:00 – 22:30	Poster session 1 [Sponsor: MDPI Journals – Cells, Biomolecules, Non-Coding RNA	Pauley Ballroom & Patio

THURSDAY MAY 31

07:45 – 08:45	Sponsored Seminar (page 27) [Sponsor: Lexogen]	Berkeley City Club
09:00 – 11:45	Plenary session 2: From Oligo to RNP (52-62, 135) [Andrea Rentmeister]	Zellerbach Auditorium
11:45 – 12:30	Keynote 2 (63) [Geraldine Seydoux]	Zellerbach Auditorium
12:30 – 14:00	Lunch	Crossroads Dining Hall
12:30 – 14:00	Mentoring Lunch	International House & Anna Head Alumnae Hall
14:00 – 16:00	Concurrent session 3: Splicing Mechanisms (64-72) [Stephen Rader]	Zellerbach Auditorium
	Concurrent session 4: Interconnected RNA Processes (73-81) [Mary O'Connell]	Zellerbach Playhouse
16:30 – 18:30	Industry Session: Careers Beyond Academia (page 23) [Junior Scientists]	Hertz Concert Hall
18:30 – 20:00	Dinner	Crossroads Dining Hall
18:30 – 20:30	Board of Directors dinner/meeting	Anna Head Alumnae Hall
20:00 – 22:30	Poster session 2	Pauley Ballroom & Patio

FRIDAY JUNE 1

07:30 – 08:30	Sponsored Seminar (page 29) [Sponsor: Cellecta]	Berkeley City Club
09:00 – 11:45	Plenary session 3: The Life and Times of a Ribosome (82-92) [Kristin Koutmou]	Zellerbach Auditorium
11:45 – 12:30	Keynote 3 (93) [Jonathan Weissman]	Zellerbach Auditorium
12.30 – 14:00	Lunch	Crossroads Dining Hall
14:00 – 16:45	Concurrent session 5: Splicing Regulation (94-101) [Julia Salzman]	Zellerbach Auditorium
	Concurrent session 6: Emerging Technologies (102-111) [Jane Jackman]	Zellerbach Playhouse
17:00 – 18:30	Workshop 4: Single-molecule Analysis (112-117) [Dan Larson]	Hertz Concert Hall
	Workshop 5: RNA Editing and Modification [Wendy Gilbert] (118-120, 149, 392, 121, 123)	Zellerbach Auditorium
	Workshop 6: Transcript Isoform Analysis (124-127) [Qingqing Wang]	Zellerbach Playhouse
18:30 – 20:00	Dinner	Crossroads Dining Hall
20:00 – 22:30	Poster session 3	Pauley Ballroom & Patio

SATURDAY JUNE 2

09:00 – 10:30	Plenary session 4: 3'-End Formation and RNA Decay (128-134) [Bobby Hogg]	Zellerbach Auditorium
11:00 – 12:30	Plenary session 5: Coordinated RNA Processes (136-140) [Gloria Brar]	Zellerbach Auditorium
12:30 – 14:00	Lunch	Crossroads Dining Hall
14:00 – 17:00	Concurrent session 7: RNA and Disease (141-148, 150-153) [Julia Kenyon]	Zellerbach Auditorium
	Concurrent session 8: RNA Structure (154-165) [Yanli Wang]	Zellerbach Playhouse
17:15 – 18:45	Awards Ceremony	Zellerbach Auditorium
19:00 – 23:30	Reception/Dinner/Dance	The Faculty Club

SUNDAY JUNE 3

Conference concludes