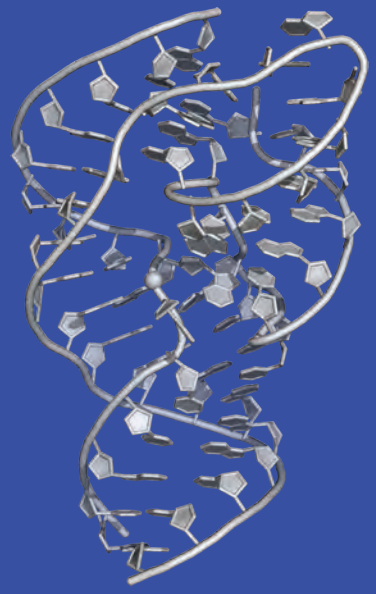
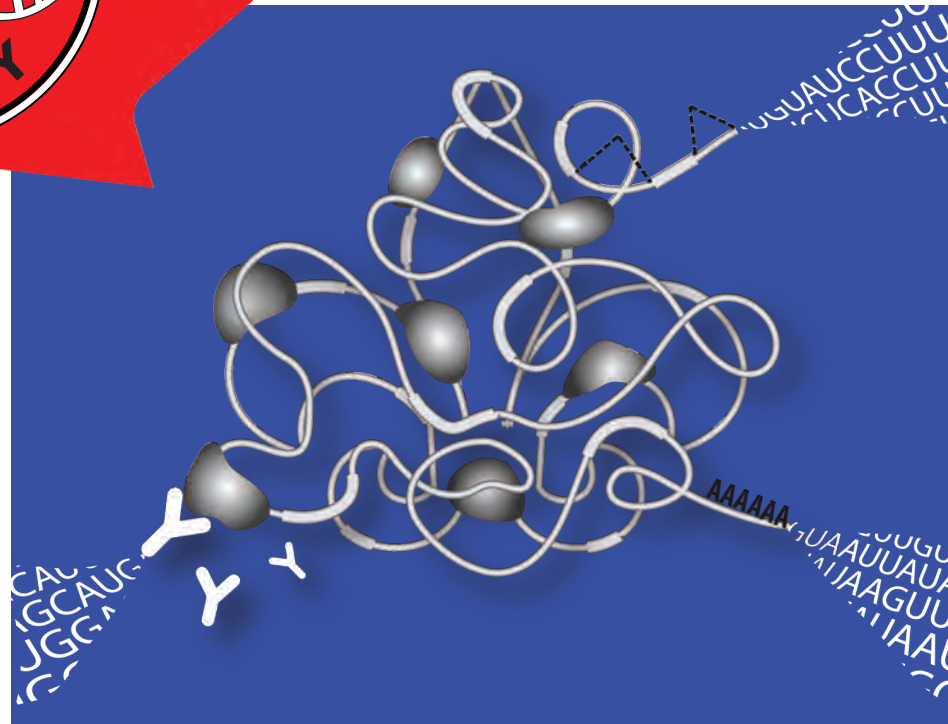
RNA 2014

The Nineteenth Annual Meeting of the RNA Society

June 3–8, 2014
Quebec City, Canada





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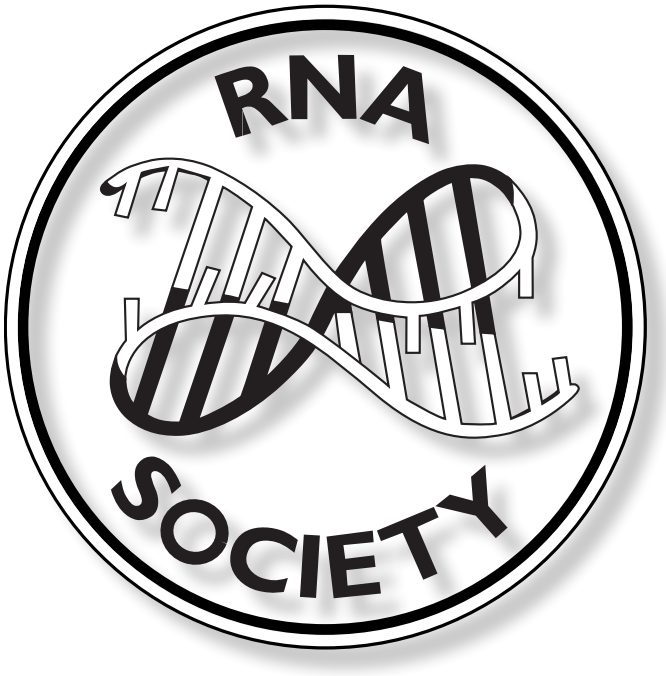
RNA 2014

The Nineteenth Annual Meeting of the RNA Society

PROGRAM & ABSTRACTS



June 3-8, 2014
Centre des Congrès de Québec
Quebec City, Canada

Benoit Chabot, *Université de Sherbrooke*
Martin Simard, *Université Laval, CHU de Québec*
Elena Conti, *Max Planck Institute of Biochemistry*
Fátima Gebauer, *Centre for Genomic Regulation*
Barbara Golden, *Purdue University*
Sean Ryder, *UMass Medical School*

ACKNOWLEDGEMENTS

Our thanks go out to all the volunteers who worked to make this conference possible, especially David Lilley (Univ of Dundee) for guiding the selection of the venue as the Chair of the Meetings Committee. We thank the keynote speakers for setting the perfect tone for the conference. Thanks to the session chairs, who helped select, order, introduce and run such stimulating oral sessions. Thanks, also, to the Junior Scientists Representatives Jo Marie Bacusmo, Michael Meers, Oussama Meziane, and Callie Wigington, along with their faculty advisors, Katrin Karbstein and Beth Tran, for organizing another great set of Junior Scientist workshops and activities.

Throughout the program listing, the numbers next to the titles refer to corresponding Oral or Poster 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.

To encourage sharing of unpublished data at the RNA Society Meeting, taking of photographs and/or videos during scientific sessions (oral or posters), 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.

Front Cover

Top right. A structure from the 3' UTR of flaviviruses that resists degradation by host cell exonuclease Xrn1, thereby producing a pathogenic and cytopathic subgenomic RNA. Reference: Chapman, E.G., Costantino, D.A., Rabe, J.L., Moon, S.L., Wilusz, J., Nix, J., & Kieft, J.S. (2014) The structural basis of pathogenic subgenomic flavivirus RNA (sfRNA) production. Science, in press. See abstract #93.

Bottom left. Crystal structure of the twister ribozyme at 2.3 Å resolution. The RNA adopts a novel compact fold based on a double pseudoknot structure, with the active site at its center. See abstracts #73 and #334.

Bottom right. A rendering of RNAs wound in an undecipherable knot bound by RNA binding proteins, describing the intractable problem of RNA processing and regulation as a "Gordian Knot." As Alexander the Great discovered, the best way to unravel such a knot is a brute force approach, and thus high-throughput RNA sequencing approaches attack this puzzle through multiple, genome-wide swords. The sequence coming from the CLIP represents a RBFOX2 binding site extracted from the ENAH gene, the sequences coming from the alternative splicing event are canonical splice sites, and the sequence coming from the poly-A is derived from the 3'UTR of HNRNPA2B1. (Designed by Stephanie Huelga, laboratory of Gene Yeo, UCSD). See abstract #518.

Cover design and layout by Barbara Golden.

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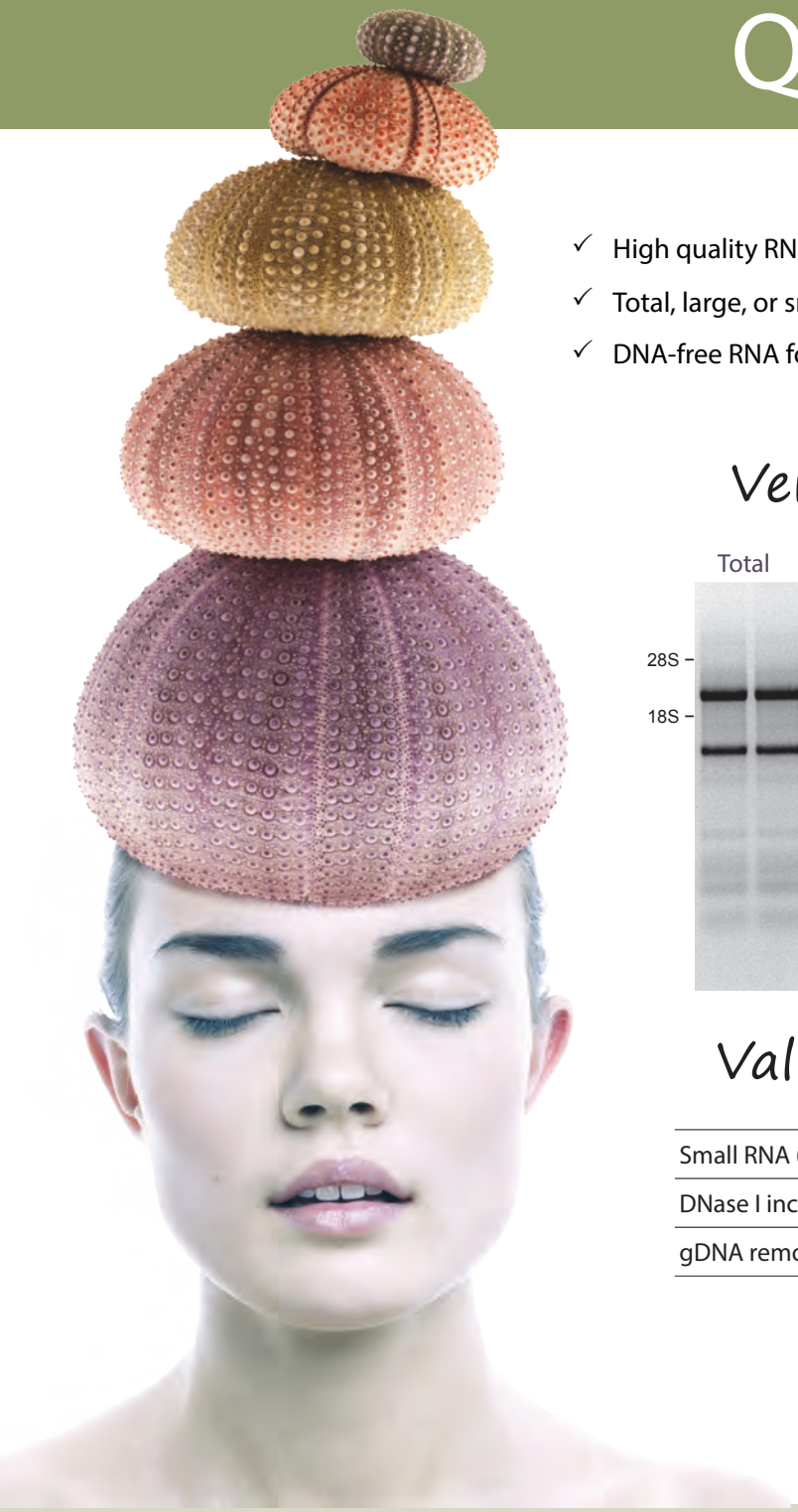

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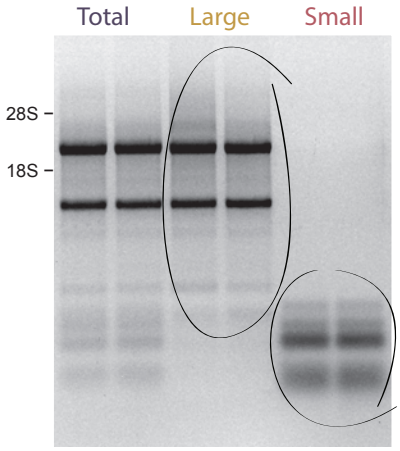
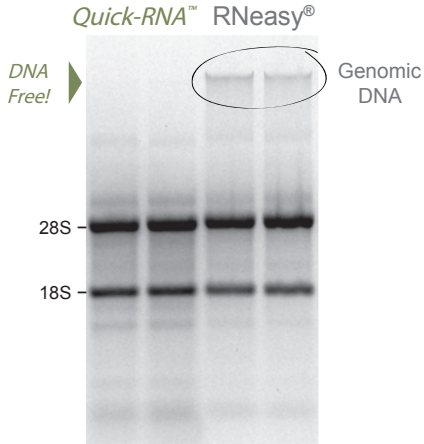


Quality



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Quick-RNA™ MidiPrep	10^6 - 10^8 cells 250 mg tissue	1 mg	≥ 200 µl	R1056	25 Preps
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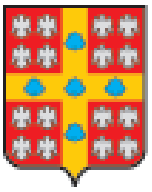

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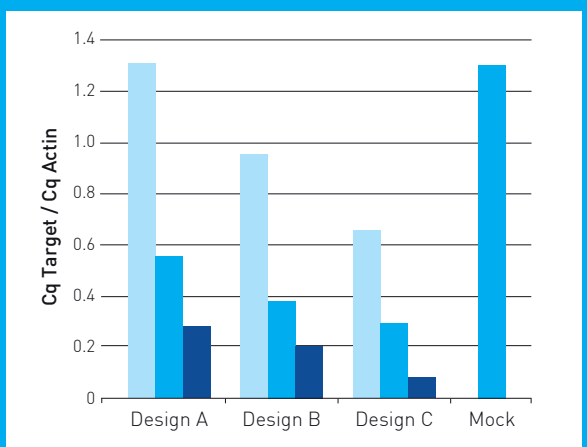

LNA™ longRNA GapmeRs were transfected into BT474 cells at 1nM, 5nM and 25nM (light, medium and dark blue respectively). The effect on mRNA levels was measured by qPCR using actin as a control. All three designs show an excellent dose response compared to the mock transfection.

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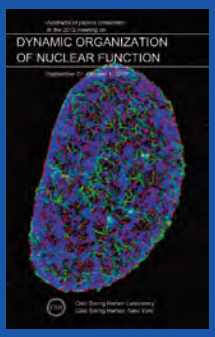
August 19 - 23
abstracts due June 6
Edith Heard, Martin Hetzer, David Spector



Regulatory & Non-Coding RNAs

August 26 - 30 abstracts due June 13
Gregory Hannon, Elisa Izaurralde, Michael Terns

Topics

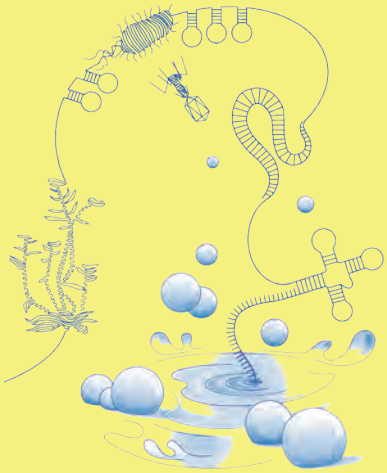
- **Biological Function of miRNAs**
- **Mechanisms of miRNA-mediated Gene Silencing**
- **siRNAs and piRNAs**
- **Non-coding RNAs in Prokaryotes**
- **Silencing Pathways in Plants**
- **Transcriptional Gene Silencing**
- **Catalytic RNAs**
- **Long Non-Coding RNAs**

Keynote Speakers

David Bartel, John Rinn

Discussion Leaders

Alexei Aravin,
Marc Büehler,
Xuemei Chen,
Jennifer Doudna,
Steve Jacobsen,
Rene Ketting,
Narry Kim,
Alan Lambowitz,
Donal O'Carroll, Nicholas Proudfoot,
Mikiko Siomi, Joerg Vogel



Translational Control

September 2 - 6
abstracts due June 20
Thomas Dever, Rachel Green,
Robert Schneider

Epigenetics & Chromatin

September 9 - 13
abstracts due June 27
Shelley Berger, Robert Kingston,
Juerg Mueller

CSHL Genentech Center 2014 Conference on the History of Molecular Biology and Biotechnology

Messenger RNA: From Discovery to Synthesis & Regulation in Bacteria & Eukaryotes

August 9 - 11 no abstracts due James Darnell, Adrian Krainer, Mila Pollock

2015 RNA Meetings

RNA & Oligonucleotide Therapeutics
April 8 - 11, 2015 abstracts due Jan 30
Retroviruses
May 18 - 23, 2015 abstracts due March 6

Eukaryotic mRNA Processing
August 18 - 22, 2015 abstracts due June 5
Mechanisms of Eukaryotic Transcription
August 25 - 29, 2015 abstracts due June 12

www.cshl.edu/meetings

The RNA Society

Officers of the RNA Society FY 2014

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Meetings
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University of Dundee

Membership
Kristian Baker
Case Western Reserve Univ.

RNA 2015

The 20th Annual Meeting of the RNA Society will be held in Madison, Wisconsin, USA from May 26-31, 2015, on the University of Wisconsin–Madison campus.

2015 Organizers

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Matthias Hentze, *EMBL*
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Anna Pyle, *Yale Univ*

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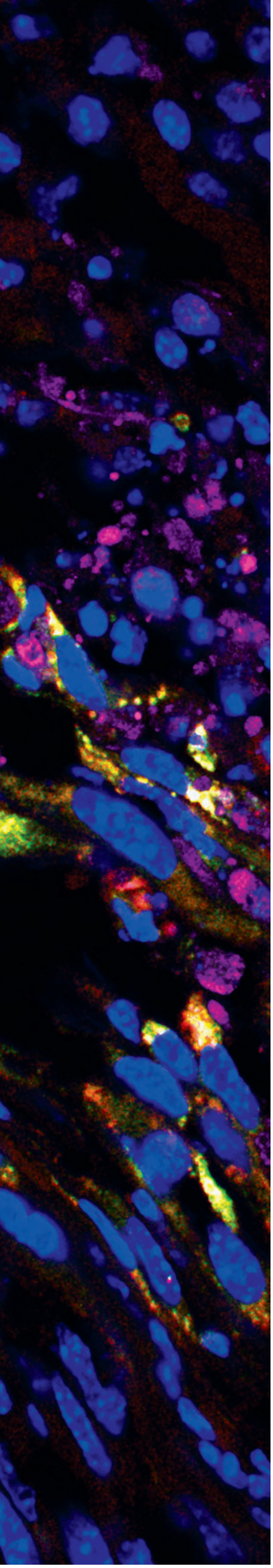

SCIENTIFIC CONFERENCES 2014-2015

American Association for Cancer Research

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

Marsha Rivkin Center for Ovarian Cancer Research-AACR 10th Biennial Ovarian Cancer Research Symposium
Co-Chairpersons: Kathleen Cho, Sandra Orsulic, Mary L. "Nora" Disis, and Saul E. Rivkin
September 8-9, 2014
Seattle, WA

Targeting PI3K-mTOR Networks in Cancer
Co-Chairpersons: Lewis C. Cantley, Jose Baselga, Joan S. Brugge, Brendan D. Manning, and Malte Peters
September 14-17, 2014
Philadelphia, PA

Hematologic Malignancies: Translating Discoveries to Novel Therapies
Chairperson: Kenneth C. Anderson
Co-Chairpersons: Scott Armstrong and Riccardo Dalla-Favera
September 20-23, 2014
Philadelphia, PA

Advances in Melanoma: From Biology to Therapy
Co-Chairpersons: Suzanne L. Topalian, Keith T. Flaherty, and Levi A. Garraway,
September 20–23, 2014
Philadelphia, PA

13th Annual International Conference on Frontiers in Cancer Prevention Research
Program Committee Chairperson: Phillip A. Dennis
September 28-October 1, 2014
New Orleans, LA

Seventh AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and Medically Underserved
Co-Chairpersons: Ethan Dmitrovsky, Rick A. Kittles, Electra D. Paskett, and Victoria L. Seewaldt
November 9-12, 2014
San Antonio, TX

EORTC-NCI-AACR International Symposium on Molecular Targets and Cancer Therapeutics
Scientific Committee Co-Chairpersons: Jean-Charles Soria, Lee J. Helman, and Jeffrey A. Engelman
November 18-21, 2014
Barcelona, Spain

Tumor Immunology and Immunotherapy: A New Chapter
Co-Chairpersons: Robert H. Vonderheide, Nina Bhardwaj, Stanley Riddell, and Cynthia L. Sears
December 1-4, 2014
Orlando, FL

San Antonio Breast Cancer Symposium
Co-Directors: Carlos L. Arteaga, Ismail Jatoi, and C. Kent Osborne
December 9-13, 2014 • San Antonio, TX

Myc: From Biology to Therapy
Co-Chairpersons: James E. Bradner, Martin Eilers, Dean W. Felsher, and Carla Grandori
January 7-10, 2015 • La Jolla, CA

Translation of the Cancer Genome
February 7-9, 2015
Co-Chairpersons: William Hahn, Lynda Chin, and William Sellers

Computational and Systems Biology of Cancer
February 9-11, 2015
Co-Chairpersons: Andrea Califano, Brenda Andrews, and Peter Jackson
The Fairmont, San Francisco, CA

AACR-Society of Nuclear Medicine and Molecular Imaging Joint Conference: Molecular Imaging in Cancer Biology and Therapy
Co-Chairpersons: Carolyn J. Anderson, Christopher H. Contag, and David Piwnica-Worms
February 11-14, 2015 • San Diego, CA

Invitation to Membership

The RNA Society was established in 1993 to facilitate sharing and dissemination of experimental results and emerging concepts in RNA research. The Society is an interdisciplinary, cohesive intellectual home for those interested in all aspects of RNA Science. 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

Our members receive:

- Subscription to the Society journal, *RNA* (IF 6.051) with
  - 50% discount on page charges
  - 50% discount on first color figure charge (a savings of $225)
  - For those members who wish to have their articles completely open access immediately upon publication can do so at a reduced cost of $1500 (a $500 savings from non-member fee)
- Reduced registration fees for the annual meeting of the Society (a savings of $175)
- The RNA Society Newsletter, a forum for disseminating information to members and discussing issues affecting the Society and RNA Science
- Numerous opportunities for junior scientists to become involved in the Society
- The Directory of Members, available online
- Free job postings on the Society website
- Opportunities to request Travel Fellowships and Meeting Support for RNA-related meetings you are organizing

These member savings more than offset the cost of a one-year membership in the Society.

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http://rnasociety.org/become-a-member

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PROGRAM–RNA 2014

The Nineteenth Annual Meeting of the RNA Society
Quebec City, Canada
June 03–08, 2014

(See room locations floor plan at the back of the book.)

Tuesday June 3

14:00 – 20:00	Registration	Foyer 4
17:30 – 20:00	Welcoming reception and dinner party	Foyer 4
20:00 – 20:10	Welcoming remarks	200AB
20:10 – 21:10	**Keynote address: Phillip Zamore, UMass Medical School**	
21:10 – 22:10	**Keynote address: Robert Schneider, NYU School of Medicine**	

Wednesday June 4

07:30 – 20:00	Registration	Foyer 4
08:30 – 10:30	**Plenary session 1: RNA-Protein Interactions (1-15)** *Chair: Kathy Collins, UC Berkeley*	200AB
10:30 – 11:00	Coffee break	Foyer 4
11:00 – 12:30	**Plenary session 1 (continued)**	200AB
12:30 – 14:00	Lunch	400B
14:00 – 16:45	**Plenary session 2: Splicing Regulation (16-25)** *Sponsored by RiboClub* *Chair: Ben Blencowe, University of Toronto*	200AB
16:45 – 17:15	Coffee break	Foyer 4
17:15 – 18:45	**Workshop 1: RNA Chemistry (26-31)** *Chair: Scott Strobel, Yale University*	200A
	Workshop 2: RNA Regulation in Protozoa (32-37) *Sponsored by Burroughs Wellcome Fund* *Chair: Barbara Papadopoulou, Université Laval*	301AB
	Workshop 3: RNA Programmable Genome Editing (38-42) *Sponsored by CRISPRTX and Sigma Life Science* *Chair: Emmanuelle Charpentier, Helmholtz Center for Infectious Research*	200B
18:45 – 20:30	Dinner	400B
18:45 – 20:30	Meetings Committee dinner/meeting	202

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

19:30 – 20:30	Junior Scientists Social	Solarium
20:30 – 23:00	**Poster Session 1**	400A

(20:30 – 21:45 even numbers; 21:45 – 23:00 odd numbers)
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Abstracts	Topic
(173 – 183)	3′ End Processing
(184 – 203)	Bioinformatics
(204 – 219)	Emerging & High-throughput Techniques
(220 – 235)	Interconnections between Gene Expression Processes
(236 – 240)	Mechanisms of RNA Interference
(241 – 282)	Non-coding and Regulatory RNAs
(283 – 283)	Riboregulation in Development
(284 – 314)	Ribosomes and Translation
(315 – 321)	RNA and Epigenetics
(322 – 334)	RNA Catalysis and Riboswitches
(335 – 340)	RNA Chemistry
(341 – 365)	RNA Editing and Modification
(366 – 400)	RNA Structure and Folding
(401 – 405, 708)	RNA System Biology
(406 – 418)	RNA Transport and Localization
(419 – 448)	RNA Turnover
(449 – 491)	RNA-protein Interactions
(492 – 519)	RNA in Disease
(520 – 536)	RNP Structure, Function and Biosynthesis
(537 – 562)	Small RNAs
(563 – 587)	Splicing Mechanisms
(588 – 636)	Splicing Regulation
(637 – 642)	Therapeutic RNAs
(643 – 676)	Translational Regulation
(677 – 696)	tRNA, snRNA, snoRNA, rRNA
(697 – 707)	Viral RNAs

Thursday June 5

08:00 – 19:00	Registration	Foyer 4
08:30 – 10:15	**Plenary session 3: Emerging and High-Throughput Techniques (43-54)** *Sponsored by Genome Canada* *Chair: Gene Yeo, UC San Diego*	200AB
10:15 – 10:45	Coffee break	Foyer 4
10:45 – 11:45	**Plenary session 3 (continued)**	200AB
11:45 – 13:15	Lunch	400B
11.45 – 13:15	Mentor/Mentee luncheon	400B

13:15 – 15:30	**Concurrent session 1: RNA Silencing (55-63)** *Chair: Yukihide Tomari, University of Tokyo*	200A
	Concurrent session 2: Ribosome Mechanisms and Assembly (64-72) *Chair: Katrin Karbstein, Scripps Research Institute*	200B
15:30 – 16:00	Coffee break	Foyer 4
16:00 – 17:00	**Keynote address: Anne Ephrussi, EMBL**	200AB
17:00 – 17:30	Break	Foyer 4
17:30 – 19:00	**Concurrent session 3: Ribozymes and Riboswitches (73-78)** *Chair: Ron Breaker, Yale University*	200A
	Concurrent session 4: RNA Transport and Localization (79-84) *Chair: Anita Corbett, Emory School of Medicine*	200B
	Concurrent session 5: 3′ End Processing (85-90) *Chair: Elmar Wahle, University of Halle*	301AB
19:00 –	Dinner and evening in Quebec City on own – tours and other activities will be offered	

Friday June 6

08:00 – 18:30	Registration	Foyer 4
08:30 – 10:30	**Plenary session 4: RNA in Disease (92-105)** *Chair: Claudia Bagni, VIB/KU Leuven*	200AB
10:30 – 11:00	Coffee break	Foyer 4
11:00 – 12:30	**Plenary session 4 (continued)**	200AB
12:30 – 14:00	Lunch	400B
14:00 – 16:15	**Plenary session 5: Non-coding and Regulatory RNAs (106-114)** *Chair: Jørgen Kjems, Aarhus University*	200AB
16:15 – 16:45	Coffee break	Foyer 4
16:45 – 19:00	**Plenary session 6: RNA Technologies and Therapeutic RNAs (115-123)** *Chair: Matt Disney, Scripps Research Institute*	200AB
19:00 – 20:30	Dinner	400B
19:00 – 20:30	Board of Directors dinner/meeting	202
20:30 – 23:00	**Poster session 2** **(20:30 – 21:45 odd numbers; 21:45 – 23:00 even numbers)** *Sponsored by Génome Québec*	400A

Abstracts	**Topic**
(173 – 183)	3′ End Processing
(184 – 203)	Bioinformatics
(204 – 219)	Emerging & High-throughput Techniques
(220 – 235)	Interconnections between Gene Expression Processes
(236 – 240)	Mechanisms of RNA Interference

(241 – 282)	Non-coding and Regulatory RNAs
(283 – 283)	Riboregulation in Development
(284 – 314)	Ribosomes and Translation
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(520 – 536)	RNP Structure, Function and Biosynthesis
(537 – 562)	Small RNAs
(563 – 587)	Splicing Mechanisms
(588 – 636)	Splicing Regulation
(637 – 642)	Therapeutic RNAs
(643 – 676)	Translational Regulation
(677 – 696)	tRNA, snRNA, snoRNA, rRNA
(697 – 707)	Viral RNAs

Saturday June 7

08:00 – 18:30	Registration	Foyer 4
08:30 – 10:00	**Concurrent session 6: RNA Decay (124-129)** *Chair: Jeff Coller, Case Western Reserve University*	200A
	Concurrent session 7: RNA Interconnections (130-135) *Chair: Karla Neugebauer, Yale University*	200B
10:00 – 10:30	Coffee break	Foyer 4
10:30 – 12:00	**Workshop 4: Bioinformatics of RNA Interactions (136-141, 141a–141d)** *Chairs: Eric Westhof, IBMC-CNRS, Janusz Bujnicki, IIMBC, and François Major, Université de Montréal*	200A
	Workshop 5: RNA Editing in Cellular Function (142-148) *Chair: Marie Öhman, Stockholm University*	200B
	Career Development Workshop *Presenter: Michael Matrone, Scripps Research Institute*	301AB
12:00 – 13:30	Lunch	400B
13:30 – 15:00	**Concurrent session 8: Translational Control (149-154)** *Chair: Wendy Gilbert, MIT*	200A
	Concurrent session 9: Splicing Mechanisms (155-160) *Chair: Jonathan Staley, University of Chicago*	200B
15:00 – 15:30	Coffee break	Foyer 4

15:30 – 18:15	**Plenary session 7: RNA Architecture: Structure, Folding and Modification (161-172)** *Chair: Michael Sattler, Helmholtz Zentrum München/TU München*	200AB
18:30 –	Apero/Banquet/Awards/Dance	Hilton Hotel, First floor foyer and ballroom

Sunday June 8

Conference concludes

RNA 2014 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), and Phillip Sharp (2013).

*Congratulations to **Reinhard Lührmann** who is the winner of the 2014 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), and Brenda Peculis (2012). There was no recipient for 2013.

*Congratulations to **Ann Marie Micenmacher** who is the winner of the 2014 RNA Society Service 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. 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) and Wenwen Fang (2013). 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), and Je-Hyun Yoon (2013).

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*Congratulations to graduate student **David Weinberg**, and postdoctoral fellow **Jinwei Zhang**, who are the winners of the 2014 RNA Society/Scaringe Award.*

The ACS Chemical Biology Poster Prize

The journal *ACS Chemical Biology* is pleased to recognize junior scientists with a poster prize to be awarded at RNA 2014. The prize is for 'innovative use of chemical biology applied to the study of RNA', and consists of a $250 cash prize. All graduate students and postdoctoral fellows presenting posters at the meeting are eligible.

The Biochemistry Poster Prize

The journal *Biochemistry* is pleased to recognize junior scientists with a poster prize to be awarded at RNA 2014. The prize is for 'innovation in the study of RNA biochemistry', and consists of a $250 cash prize. All graduate students and postdoctoral fellows presenting posters at the meeting are eligible.

The NRMCB Poster Prize

Nature Reviews Molecular Cell Biology (NRMCB) is pleased to sponsor a poster prize to be awarded at the 2014 RNA Society Meeting. The prize is for 'innovation and interdisciplinary research,' and consists of a free one-year print and online subscription to NRMCB and a $200 cash award. All graduate students and postdoctoral fellows presenting posters at the meeting are eligible.

The NSMB Poster Prize

Nature Structural & Molecular Biology (NSMB) is pleased to sponsor three poster prizes to be awarded at the 2014 RNA Society Meeting. The prizes, one in the area of molecular biology and biochemistry, one in genetics and development, and one in biophysics and structural biology, consist of a free one-year print and online subscription to NSMB and a $200 cash award. All graduate students and postdoctoral fellows presenting posters at the meeting are eligible.

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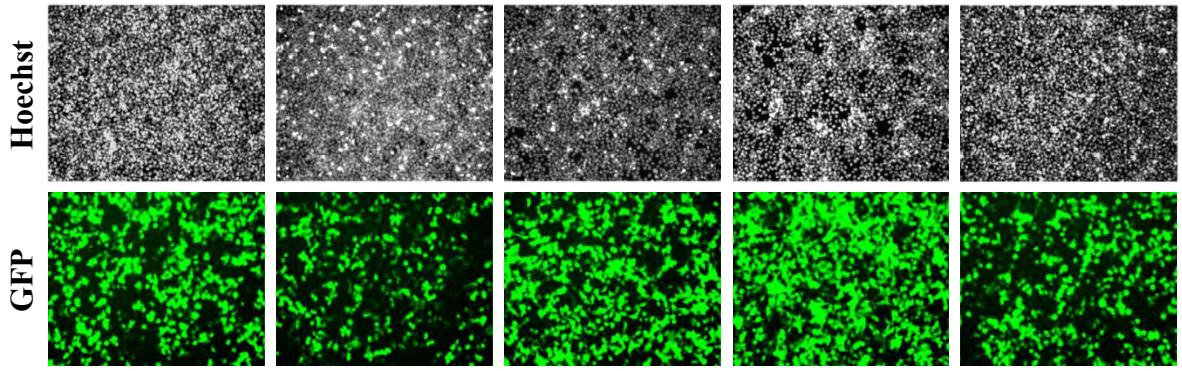

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ADDITIONAL SCHEDULED EVENTS

Tuesday, June 3

11:00 – 12:30 **Junior Scientists - Guided Maritime Cruise**

- Open to all attendees
- $30 per person (tax not included), refreshments are available for purchase on board
- Save yourself a spot by completing this brief survey: https://www.surveymonkey.com/s/V2BSN3M

A captivating boat cruise along the St. Lawrence river navigating from Cap Diamant to the breathtaking Montmorency falls (30 meters higher than Niagara). A costumed guide will enlighten us on the history of Quebec as we enjoy this scenic route.
For more information on the tour: http://www.croisieresaml.com/en/plan-your-cruise/quebec/guided-sightseeing-cruise-1/detail/

Wednesday, June 4

18:45 – 20:30 **Meetings Committee Meeting** 202

- 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 the venues 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, David Lilley (d.m.j.lilley@dundee.ac.uk).

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

- Open to all graduate students and post docs
- No additional charge, no registration required

The social is a casual setting to socialize with your fellow colleagues and talk some science over drinks.

Thursday, June 5

11:45 – 13:15 **Mentor/Mentee Lunch** 400B

- Open to all attendees
- No additional charge, but advance registration is required

This lunch is an informal gathering that brings together 6-7 graduate students and post docs with one to two academic and industry mentors to answer student 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.

19:00 **Free Evening**

Enjoy your free evening in Québec City, and all that it has to offer. Short walking tours of Old Québec will be offered for anyone interested in informally exploring the area, or

set out on your own to one of the many cafes within walking distance of the Congress Centre for some of Québec's well known gourmet cuisine.

Friday, June 6

19:00 – 20:30 **Board of Directors Meeting** 202

- Open to the Board of Directors and (due to space constraints) a small number of additional observers

This is the business meeting of the 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 (mcswigj@comcast.net).

Saturday, June 7

10:30 – 12:00 **Junior Scientists Career Development Workshop** 301AB

- Open to all attendees, but tailored for junior scientists

The Career Development Workshop is an annual fixture of the meeting that discusses strategies for professional development and career advancement for graduate and postdoctoral RNA scientists. We're proud to bring you a fantastic session this year, entitled "Time Management: Doing More Than Surviving", that will delve into the unique challenges posed by the intricacies of managing one's personal and professional schedules, and the conflicts that can stem from them. The workshop will feature a keynote presentation by ***Michael Matrone****, Program Coordinator in the Office of Career and Postdoctoral Services at Scripps Research Institute in Jupiter, FL, USA, that will address ideas and strategies for managing time demands with a view toward both short- and long-term personal and professional goals. Attendees will also enjoy a small group discussion session focused on using time management strategies to analyze case studies from common scientific settings. We look forward to bringing you an informative and engaging workshop!*

Conference Closing Events

Open to all attendees who pre-registered by May 15

18:30 – 19:30 **Reception** Hilton Hotel Foyer (first floor)

19:30 – 21:30 **Conference Banquet & Awards** Hilton Hotel Ballroom (first floor)

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

- Reinhard Lührmann; RNA Society Lifetime Achievement Award
- Ann Marie Micenmacher; RNA Society Service Award
- RNA Society/Scaringe Award winners
  - *David Weinberg, Graduate Student Award*
  - *Jinwei Zhang; Post-Doctoral Award*
- Poster prize winners

21:30 – **Dance** Hilton Hotel Ballroom (first floor)

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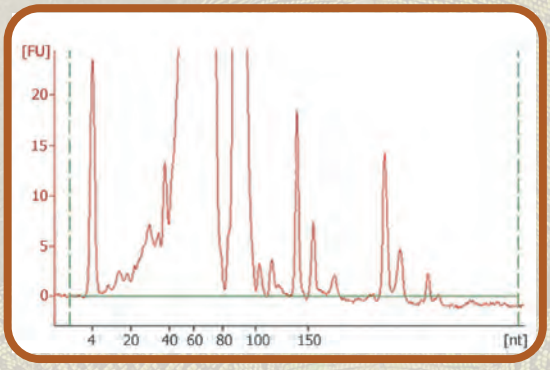

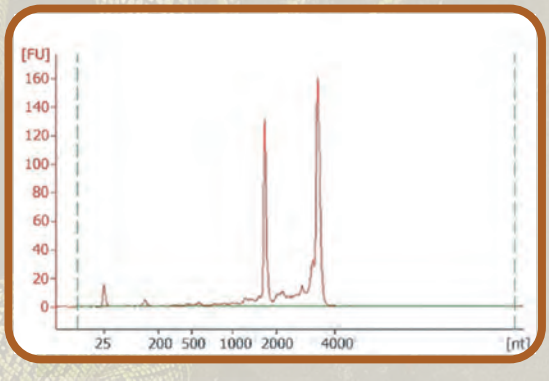

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* Piotr Chomczynski, US and international patents

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ORAL ABSTRACT LISTING

WEDNESDAY, JUNE 4, 2014: 8:30 – 12:30
Plenary Session 1: RNA-Protein Interactions, 200AB

Kathy Collins, Chair

Abstracts 1 – 15

1 **Recognition of budding yeast telomerase RNA by TERT and its role in template boundary definition**
Jinqiang Liu, Hyun-Ik Jun, Catherine Meyers, Feng Qiao

2 **A self-regulating template in human telomerase**
Andrew Brown, Xiaodong Qi, Yinnan Chen, Joshua Podlevsky, Mingyi Xie, Julian J.-L. Chen

3 **NMR and X-Ray structure-function analyses of box C/D ribonucleoprotein particles assembly complexes at atomic level**
Marc Quinternet, Christophe Charron, Benjamin Rothé, Régis Back, Jonathan Bizarro, Decebal Tiotu, Jean Michel Saliou, Sarah Sanglier-Cianférani, Cyril Dominguez, Frédéric Allain, Séverine Massenet, Edouard Bertrand, Xavier Manival, Bruno Charpentier, Christiane Branlant

4 **Roles of Eukaryote-Specific rRNA Expansion Segments in Ribosome Biogenesis**
Madhumitha Ramesh, John Woolford

5 **The DEAH-box helicase Ecm16 Dissociates U3 snoRNA from the pre-rRNA to promote rRNA folding**
Richa Sardana, Xin Liu, Sander Granneman, Jieyi Zhu, Michael Gill, David Tollervey, Carl Correll, Arlen Johnson

6 **Enzymatic Regulation of a DEAD-box RNA Helicase Promotes Efficient mRNP Assembly During Transcription**
Wai Kit Ma, Elizabeth Tran

7 **Mixed Doubles: The structural basis for dsRNA recognition by NF90/ILF3**
Heather Grey, Elizabeth Petfalski, Sander Granneman, David Tollervey, Atlanta Cook

8 **Comprehensive Analysis of RNA-Protein Interactions by High Throughput Sequencing-RNA Affinity Profiling**
Jacob Tome, Abdullah Ozer, John Pagano, Gary Schroth, John Lis

9 **Mechanism of U4/U6 di-snRNA recognition by a novel RNA-binding domain in spliceosomal protein Prp3 and its role in U4/U6•U5 tri-snRNP assembly**
Sunbin Liu, Sina Mozaffari-Jovin, Patrizia Fabrizio, Stanislaw Dunin-Horkawicz, Janusz M. Bujnicki, Reinhard Lührmann, Markus C. Wahl

10 **An in vitro peptide complementation assay for CYT-18-dependent group I intron splicing reveals a new role for the N-terminus**
Chun Geng, Paul Paukstelis

11 **Structural investigation of the spliceosomal helicase Aquarius**
Inessa De, Sergey Bessonov, Romina Höfele, Henning Urlaub, Reinhard Lührmann, Vladimir Pena

12 **A New Molecular Signature for Activation of Human 2'-5' Oligoadenylate synthetase-1 (hOAS1)**
Virginia Vachon, Graeme Conn

13 **RNA regulation by the *Caenorhabditis elegans* oocyte maturation determinant, OMA-1**
Ebru Kaymak, Sean Ryder

14 **A network of prion-like domains in RNA-binding proteins underpins paraspeckles: subnuclear RNP granules**
Sven Hennig, Geraldine Kong, Alwin Lian, Agata Sadowska, Tetsuro Hirose, Charlie Bond, Archa Fox

15 Defining the protein-protein interactions of the polyadenylate-binding protein nuclear 1 (PABPN1), the protein mutated in Oculopharyngeal Muscular Dystrophy
Ayan Banerjee, Duc Duong, Grace Pavlath, Anita Corbett

WEDNESDAY, JUNE 4, 2014: 14:00 – 16:45
Plenary Session 2: Splicing Regulation, 200AB
Sponsored by RiboClub
Ben Blencowe, Chair
Abstracts 16 – 25

16 Alternative Splicing of MKK7 forms a Novel Feed-Forward Loop to Promote T cell Activity
Nicole M Martinez, Kristen W Lynch

17 Regulation of cell-type specific and activation-dependent Traf3 alternative splicing
Astrid-Solveig Schultz, Monika Michel, Florian Heyd

18 Mechanistic insights into alternative splicing regulation by the neural-specific SR-related protein nSR100/SRRM4
Bushra Raj, Manuel Irimia, Ulrich Braunschweig, Tim Sterne-Weiler, Dave O'Hanlon, Zhen Yuan-Lin, Laura Easton, Jernej Ule, Anne-Claude Gingras, Eduardo Eyras, Benjamin Blencowe

19 Functional characterization of PTBP1 regulated alternative splicing events during neuronal differentiation
Anthony Linares, Douglas Black

20 The splicing factor Rbfox2 coordinated splicing is required for myoblast fusion
Ravi Singh, Xia Zheng, Chris Bland, Auinash Kalsotra, Marissa Ruddy, Tomaz Curk, Wei Li, Thomas Cooper

21 Independent regulation of transcription and alternative pre-mRNA splicing by a histone methyltransferase
Maayan Salton, Ty Voss, Tom Misteli

21A RBFox2 Provides Essential Cardiac Function by Regulating Alternative Splicing, Modulating MicroRNA Function, and Transcriptional Repression
Chaoliang Wei, Yu Zhou, Rui Xiao, Xiang-Dong Fu

22 Drosha Promotes Splicing of a Pre-microRNA-like Alternative Exon
Mallory Havens, Ashley Reich, Michelle Hastings

23 SRSF2 and ZRSR2: Spliceosomal RNA-binding Proteins and Myelodysplastic Syndrome Factors
Lindsey Skrdlant, Zhaojun Qiu, Emilee Bargoma, Ren-Jang Lin

24 Exitron Splicing, a New Type of Alternative Splicing Event
Yamile Marquez, Markus Höpfler, Zahra Ayatollahi, Andrea Barta, Mariya Kalyna

25 Evidence for regulatory splicing in nature's smallest spliceosome
Cameron Grisdale, Martha Stark, Stephen Rader, Naomi Fast

WEDNESDAY, JUNE 4, 2014: 17:15 – 18:45
Workshop 1: RNA Chemistry, 200A
Scott Strobel, Chair
Abstracts 26 – 31

26 The Spinach RNA aptamer contains a G-quadruplex and activates fluorescence in a GFP-like fluorophore
Hao Huang, Nikolai Suslov, Nan-sheng Li, Sandip Shelke, Molly Evans, Yelena Koldobskaya, Phoebe Rice, Joseph Piccirilli

27 RNA Mango: a tool for real-time visualization of RNA in living cells
Elena Dolgosheina, Sunny Jeng, Razvan Cojocaru, Peter Wilson, Nancy Hawkins, Paul Wiggins, Peter Unrau

28 **RNA Structure-Function Relationships in a Dengue Virus Genome**
Elizabeth Dethoff, Kevin Weeks

29 **Molecular basis for discrimination in RNA 3′-termini binding**
Kevin Desai, Craig Bingman, George Phillips Jr., Ronald Raines

30 **RNA crosslinking using Pt(II) complexes to probe the tertiary structure of the HDV Ribozyme**
Kory Plakos, Erich Chapman, Elaine Chase, Barbara Golden, Victoria DeRose

31 ***In vitro* evolution of self-cleaving ribozymes in the presence of iron - implications for RNA function on the early earth**
Milena Popovic, Mark Ditzler

WEDNESDAY, JUNE 4, 2014: 17:15 – 18:45
Workshop 2: RNA Regulation in Protozoa; 301AB

Sponsored by Burroughs Wellcome Fund
Barbara Papadopoulou, Chair
Abstracts 32 – 37

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32 **Networks of post-transcriptional control in trypanosomes**
Abeer Fadda, Esteban Erben, Mark Ryten, Dorothea Droll, Federico Rojas, Jurgen Haanstra, Smiths Lueong, Valentin Faerber, Keith Matthews, Joerg Hoheisel, Barbara Bakker, Christine Clayton

33 **The spliced leader RNA of *Trypanosoma brucei* determines the life and death and social motility of the parasites**
Shulamit Michaeli, Ronen Hope, Dror Eliaz

34 **Cross-linking Mass Spectrometry and Random Mutagenesis Approaches Reveal Detailed Insights into the Functional Architecture of *Trypanosoma brucei* Editosomes**
Suzanne McDermott, Jie Luo, Jason Carnes, Jeff Ranish, Ken Stuart

35 **Biology of small RNAs in trypanosome mitochondria**
Ruslan Aphasizhev, Takuma Suematsu, Lan Huang, Inna Aphasizheva

36 **New and old players in the translation apparatus of Leishmania**
Shimi Meleppattu, Dikla Kamus-Elimeleh, Dana Raz, Alexandra Zinoviev, Irit Orr, Michal Shapira

37 **A *Leishmania* DEAD-box RNA helicase homolog plays a key role in translational control under stress and during the intracellular parasite development**
Barbara Papadopoulou, Prasad K Padmanabhan, Ouafa Zghidi-Abouzid, Mukesh Samant, Carole Dumas

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WEDNESDAY, JUNE 4, 2014: 17:15 – 18:45
Workshop 3: RNA Programmable Genome Editing; 200B

Sponsored by CRISPRTX and Sigma Life Science
Emmanuelle Charpentier, Chair
Abstracts 38 – 42

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38 **Development and Applications of CRISPR-Cas9 for Genome Editing**
Feng Zhang

39 **Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients**
Bon-Kyoung Koo, Hans Clevers

40 **Repurposing Cas9 as a Multifunctional DNA binding protein**
Luke Gilbert, Lei S. Qi, Baohui Chen, Elizabeth Blackburn, Jennifer A. Doudna, Wendell A. Lim, Bo Huang, Jonathan Weissman

41 An mRNA delivery approach for HIV gene therapy
Maggie Bobbin, Anton McCaffrey, John Burnett, John Rossi

42 Next-Generation CRISPR-Cas Nucleases with Improved Specificities
James Angstman, Yanfang Fu, Shengdar Q Tsai, J. Keith Joung

THURSDAY, JUNE 5, 2014: 08:30 – 11:45
Plenary session 3: Emerging and High-Throughput Techniques, 200AB

Sponsored by Genome Canada
Gene Yeo, Chair
Abstracts 43 – 54

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43 Systems Level Analysis of Alternative Pre-mRNA Splicing: The roles of RNA structure and RNA Chaperone Proteins
Yeon Lee, Ming Hammond, Don Rio

44 High-Throughput Sequencing of Lariat Branch Sites and 5′ Splice Sites Identifies Dozens of Novel Branch Points and Diverse Alternative Splicing in the *Saccharomyces cerevisiae* Genome
Genevieve Gould, Joseph Paggi, Eric Wang, Christopher Burge

45 Interrogating the contribution of alternative splicing to neuronal physiology through genome-wide analyses of actively translating mRNAs in diverse cell types
Xicotencatl Gracida, Michael Dion, John A. Calarco

46 MOHCA-seq: Nucleotide-precision RNA proximity mapping from single multiplexed experiments
Clarence Cheng, Fang-Chieh Chou, Wipapat Kladwang, Siqi Tian, Pablo Cordero, Rhiju Das

47 Uridylation pattern of coding and noncoding RNAs in human cells
Dmytro Ustianenko, Lukas Bednarik, Biter Bilen, Mihaela Zavolan, Stepanka Vanacova

48 TORC1 orchestrates ribosome biogenesis and nitrogen catabolism at the transcriptional level to optimize cell growth
David Gresham

49 Extensive translation of small ORFs revealed by 'Polysomal-RiboSeq'
Julie Aspden, Ying Chen Eyre-Walker, Rose Philips, Michele Brocard, Unum Amin, Juan Pablo Couso

50 Global Identification of RNA-protein complexes with density gradient centrifugation and SILAC-MS
Rachel Knoener, Mark Scalf, Audrey Gasch, Lloyd Smith

51 Quantitative Differential Proteomics Analyses Dissect the Human LINE-1 Retrotransposon Ribonucleoprotein Physical Interactome
John LaCava, Kelly R. Molloy, David Fenyö, Martin S. Taylor, Hua Jiang, Jef D. Boeke, Brian T. Chait, Michael P. Rout

52 Massive parallel sequencing based hydroxyl radical probing of RNA accessibility (HRF-Seq) using Fenton chemistry and synchrotron irradiation
Lukasz Jan Kielpinski, Jeppe Vinther

53 mRNAseq procedures for the detection of subtle differences in gene expression
Irina Mohorianu, Damian Smith, Amanda Bretman, Wayne Rostant, Tamas Dalmay, Tracey Chapman

54 Modeling local splice variations from RNA-Seq data
Juan González-Vallinas, Jorge Vaquero-Garcia, Alejandro Barrera, Brian Cole, Kristen Lynch, Yoseph Barash

THURSDAY, JUNE 5, 2014: 13:15 – 15:30
Concurrent session 1: RNA Silencing, 200A
Yukihide Tomari, Chair
Abstracts 55 – 63

55 Structure models of bacterial Sm protein Hfq in complex with *rpoS* mRNA
Yi Peng, Joseph E. Curtis, Xianyang Fang, Sarah A. Woodson

55A Mammalian 5′-capped microRNA precursors that generate a single microRNA
Mingyi Xie, Mingfeng Li, Anna Vilborg, Nara Lee, Mei-Di Shu, Valeria Yartseva, Nenad Šestan, Joan Steitz

56 Turning catalytically inactive human Argonaute proteins into active slicer enzymes
Judith Hauptmann, Lukas Kater, Simone Harlander, Patrick Löffler, Rainer Merkl, Gunter Meister

57 A new post-translation modifier implicated in microRNA regulation in *C. elegans*
Gabriel Bossé, Lucile Fressigné, Guillaume Landry-Proulx, Mandy Ducy, Sandra Piquet, Martin Simard

58 Alternative Slit Promoters Drive Motor Neuron-Specific MicroRNA (miR-218) Necessary for Viability
Neal Amin, Ge Bai, Shawn Driscoll, Wesley Gifford, Samuel Pfaff

59 Coupled pri-miR-17~92 processing and pre-miRNA stabilization in cancer
Ariel Donayo, Thomas Duchaine

60 Dissecting the role for microRNA and circular RNA in the development of mammalian brain
Jørgen Kjems, Morten T. Venø

61 Specific Delivery Of Nucleic Acids To Cancer Cells By β-hairpin Forming Cell Penetrating Peptides
Kshitij Gupta, Kirill Afonin, Mathias Viard, Katelyn Nagy, Wojciech Kasparzak, Anu Puri, Alexey Zakharov, Mark C. Nicklaus, Robert Blumenthal, Joel Schneider, Bruce A. Shapiro

62 Dissecting the molecular mechanisms of small RNA mediated gene activation in *C. elegans*
Christopher Wedeles, Michelle Francisco, Monica Wu, Julie Claycomb

63 Multiple evolutionarily distinct RNA dependent RNA polymerase pathways compensate for the loss of piRNAs in multiple independent nematode lineages
Peter Sarkies, Murray Selkirk, Mark Taylor, John Jones, Eric Miska

THURSDAY, JUNE 5, 2014: 13:15 – 15:30
Concurrent session 2: Ribosome Mechanisms and Assembly, 200B
Katrin Karbstein, Chair
Abstracts 64 – 72

64 Multiple pathways for ribosomal small subunit biogenesis *in vivo*
Neha Gupta, Gloria Culver

65 Direct binding of Bms1p to the endonuclease Rcl1p is critical for Rcl1p recruitment into pre-ribosomes and early processing of the pre-rRNAs
Anna Delprato, Yasmine Al Kadhri, Natacha Péréaskine, Cécile Monfoulet, Yves Henry, Anthony K. Henras, Sébastien Fribourg

66 Rio1 mediates ATP-dependent final maturation of 40S ribosomal subunits
Tomasz Turowski, Simon Lebaron, David Tollervey

67 Eukaryotic Ribosome Assembly Samples Hybrid and Classical States as a Quality Control Mechanism
Hari Bhaskaran, Juliette Trepreau, Bethany Strunk, Katrin Karbstein

68 **New structural insights into mechanisms of cap-independent translation initiation**
Cha San Koh, Axel F. Brilot, Nikolaus Grigorieff, Andrei Korostelev

69 **Structure of the 80S initiation complex bound to histone H4 mRNA**
Angelita Simonetti, Jean-François Ménétret, Bruno Klaholz, Gilbert Eriani, Franck Martin

70 **Redefining the Translational Status of 80S Monosomes**
Erin Heyer, Melissa Moore

71 **The β-actin mRNA zipcode controls β-actin translation driving spatially localized actin cytoskeleton remodeling and adherens junction assembly to regulate 2D & 3D epithelial structure and function**
Natasha Gutierrez, Pavan Vedula, Lissette Cruz, Itua Eromobor, Ryan Petrie, Alexis Rodriguez

72 **Oxidative and alkylative RNA damage stall the translational machinery and induce No-Go Decay**
Benjamin Hudson, Carrie Simms, Hani Zaher

THURSDAY, JUNE 5, 2014: 17:30 – 19:00
Concurrent session 3: Ribozymes and Riboswitches, 200A
Ron Breaker, Chair
Abstracts 73 – 78

73 **The crystal structure and a catalytic mechanism of the twister ribozyme**
Yijin Liu, Timothy Wilson, Scott McPhee, David Lilley

74 **Biological impact of transcripts terminated by HDV-like ribozymes**
Randi Jimenez, Dana Ruminski, Andrej Luptak

75 **Direct evaluation of tRNA aminoacylation status by the T-box riboswitch using intermolecular stacking and steric readout**
Jinwei Zhang, Adrian Ferré-D'Amaré

76 **Riboswitch and small RNA-induced translation inhibition lead to mRNA degradation using different mechanisms**
Laurène Bastet, Anne-Marie Lamontagne, Eric Massé, Daniel Lafontaine

77 **Ligand binding by the tandem glycine riboswitch depends on aptamer dimerization but not double ligand occupancy**
Karen M Ruff, Scott A Strobel

78 **Bacterial riboswitches cooperatively bind Ni^{2+} or Co^{2+} ions and control expression of heavy metal transporters**
Zhiyuan Zhou, Kazuhiro Furukawa, Arati Ramesh, Zasha Weinberg, Tenaya Vallery, Wade Winkler, Ronald Breaker

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79 **U11 snRNP binding to 3′-UTR leads to nuclear retention**
Jens Verbeeren, Elina H. Niemelä, Mikko J. Frilander

80 **Developmentally-Regulated Elimination of Damaged Nuclei Involves a Chk2-Dependent Mechanism of mRNA Nuclear Retention**
Carole Iampietro, Julie Bergalet, Xiaofeng Wang, Neal Cody, Ashley Chin, Fabio Alexis Lefebvre, Melanie Douziech, Henry Krause, Eric Lecuyer

81 **Identification of mRNA export targets of SR protein family members reveals variation in cargo and shuttling**
Michaela Müller-McNicoll, Michaela Steiner, Holger Brandl, Valentina Botti, Antonio Domingues, Karla Neugebauer

82 **How mRNAs are Localized to the Endoplasmic Reticulum**
Alexander Palazzo, Xianying Cui

83 **Pheromone-induced transport of the yeast MFA2 mRNA to the mating projection is mediatedby specific RNP granules**
Stella Aronov, Saray Dover, Polina Geva, Enav Halperin, Mordechai Choder

84 **In vitro biogenesis of an mRNA-transport complex from yeast**
Annika Niedner, Roland Heym, Marisa Müller, Dennis Zimmermann, Ralf-Peter Jansen, Zeynep Oekten, David Kovar, Dierk Niessing

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Concurrent session 5: 3′ End Processing, 301AB
Elmar Wahle, Chair
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85 ***In vitro*** **reconstitution of the mammalian cleavage and polyadenylation specificity factor (CPSF)**
Lars Schönemann, Uwe Kühn, Georges Martin, Andreas R. Gruber, Mihaela Zavolan, Elmar Wahle

86 **An mRNA alternative polyadenylation regulation network promotes stem cell self-renewal**
Chengguo Yao, Brad Lackford, Lingjie Weng, Guang Hu, Yongsheng Shi

87 **The mTOR pathway regulates alternative cleavage and polyadenylation for transcriptome-wide control of 3′UTR length**
Jae-Woong Chang, Wei Zhang, Semo Jun, Kwan-Hyun Kim, Hsin Sung Yeh, Do-Hyung Kim, Rui Kuang, Jeongsik Yong

88 **Genome-wide Investigation of Poly(A) Tail Length and 3′ End Modifications by TAIL-seq**
Hyeshik Chang, Jaechul Lim, Minju Ha, Narry Kim

89 **The Histone Locus Body functions to increase the rate of 3′end formation and couple transcription and processing in vivo**
Deirdre Tatomer, Ivan Sabath, Zbigniew Dominski, Robert Duronio, William Marzluff

90 **Changing nature of the CTD structure**
Olga Jasnovidova, Karel Kubicek, Richard Stefl

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Claudia Bagni, Chair
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92 **Processing of HIV-1 short transcripts into miRNA**
Alex Harwig, Ben Berkhout, Atze Das

93 **Xrn1-resistant RNA structures present in the 3′ untranslated region of Flaviviruses**
Erich Chapman, David Costantino, Jennifer Rabe, Stephanie Moon, Jay Nix, Jeff Wilusz, Jeffrey Kieft

94 **Mechanism of regulated RNA cleavage during stress and immune surveillance by kinase-linked receptors RNase L and Ire1**
Yuchen Han, Jesse Donovan, Sneha Rath, Gena Whitney, Alisha Chitrakar, Alexei Korennykh

95 The sense and antisense of toxic RNA in neurodegeneration – human iPSC models to study misregulated RNA processing in *C9ORF72* ALS/FTD
Sebastian Markmiller, Anthony Vu, Patrick Liu, Thai Nguyen, Leen Jamal, Clotilde Lagier-Tourenne, Michael Baughn, Frank Rigo, Frank Bennett, John Ravits, Don Cleveland, Gene Yeo

96 Identification of genes in trinucleotide repeat RNA toxicity pathways in *C. elegans*
Susana Garcia, Yuval Tabach, Guinevere Lourenco, Maria Armakola, Gary Ruvkun

97 Dysregulation of microRNA bioprocessing in neurodegeneration
Anna Emde, Irit Reichenstein, Thomas Möller, Scott Hammond, Robert Sons, John Ravits, Eran Hornstein

98 A Novel Role for the Arginine Methyltransferase CARM1 in Nonsense Mediated Decay: Implications for Spinal Muscular Atrophy
Gabriel Sanchez, Emma Bondy-Chorney, Janik Laframboise, Geneviève Paris, Jocelyn Côté

99 The Fragile X Mental Retardation Protein regulates mRNA metabolism during corticogenesis affecting circuitry in the developing cortex
Giorgio La Fata, Annette Gärtner, Nuria Dominguez-Iturza, Tom Dresselaerts, Julia Dawitz, Rogier B. Poorthuis, Michele Averna, Tilmann Achsel, Uwe Himmelreich, Rhiannon M. Meredith, Carlos Dotti, Claudia Bagni

100 The oncogenic kinase NPM-ALK-dependant repression of miR150 level promotes lymphoma cells growth
Coralie Hoareau-Aveilla, Thibaud Valentin, Pierre Brousset, Fabienne Meggetto

101 Oncogenic properties of the RNA-binding protein UNR: Targets in Melanoma
Laurence Wurth, Panagiotis Papasaikas, Maria Garcia, Marisol Soengas, Fatima Gebauer

102 An RNA binding protein hnRNPM promotes breast cancer metastasis via regulating alternative splicing
Yilin Xu, Xin D Gao, Jae-Hyung Lee, Huilin Huang, Haiyan Tan, Jaeyoon Ahn, Lauren Reinke, Marcus Peter, Yue Feng, David Gius, Kalliopi Siziopikou, Junmin Peng, Xinshu G Xiao, Chonghui Cheng

102A Splicing factor hnRNP A2/B1 modulates breast cancer metastasis controlling alternative splicing of invasion genes
Regina Golan-Gerstl, Ilana Lebenthal-Loinger, Pushkar R Malaka, Jasmine Jacob, Gideon Rechavi, Ben Davidson, Reuven Reich, Rotem Karni

103 Non-redundant functions of splicing factors in breast-cancer initiation and metastasis
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104 Attenuation of NMD activity during chemotherapeutic treatment
Max Popp, Lynne Maquat

105 PGRN Network-wide Project: Transcriptome Analysis of Pharmacogenes in Human Tissues
Courtney E. French, Aparna Chhibber, Eric R. Gamazon, Sook Wah Yee, Xiang Qin, Elizabeth Theusch, Amy Webb, Scott T. Weiss, Marisa W. Medina, Ronald M. Krauss, Steven E. Scherer, Nancy J. Cox, Kathleen M. Giacomini, Steven E. Brenner

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Jørgen Kjems, Chair
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106 DGCR8 acts as a novel adaptor for the exosome complex to degrade double-stranded structured RNAs
Sara Macias, Ross Cordiner, Javier F. Caceres

107 piRNA- and lncRNA-mediated chromosomal fusions in the ciliate *Oxytricha*
Xing Wang, John Bracht, Keerthi Shetty, Sierra McCloud, Xiao Chen, Mariusz Nowacki, Laura Landweber

108 Human colorectal cancer-specific *CCAT1-L* lncRNA regulates long-range chromatin interactions in the *MYC* locus
Jian-Feng Xiang, Qing-Fei Yin, Tian Chen, Yang Zhang, Xiao-Ou Zhang, Li Yang, Ling-Ling Chen

109 Genome-wide screen identifies pathways that govern tRNA splicing and intron turnover
Jingyan Wu, Yao Wan, Anita Hopper

110 The modular transcriptome: resolving the extensive library of functional, structured non-coding RNA domains in the human genome
Martin Smith, Stefan Seemann, Tanja Gesell, Peter Stadler, John Mattick

111 Hypoxia regulated long non-coding RNAs in breast cancer: *Novel insights of hypoxic non-coding transcriptome*
Hani Choudhry, Johannes Schodel, Ashwag Albukhari, Spyros Oikonomopoulos, Francesca Buffa, Syed Haider, Peter Ratcliffe, David Mole, Adrian Harris, Joannis Ragousis

112 DNA damage response RNAs (DDRNAs) are novel necessary components of DNA damage response foci
Flavia Michelini, Sethuramasundaram Pitchiaya, Nils Walter, Fabrizio d'Adda di Fagagna

113 Primate-specific lncRNA genes as causes of human disease: evidence from GWAS and reverse genetics
Leonard Lipovich, Fabien Dachet, Donghong Ju, Juan Cai, Mary Ann Kosir, Cynthia A. Kalita, Chin-Yo Lin, James B. Brown

114 tRNA intronic circular (tric)RNAs: biogenesis, biology and biotechnology
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115 Strategies for Targeting the RNA in Expanded Repeat Disorders
Suzanne Rzuczek, Matthew Disney

116 In vitro and in vivo correction of nonsense mutations by amlexanox
Jieshuang Jia, David Tulasne, Fabrice Lejeune

117 A unique RNA structure formed by a long-distance interaction uncovers the therapeutic potential of a deep intronic sequence
Natalia Singh, Mariah Lawler, Matthew Howell, Eric Ottesen, Daya Upreti, Jennifer Kaczynski, Ravindra Singh

118 RECTAS, a candidate of the therapeutic drug for Familial dysautonomia, rectifies aberrant splicing of *IKBKAP* gene
Mayumi Yoshida, Naoyuki Kataoka, Kenjyo Miyauchi, Kei Iida, Tomomi Usui, Kenji Ohe, Suguru Yoshida, Takayuki Nojima, Yukiko Okuno, Hiroshi Onogi, Akihide Takeuchi, Takamitsu Hosoya, Tsutomu Suzuki, Masatoshi Hagiwara

119 Identification of Multiple Small Molecule Modulators of HIV-1 RNA Processing and Gene Expression
Ahalya Balachandran, Raymond Wong, Peter Stoilov, Alan Cochrane

120 RNA: The new revolution in nucleic acid vaccines
Andrew Geall

121 Inhibition of Individual 14q32 MicroRNAs Drastically Increases Neovascularization and Blood Flow Recovery after Ischemia
Sabine Welten, Teun Bastiaansen, Rob de Jong, Margreet de Vries, Erna Peters, Soren Sheikh, Nicola La Monica, Ekambar Kandimalla, Paul Quax, Yael Nossent

122 Development of therapeutic RNA switches selectively activated in diseased cells
Kirill Afonin, Eckart Bindewald, Bruce Shapiro

123 Targeting Structured Oncogenic RNAs with Antibodies
Jennifer Archer, Eileen Sherman, Jingdong Ye

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124 The structure of a yeast Pan2-Pan3 core complex reveals insights into its architecture and regulation
Ingmar Schäfer, Fabien Bonneau, Steffen Schüssler, Michaela Rode, Elena Conti

125 Assembly of the PAN2-PAN3 complex involves an asymmetric bipartite interface
Stefanie Jonas, Mary Christie, Daniel Peter, Dipankar Bhandari, Belinda Loh, Eric Huntzinger, Oliver Weichenrieder, Elisa Izaurralde

126 New insights in free m7G cap metabolism
Valerio Taverniti, Bertrand Seraphin

127 Codon optimization is a major determinant of mRNA half-life in *Saccharomyces cerevisiae*
Vladimir Presnyak, Ying-Hsin Chen, David Weinberg, Najwa Al Husaini, Sarah Olson, Kristian Baker, Brenton Graveley, Jeff Coller

128 Magnetic tweezers reveal Upf1 as a highly processive translocase
Francesca Fiorini, Debjani Bagchi, Vincent Croquette, Hervé Le Hir

129 Identification of cis-regulatory element within UPF1 target transcripts
Naoto Imamachi, Hidenori Tani, Rena Mizutani, Katsutoshi Imamura, Takuma Irie, Yutaka Suzuki, Nobuyoshi Akimitsu

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Concurrent session 7: RNA Interconnections, 200B
Karla Neugebauer, Chair
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130 CLAMPing the transcriptome
Laura Milligan, Grzegorz Kudla, Jean Beggs, David Tollervey

131 Post-transcriptional regulation of meiotic genes by a nuclear RNA silencing complex
Emily D. Egan, Craig R. Braun, Steven P. Gygi, Danesh Moazed

132 A third α-helix in Cyp33-RRM acts as an allosteric switch in MLL mediated transcription regulation
Markus Blatter, Charlotte Meylan, Frederic Allain

133 The spliceosomal U1 snRNP component Mud1 is autoregulated by promoting premature cleavage and polyadenylation of its own transcript
Madhura Raghavan, Hansen Xu, Jeff Pleiss

134 Detained introns are a novel, widespread class of introns posttranscriptionally spliced to regulate gene expression
Paul Boutz, Arjun Bhutkar, Phillip Sharp

135 Profiling the RNA maturation landscape in yeast
Alexander Ratushny, Karen E Wei, Peter Fridy, Wei-Ming Ming, Richard Rogers, Burak Kutlu, Ramsey Saleem, Michael P Rout, John Aitchison, Marlene Oeffinger

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Eric Westhof, Janusz Bujnicki, and François Major, Chairs
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136 Folding RNA by a Hierarchical Graph Sampling Approach
Namhee Kim, Shereef Elmetwaly, Mai Zahran, Tamar Schlick

137 Nearest neighbor parameters for RNA from accurate atomistic simulations
Maria Darvas, Giovanni Bussi

138 Computational Strategies for Analyzing RNA-Protein Complexes and Predicting RNA-Protein Interaction Networks
Rasna R. Walia, Li C. Xue, Usha K. Muppirala, Benjamin A. Lewis, Vasant G. Honavar, Drena Dobbs

139 A RNA Binding Score for predicting RNA binding probabilities of protein residues
Zhichao Miao, Eric Westhof

140 Harnessing the Mutational Landscape of Structured RNA
Vladimir Reinharz, Yann Ponty, Jérôme Waldispühl

141 New tools and resources for RNA structure analysis, comparison, and prediction
Craig Zirbel, Blake Sweeney, Anton Petrov, Neocles Leontis

141A RNA Bricks - a database of RNA 3D motifs and their interactions
Grzegorz Chojnowski, Tomasz Walen, Janusz M. Bujnicki

141B Staufen and Stau1 binding preferences defined using a new computational method applied to in vivo binding data
Xiao Li, Jerome Waldispühl, Howard Lipshitz, Quaid Morris

141C *RNA-Puzzles* Round II: Assessment of RNA structure prediction of two large riboswitches
Zhichao Miao, Marc-Frédérick Blanchet, Michal Boniecki, Janusz M. Bujnicki, Shi-Jie Chen, Clarence Cheng, Fang-Chieh Chou, Pablo Cordero, José Almeida Cruz, Rhiju Das, Feng Ding, Nikolay V. Dokholyan, Stanislaw Dunin-Horkawicz, Adrian Ferre-D'Amare, Wipapat Kladwang, Andrey Krokhotin, Marcin Magnus, François Major, Thomas H. Mann, Dorota Matelska, Alla Peselis, Alexander Serganov, Arpit Tandon, Siqi Tian, Xiaojun Xu, Jinwei Zhang, Peinan Zhao, Eric Westhof

141D RNA design rules from a massive open laboratory
Jeehyung Lee, Wipapat Kladwang, Minjae Lee, Daniel Cantu, Martin Azizyan, Hanjoo Kim, Alex Limpaecher, Sungroh Yoon, Adrien Treuille, Rhiju Das

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142 Adenosine to Inosine Editing Frequencies Controlled by the Speed of Splicing
Konstantin Licht, Michael Jantsch

143 N6-methyl-adenosine RNA modification controls cell fate transition in mamallian embryonic stem cells
Pedro Batista, Kun Qu, Jiajing Zhang, Lingjie Li, Donna Bouley, Ernesto Lujan, Ava Carter, Ryan Flynn, Marius Wernig, Howard Chang

144 Genome-wide pseudouridine sequencing (Pseudo-Seq) reveals regulated pseudouridinylation of mRNAs in yeast and humans
Thomas Carlile, Maria Rojas-Duran, Kristen Bartoli, Boris Zinshteyn, Kris Shin, Wendy Gilbert

145 Pseudouridylation of yeast U2 directly affects the ATPase activity of Prp5p during pre-mRNA splicing
Guowei Wu, David Stephenson, Chunxing Yang, Charles Query, Yi-Tao Yu

146 Proteins that contain a functional Z-DNA binding domain localize to cytoplasmic stress granules
Siew Kit Ng, Rebekka Weissbach, George Ronson, Dee Scadden

147 Two methylase paralogs, two diverging functions: from tRNA biogenesis to ribosome assembly
Ian Fleming, Mary Anne Rubio, Zdeněk Paris, Kirk Gaston, Juan Alfonzo

148 Diverse roles of the prion-like protein, Mod5, in tRNA-modification and RNA-silencing
Philip Smaldino, David Read, Matthew Pratt-Hyatt, Paul Good, David Engelke

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149 A specialized mechanism of miRNA mediated translation in cellular Quiescence
Syed Irfan Bukhari, S Spencer Truesdell, Shobha Vasudevan

150 Translational regulation by the mRNA-binding protein Cpeb4 controls terminal differentiation of erythroid cells
Wenqian Hu, Bingbing Yuan, Harvey Lodish

151 Folding and function of a preQ$_1$ riboswitch-regulated messenger RNA at the single molecule level
Paul Lund, Arlie J. Rinaldi, May Daher, Mario R. Blanco, Krishna C. Suddala, Nils G. Walter

152 Evolutionary conserved patterns of sequence complementarity between eukaryotic mRNA UTRs and rRNAs and their implication for gene translation regulation
Josef Pánek, Leoš Valášek

153 What makes an efficient ribosome binding site an efficient ribosome binding site? A study of the randomized libraries exceeding 1 000 000 5′-UTRs
Ilya Osterman, Sergey Evfratov, Ekaterina Andreyanova, Maria Rubtsova, Ekaterina Kostryukova, Vadim Govorun, Alexey Bogdanov, Petr Sergiev, Olga Dontsova

154 Accurate measurements of ribosome footprints and mRNA abundances reveal the limited extent of translational control
David Weinberg, Premal Shah, Stephen Eichhorn, Jeffrey Hussmann, Joshua Plotkin, David Bartel

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Concurrent session 9: Splicing Mechanisms, 200B
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155 Core structure of the U6 snRNP and investigation of U4/U6 di-snRNP biogenesis
Eric Montemayor, Allison Didychuk, Elizabeth Curran, Hong Hong Liao, Kristie Andrews, Christine Treba, Samuel Butcher, David Brow

156 An early assembly intermediate containing tri-snRNP forms during both exon- and intron-defined splicing, and its conversion to a stable B complex involves structural rearrangements triggered by tri-snRNP/5′ splice site interaction
Carsten Boesler, Cindy L. Will, Berthold Kastner, Norbert Rigo, Prakash Dube, Dmitry Agafonov, Ralf Ficner, Henning Urlaub, Reinhard Lührmann

157 Brr2 and Prp8 retinitis pigmentosa alleles impact the efficiency and fidelity of multiple steps in the splicing cycle
Megan Mayerle, Christine Guthrie

158 Crystal structure of a eukaryotic group II intron lariat
Aaron Robart, Russell Chan, Jessica Peters, Kanagalaghatta Rajashankar, Navtej Toor

159 The spliceosomal DExD/H-box ATPase Prp16 chaperones splice site selection
Daniel Semlow, Mario Blanco, Nils Walter, Jonathan Staley

160 Identification of the 3'SS substrate binding site in the spliceosome second-step active site
Charles Query, Magda Konarska

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Michael Sattler, Chair
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161 Transient RNA structure features are evolutionarily conserved and can be computationally predicted
Jing Yun A. Zhu, Adi Steif, Jeff R. Proctor, Irmtraud M. Meyer

162 Accurate and generic approaches for novel motif discovery in large RNAs using differential SHAPE
Greggory M. Rice, Nathan A. Siegfried, Steven Busan, Julie A.E. Nelson, Kevin M. Weeks

163 Three classes of conformations are a necessary structural feature of the Retinoblastoma 5' UTR
Katrina Kutchko, Wes Sanders, Amanda Solem, Matthew Halvorsen, Alain Laederach

164 Three-dimensional structure determination of large RNAs using small-angle X-ray scattering
Yuba Bhandari, Jinbu Wang, Xianyang Fang, Ping Yu, Yun-Xing Wang

165 Characterizing transient base-pair rearrangements in RNAs by Nitrogen relaxation dispersion NMR
Yi Xue, Mitchell McBrairty, Hashim Al-Hashimi

166 Kinetics and Thermodynamics of Domain Docking in the Juctionless Hairpin Ribozyme
Neil White, Charles Hoogstraten

167 Quantitative analysis of RNA modifications during bacterial ribosome assembly
Anna Popova, James Williamson

168 Widespread N^6-methyladenosine-dependent RNA Structural Switches Regulate RNA-Protein Interactions
Nian Liu, Marc Parisien, Qing Dai, Chuan He, Tao Pan

169 Structure and function of 23S rRNA m^6A methyltransferase RlmJ
Avinash Punekar, Josefine Liljeruhm, Tyson Shepherd, Anthony Forster, Maria Selmer

170 A comprehensive catalogue of the *C. elegans* dsRNAome
Joseph Whipple, Osama Youssef, David Nix, Changjin Hong, W. Evan Johnson, Brenda Bass

171 The RNA editing enzyme ADAR1 is a key regulator of innate immune responses to RNA
Niamh Mannion, Sam Greenwood, Liam Keegan, Mary O' Connell

172 Editing of miRNAs fine-tunes synaptic gene regulation
Mikaela Behm, Ylva Ekdahl, Marie Öhman

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174 Pin1 regulates mRNA 3′ end processing during the DNA damage response
Jorge Baquero, Frida Kleiman

175 Genome-wide analysis of RNA polymerase II distribution in CDS-associated polyadenylation sites in *Arabidopsis*
Laura de Lorenzo, Arthur G. Hunt

176 Post-transcriptional control of gene expression including alternative polyadenylation plays a major and unexpected role in the cellular stress response
Ina Hollerer, Tomaz Curk, Vladimir Benes, Matthias W. Hentze, Andreas E. Kulozik

177 Regulation of COX-2 expression by post-transcriptional mechanisms
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178 Mis-regulation of Alternative Polyadenylation in a Microsatellite Expansion Disease
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179 Molecular Characterization of the pre-mRNA 3′ end core cleavage factor: Interactions, assembly and activity of CPSF73, CPSF100, and Symplekin
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180 hnRNPs repress alternative polyA sites to promote formation of longer 3′UTRs
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181 The Poly(A)-Binding Protein Nuclear 1 (PABPN1) and the Nuclear Exosome Promote 3′ End Maturation of the Human Telomerase RNA
Duy Nguyen, Valérie Grenier St-Sauveur, Francois Bachand

182 Hydrogen Deuterium Exchange Mass Spectrometry (HDXMS) and cross-linking in structural studies on the FLASH-Lsm11 complex and its interaction with polyadenylation factors
Aleksandra Skrajna, Krzysztof Tarnowski, Xiao-cui Yang, Kinga Fituch, Ivan Sabath, William F. Marzluff, Zbigniew Dominski, Michal Dadlez

183 U1 snRNP-dependent Regulation of Intronic Polyadenylation Linked to Tumor Dissemination
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184 Rational experiment design for sequencing-based RNA structure characterization
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185 Long-range Nucleotide Covarations in Drosophila Genomes Detected By A Novel Computational Approach
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186 Abstract Withdrawn

187 RNA Bricks - a database of RNA 3D motifs and their interactions
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188 Nucleic Acid Database (NDB): Redesign and focus on RNA
Buvaneswari Coimbatore Narayanan, John Westbrook, Saheli Ghosh, Anton Petrov, Blake Sweeney, Craig Zirbel, Neocles Leontis, Helen Berman

189 Identification and visualization of alternative events from RNA-Seq data using EASANA: Impact of SF3B1 mutations in uveal melanoma
Frédéric Lemoine, Caroline Garnier, Audrey Rapinat, Marc Rajaud, David Gentien, Pierre de la Grange

190 Consistent alternative splicing isoform switches in tumor samples provide novel signatures in 10 cancer types
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191 Common Secondary Structure Prediction for RNA Homologs with Domain Insertions: Dynalign II
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192 Detecting cis-antisense elements using RNA sequencing and comparative genomics in the Archaea
Andrew Holmes, David Bernick, Todd Lowe

193 3D Modeling of Group I Intron structures by comparative modeling with ModeRNA and de novo RNA folding with SimRNA
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194 RNA transcript complexity is associated with Chronic Obstructive Pulmonary Disease
Lela Lackey, Alain Laederach

195 eRNA: a collection of web servers for comparative RNA structure prediction and visualisation
Daniel Lai, Irmtraud Meyer

196 Improved quality assessment of RNA 3D models
Marcin Magnus, Albert Bogdanowicz, Janusz M. Bujnicki

197 Computational analysis of the key components of the splicing and NMD machinery in *Ustilago maydis*
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199 Abstract Withdrawn

200 Sailfish: Software for Rapid, Alignment-free Quantification of Isoform Abundance
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201 Estimating Intron/Exon retention levels using IntEREst
Ali Oghabian, Elina Niemelä, Mikko Frilander

202 Principal component decomposition to visualize the RNA suboptimal ensemble
Chanin Tolson, Alain Laederach

203 Developing computational approach to predict coincidence of structural and primary signatures in mRNAs
Olivia Zhang, Yann Ponty, Quaid Morris, Mathieu Blanchette, Eric Lecuyer, Jerome Waldisphul

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205 Massive parallel sequencing based hydroxyl radical probing of RNA accessibility (HRF-Seq) using Fenton chemistry and synchrotron irradiation
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206 Large scale variant discovery in microRNA genes to identify microRNAs regulating neuronal processes
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207 Deoxyribozymes for Preparation of Fluorescent Pre-mRNAs without Splinted Ligation
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208 Single Molecule, Real-Time Sequencing of Full-length cDNA Transcripts Uncovers Novel Alternatively Spliced Isoforms
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209 Integrating mRNA-protein interactions, translation efficiency and protein mass spectrometry to measure cellular stress response
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210 *Cryptococcus neoformans* var. *grubii* transcriptome structure
Damien Paulet, Quiandong Zeng, Hon Chung-Chau, François Brunel, Claude Gaillardin, Carolin Wollschlaeger, Sara Gonzalez-Hilarion, Sharvari Gujja, Tristan Rossignol, Jean-Yves Coppée, Cécile Neuvéglise, Christina Cuomo, Guilhem Janbon

211 Identification of the RNA targets of RBM45, a new RNA-binding protein implicated in ALS and FTLD
Yang Li, Kendall Jensen, Robert Bowser

212 Coupling demethylation to RNA sequencing reveals a missing class of modified tRNA fragments and dynamics of RNA modification
Aaron Cozen, Andrew Holmes, Eva Robinson, Erin Quartley, Eric Phizicky, Todd Lowe

213 Targets of RNA-binding proteins Identified By Editing (TRIBE); a novel technique
Aoife McMahon, Hua Jin, Michael Rosbash

214 Tumour RNA Disruption: An Effective Biomarker for Monitoring Clinical Response and Disease-free Survival After Neoadjuvant Chemotherapy in Locally Advanced Breast Cancer
Amadeo Parissenti, Baoqing Guo, Laura Pritzker, Ken Pritzker, Shirly Wang, Mu Zhu, Maureen Trudeau

215 Understanding T4 RNA ligase bias to improve small RNAseq library construction
Ryan T. Fuchs, Zhiyi Sun, Fanglei Zhuang, G. Brett Robb

216 Selective Depletion of Abundant RNAs to Enable Transcriptome Analysis of Low Input and Highly Degraded RNA from FFPE Breast Cancer Samples
Deyra N. Rodríguez, Daniela B. Munafó, Bradley W. Langhorst, Christine L. Chater, Christine J. Sumner, Salvatore Russello, Fiona J. Stewart, Dominick Sinicropi, John Morlan, Kunbin Qu, Mei-Lan Liu, Jennie Jeong, Mylan Pho, Ranjana Ambannavar, Eileen T. Dimalanta, Theodore B. Davis

217 Determining RNA and RNA•Protein conformation ensembles with Au-SAXS interferometry
Xuesong Shi, Huang Lin, David Lilley, Pehr Harbury, Daniel Herschlag

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Don Weldon, Yuko Williams, Alex Ko, Haizhen Liu

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GJ Lohman, AM Zhelkovsky, EJ Cantor, TC Evans

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220 Abstract Withdrawn

221 Transcriptome analysis reveals thousands of targets of nonsense-mediated mRNA decay that offer clues to the mechanism in different species
Courtney E. French, Gang Wei, Anna Desai, Angela N. Brooks, Thomas L. Gallagher, Li Yang, Brenton R. Gravely, Sharon L. Amacher, Steven E. Brenner

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Anna Desai, Steven E. Brenner

223 A feedback loop couples MSI1 activity to omega-9 fatty acid biosynthesis
Carina Clingman, Laura Deveau, Samantha Hay, Ryan Genga, Shivender Shandilya, Francesca Massi, Sean Ryder

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Seth B Garren, Michael O Duff, Gordon G Carmichael

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Maki Inada, Robert Nichols, Jeffrey Pleiss, Beate Schwer

226 U2 snRNP is required for transcription elongation in a gene specific manner
Daisuke Kaida, Mitsunori Koga, Takayuki Satoh

227 The NuA4 and Swr1 chromatin modification complexes are important for RNA splicing
Aron Moazamian, Jordan Martinez, Ryan Moazamian, Christine Scaduto, Tracy Kress

228 To define the role of Sam68 in p53-mediated functions
Naomi Li, Stéphane Richard

229 Exosome identification in *Lithobates catesbeianus*
Juliana Luz, Breno Caneguim, Estela Sasso-Cerri, Caren Helbing, Sandro Valentini, Carla Oliveira, Mariana Santori, Andressa Baggio

230 Autoregulatory association of a cyanobacterial DEAD-box RNA helicase with polycistronic message processing
Albert Rosana, Danuta Chamot, George Owttrim

231 Defining functions for DZF domain-containing proteins in *C. elegans*
Daniel Reich, Brenda Bass

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Francois Sement, Ruslan Afasizhev

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Craig G Simpson, Jiradet Gloggnitzer, Dominika Lewandowska, Karel Riha, John WS Brown

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Chen Wang, Ben Szaro

235 Abstract Withdrawn

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252 Interaction of the 3′ end of the non-coding RNA 7SK, a regulator of human transcription elongation, with its stabilizing partner LaRP7
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253 The Prader-Willi critical region generates non-canonical snoRNAs that regulate genes involved in lipid metabolism
Marina Falaleeva, Manli Shen, Eduardo Eyras, Pierre de la Grange, Ruth Sperling, Stefan Stamm

254 Deciphering RNA regulatory elements using co-expression graphs
Vahid Gazestani, Reza Salavati

255 Spatiotemporal dissection of cytoplasmic and nuclear miRNA function
Laurie Heinicke, Sethuramasundaram Pitchiaya, Nils Walter

256 Structural Studies of Intact Long Noncoding RNAs in Plants and Mammals
Scott Hennelly, Irina Novikova, Ashutosh Dharap, Julia Questa, Laurie Boyer, Caroline Dean, Karissa Sanbonmatsu

257 Structural insight into the molecular pathway that links miRNA target recognition to silencing
Ying Chen, Andreas Boland, Mary Christie, Duygu Kuzuoğlu-Öztürk, Belinda Loh, Praveen Bawankar, Oliver Weichenrieder, Elisa Izaurralde

258 Domain dissection of steroid receptor RNA activator (SRA) for coactivation of steroid receptor-mediated transcription
Seonghui Jang, Euihan Jung, Jungmin Lee, Younghoon Lee

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Keekwang Kim, Yanqin Yang, Jun Zhu, Robert Adelstein, Sachiyo Kawamoto

260 Promoter elements required for expression of BC200 RNA in HeLa cells
Youngmi Kim, Jungmin Lee, Heegwon Shin, Younghoon Lee

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262 Antisense RNA from the Histone Gene Clusters : Novel Regulator or Noisy Transcription?
Fabio Alexis Lefebvre, Éric Lécuyer, Neal Cody

263 Computational model of the dynamics of fourU and ROSE RNA thermometers at elevated temperatures
Filip Leonarski, Maciej Jasinski, Joanna Trylska

264 RNA-Directed Regulation of pri-miRNA Processing by a Long Noncoding RNA Transcribed from an Ultraconserved Region
Julia Liz, Anna Portela, Marta Soler, Antonio Gómez, Hui Ling, Gracjan Michlewski, George A. Calin, Sònia Guil, Manel Esteller

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Sylvain Maenner, Catherine Regnard, Tamas Schauer, Peter B. Becker

266 Widespread accumulation of circRNAs during aging
Pedro Miura, Jakub Westholm, Susan Celniker, Brenton Graveley, Eric Lai

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Ruby Yu, Gloria Jih, Nahid Iglesias, Danesh Moazed

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Birgit Nachbauer, Melanie Amort, Norbert Polacek

269 Are viroid-specific small RNAs instructive and mediate a pathogenesis network?
Rajen Julian Joseph Piernikarczyk, Jaroslav Matoušek, Gerhard Steger

270 An mRNA-derived ncRNA targets and regulates the ribosome
Andreas Pircher, Lukas Schneider, Nadja Widmer, Norbert Polacek

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Christopher Presslauer, Teshome Bizuayehu, Jorge Fernandes, Igor Babiak

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Balasubramanian Sellamuthu, Fatma Khalfaoui, Xiaoling Yang, Mohammad Reza Naghdi, Jonathan Perreault

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Jie Li, Wei Xia, Xingliang Qin, Xueting Su, Hongmei Ding, Aixue Huang, Hui Li, Lubing Hou, Shaohua Li, Qiang Zhao, Ningsheng Shao

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Sumangala Shetty, Paul Copeland

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Jenna Smith, Sarah Geisler, Juan Alvarez-Dominguez, Nicholas Kline, Nathan Huynh, Wenqian Hu, Jeff Coller, Kristian Baker

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Mansoureh Tajaddod, Konstantin Licht, Florian Huber, Sandy Schopoff, Michael. F Jantsch

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Gaelle Talhouarne, Joseph Gall

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Peter Baumann, Chi-Kang Tseng

279 The DRBD13 RNA binding protein is involved in the developmental regulation of mRNA stability in Trypanosoma brucei
Hanrong Wu, Bhaskar Anand Jha, Zhiquan Lu, Vahid H. Gazestani, Reza Salavati

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Pei-Hsuan Wu, Jiali Zhuang, Xin Zhiguo Li, Zhiping Weng, Phillip D. Zamore

281 Species-spedific alternative splicing leads to unique expression of *sno-lncRNA*s
Xiao-Ou Zhang, Qing-Fei Yin, Hai-Bin Wang, Yang Zhang, Tian Chen, Ling-Ling Chen, Li Yang

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Zohra Saci, Claire Bertrand, Marina Pinskaya, Elena Battistello, Luis Jaime Castro-Vega, José-Arturo Londoño-Vallejo, Daniel Gautheret, Antonin Morillon

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283 A knockout mouse model to define the roles of the Epithelial splicing regulatory proteins in development and epithelial cell function
Thomas Bebee, Katherine Sheridan, Benjamin Cieply, Sunder Sims-Lucas, Daniel Bushnell, Maximilian Reichert, Anil Rustgi, Carlton Bates, Russ Carstens

Ribosomes and Translation
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284 Homologous *trans*-editing factors with broad substrate specificity prevent global mistranslation
Jo Marie Bacusmo, William Cantara, Birgit Alber, Karin Musier-Forsyth

285 The DEAH-box helicase Ecm16 unwinds U3-pre-rRNA duplexes but not other substrates
Xin Liu, Jieyi Zhu, Arlen Johnson, Carl Correll

286 Thermodynamic landscape of the bacterial 30 S translation initiation complex assembly
Benoit Meyer, Guillaume Bec, Stefano Marzi, Philippe Dumas, Eric Ennifar

287 Elongation Factor G Undergoes an Extensive Structural Rearrangement during Ribosomal Translocation
Enea Salsi, Elie Farah, Jillian Dann, Dmitri Ermolenko

288 Abstract Withdrawn

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Basil Greber, Daniel Boehringer, Alexander Leitner, Philipp Bieri, Felix Voigts-Hoffmann, Jan Erzberger, Marc Leibundgut, Ruedi Aebersold, Nenad Ban

290 The telomerase inhibitor Gno1p/PINX1 activates the helicase Prp43p during ribosome biogenesis
Yan-Ling Chen, Régine Capeyrou, Odile Humbert, Saïda Mouffok, Yasmine Al Kadri, Simon Lebaron, Anthony K Henras, Yves Henry

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Maki Yoshihama, Akihiro Nakao, Shusaku Kamada, Naoya Kenmochi

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Serdal Kirmizialtin, Suna P. Gulay, Jonathan D. Dinman, Karissa Y. Sanbonmatsu

293 Atomic mutagenesis of the ribosomal Sarcin-Ricin-loop to study EF-G GTPase activation
Miriam Koch, Sara Flür, Pascal Küpfer, Christian Leumann, Ronald Micura, Norbert Polacek

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Miri Krupkin, Ella Zimmerman, Anat Bashan, Ada Yonath

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Beril Kumcuoglu, Jelena Jakovljevic, Michael Gamalinda, John Woolford

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Anne-Marie Landry-Voyer, Sarah Bilodeau, Caroline Rouleau, François-Michel Boisvert, François Bachand

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Ning Lee, Ya-Han Ting, Bo-Ru Chen, Ting-Jyun Lu, Kai-Yin Lo

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Frédéric Lessard, Véronique Bourdeau, Xavier Deschênes-Simard, Sebastian Igelmann, Marinieve Montero, Gerardo Ferbeyre

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Kelly Limoncelli, Andrei Korostelev, Nicholas Rhind, Melissa Moore

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Clarence Ling, Jillian Dann, Dmitri N. Ermolenko

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Tatsuya Maehigashi, Jack Dunkle, Stacey Miles, Christine Dunham

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Mélodie Duval, Alexey Korepanov, Olivier Fuchsbauer, Pierre Fechter, Andrea Haller, Attilio Fabbretti, Laurence Choulier, Ronald Micura, Bruno Klaholz, Pascale Romby, Mathias Springer, Stefano Marzi

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Tina McLeod, Saverio Brogna

304 Click Fluorescent Labeling and Enzymatic Mapping of RNA Targets of Platinum (II) Anticancer Therapeutics
Maire Osborn, Jonathan White, Alan Moghaddam, Victoria DeRose

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Maxim Paci, George Fox

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Jennifer Gebetsberger, Norbert Polacek

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Metodi D Metodiev, Henrik Spahr, Paola Loguercio Polosa, Caroline Meharg, Christian Becker, Janine Altmueller, Bianca Habermann, Nils-Goran Larsson, Benedetta Ruzzenente

308 Insight into mitoribosome maturation by structural methods
Patrick Scicluna, Martin Hällberg

309 Deciphering roles for small ribosomal subunit assembly factors in blocking premature translation initiation in *E.coli*
Brian Smith, Gloria Culver

310 Mechanism of regulation of GTP hydrolysis by translation elongation factors EF-Tu and EF-G as deduced from *in silico* analysis of their complexes with ribosome
Sergey Steinberg, Konstantin Bokov

311 Development of New Methods for the Study of Bacterial Ribosome Biogenesis and Characterization of its Associated Factors
Geordie Stewart, Matthew Scott, Eric Brown

312 Bcp1 is a critical checkpoint for Tif6 binding in 60S ribosome biogenesis pathway
Ya-Han Ting, Kai-Yin Lo

313 Ribosome dysfunction and erythroid failure: Analyzing the zebrafish model of Diamond-Blackfan anemia
Tamayo Uechi, Yukari Nakajima, Gnaneshwar Yadav, Yutaka Suzuki, Sumio Sugano, Naoya Kenmochi

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Ouafa Zghidi-Abouzid, Prasad Padmanabhan, Mukesh Samant, Carole Dumas, Barbara Papadopoulou

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315 Characterization of fission yeast tri-methyl guanosine synthase (tgs1)
Richard Dannebaum, Wen Tang, Peter Baumann

316 Using a histone replacement system to define co-transcriptional interactions between histone modifications and elongating RNA
Michael P. Meers, Daniel J. McKay, A. Gregory Matera

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Marisa Müller, Sylvain Maenner, Peter B. Becker

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Masatoshi Mutazono, Misato Morita, Chihiro Tsukahara, Madoka Chinen, Jun-ichi Nakayama, Kojiro Ishii, Takashi Ideue, Tokio Tani

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Matthew Simon

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Justin Wong, William Ritchie, Dadi Gao, Amy Au, Natalia Pinello, Jeff Holst, John Rasko

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322 Can deprotonated guanines promote activation of 2'-OH nucleophile in ribozymes: an insight from molecular dynamics simulations and hybrid QM/MM calculations
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323 Folding and regulation mechanism of the thiC riboswitch
Adrien Chauvier, Anne-Marie Lamontagne, Reza Naghdi, Jonathan Perreault, Daniel Lafontaine

324 Crystal structure of the Varkud Satellite Ribozyme: A peek into trans-active enzymes of the RNA World
Nikolai Suslov, Hao Huang, Saurja DasGupta, David Lilley, Phoebe Rice, Joseph Piccirilli

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Francesco Di Palma, Francesco Colizzi, Giovanni Bussi

326 Investigating the impact of the local environment on RNA function through *in vitro* evolution
James Stephenson, Milena Popovic, Thomas Bristow, Mark Ditzler

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Matthew Lau, Adrian Ferré-D'Amaré

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Joseph Liberman, Mohammad Salim, Joseph Wedekind

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Phillip J. McCown, Keith Corbino, Ronald R. Breaker

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Robert Meehan, Scott Strobel

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Manja Wachsmuth, Sven Findeiss, Robert Serfling, Ronny Lorenz, Peter Stadler, Mario Moerl

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James Nelson, Narasimhan Sudarsan, Grace Phillips, Shira Stav, Philip McCown, Ronald Breaker

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Ian Price, Ailong Ke

334 Mechanistic and structural studies on the twister ribozyme
Timothy Wilson, Yijin Liu, Scott McPhee, David Lilley

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335 Towards Optimization of a General RNA Labeling Deoxyribozyme: Analysis of Unimolecular Deoxribozyme Variants
Matthew Ashton, Tucker Carrocci, Claudia Hoebartner, Aaron Hoskins

336 Single-Molecule Fluorescence Using Nucleotide Analogs: A Proof-of-Principle
Chamaree de Silva, Elvin Aleman, Eric Patrick, Karin Musier-Forsyth, David Rueda

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Kevin Desai, Craig Bingman, George Phillips Jr., Ronald Raines

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Amanda Haas, Xiaoqin Cheng, Kristina Larson, Letitia Kwok, David Mierzejewski, Shawn Begay, Randy Rauen, Kelly Grimsley, Kaizhang He, Anja van Brabant Smith

339 DNA-mediated synthesis of site-specifically labeled RNA
Lea Büttner, Fatemeh Javadi-Zarnaghi, Claudia Höbartner

340 Synthesis and design of RNA binding molecules in enthalpy-driven manner
Nozomi Natsuhara, Yue Di, Tetsuya Tsuda, Sanjukta Mukherjee, Kazuhiko Nakatani

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341 Pseudouridylation-induced nonsense suppression is sequence context-independent
Hironori Adachi, Yi-Tao Yu

342 Architecture of the U-insertion/deletion editosome
Inna Afasizheva, Liye Zhang, Lan Huang, Stefano Monti, Ruslan Aphasizhev

343 *NCS2 links tRNA modification to pathogenic phenotypes in yeast**
Fiona Alings, Sebastian A. Leidel

344 A pseudouridine residue in the core of the spliceosome is part of the filamentous growth program in yeast
Anindita Basak, Charles Query

345 Abstract Withdrawn

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Bhaskara R. Madina, Vikas Kumar, Blaine H.M. Mooers, Ralf Bundschuh, Jorge Cruz-Reyes

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Nicolas Fossat, Karin Tourle, Tania Radziewik, Kristen Barratt, Doreen Liebhold, Joshua Studdert, Melinda Power, David Loebel, Patrick Tam

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Ryan T. Fuchs, Laurence Ettwiller, Curtis Desilets, Christopher J. Fry, G. Brett Robb

349 The Biology of Pus3 and of Ψ38 and Ψ39 Modifications in Yeast tRNAs
Lu Han, Eric Phizicky

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Michael Washburn, Emily Wheeler, Doug Rusch, Boyko Karakadov, Gene Yeo, Heather Hundley

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Mamta Jain, Maja Stulic, Michael Jantsch

352 Substrate binding by pseudouridine synthase TruB occurs in two steps and is critical for bacterial fitness
Laura Keffer-Wilkes, Ute Kothe

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Kyunghwan Kim, Shinae Suk, Younghoon Lee

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Danny D Nedialkova, Karin Buhne, Hannes Drexler, Sebastian A Leidel

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Yicheng Long, Maria Abad, Fuad Mohammad, Erik Olson, Jane Jackman

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Alborz Mazloomian, Irmtraud Meyer

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Vaibhav Mehta, Houtan Moshiri, Chun Yip, Reza Salavati

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Linda Reinhard, B. Martin Hällberg

360 Directing the timing of ribosomal RNA modification in human cells: the recruitment of late-acting snoRNAs to pre-ribosomal complexes is regulated by the RNA helicase DDX21
Katherine E. Sloan, Matthias S. Leisegang, Carmen Doebele, Ana S. Ramirez, Stefan Simm, Charlotta Safferthal, Sara Haag, Jens Kretschmer, Ingo Ebersberger, Michael Karas, Enrico Schleiff, Nicholas J. Watkins, Markus T. Bohnsack

361 Diverse roles of the prion-like protein, Mod5, in tRNA-modification and RNA-silencing
Philip Smaldino, David Read, Matthew Pratt-Hyatt, Paul Good, David Engelke

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Takuma Suematsu, Inna Aphasizheva, Lan Huang, Ruslan Aphasizhev

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William Swinehart, Jane Jackman

364 RNA repair in bacteria and beyond
Pei Wang, Kiruthika Selvadurai, Raven Huang

365 Characterising the mechanism by which Inosine-containing dsRNA suppresses interferon induction
Rebekka Weissbach, ADJ Scadden

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366 Modified Amber Force Field Correctly Models the Conformational Preference of Tandem GA pairs in RNA
Asaminew Aytenfisu, Aleksandar Spasic, Matthew Seetin, John Serafini, David Mathews

367 The DEAH-box ATPase Prp2 catalytically activates the spliceosome by rendering the first step reactants accessible for catalysis without changing the secondary structure of the spliceosomal RNA network
Penghui Bao, Patrizia Fabrizio, Klaus Hartmuth, Reinhard Lührmann

368 NMR localization of divalent metal ions in RNA using Mn^{2+}-induced paramagnetic relaxation enhancement and Cd^{2+}-induced chemical-shift perturbation of phosphorothioate RNAs
Éric Bonneau, Pascale Legault

369 RNA 3D Structure in a Nutshell
Sandro Bottaro, Francesco di Palma, Giovanni Bussi

370 Structural and Thermodynamic Studies of a Remarkably Stable Kissing-Loop Interaction Important for Substrate Recognition by the VS Ribozyme
Patricia Bouchard, Pascale Legault

371 Molecular crowding enhances folding of single ribozyme molecules
May Daher, Wendy Tay, Nils Walter

372 Transient RNA structure features are evolutionarily conserved and can be computationally predicted
Jing Yun A. Zhu, Adi Steif, Jeff R. Proctor, Irmtraud M. Meyer

373 Dehydration and cation replacement dramatically improve crystals of large RNAs
Daniel Klein, Jinwei Zhang, Adrian Ferre-D'Amare

374 Kinetic Dissection and Predictions of an RNA Assembly Process
Brant Gracia, Yi Xue, Namita Bisaria, Hashimi Al-Hashim, Rhiju Das, Dan Herschlag, Rick Russell

375 Probing the dynamics of Ribosome biogenesis in yeast
Ralph Hector, Elena Burlacu, Stuart Aitken, Thierry Le Bihan, Atlanta Cook, Sander Granneman

376 An Adaptable Pentaloop Defines a Robust Neomycin-B RNA Aptamer with Conditional Ligand-Bound Structures
Muslum Ilgu, Donald Fulton, Ragothaman Yennamalli, Monica Lamm, Taner Sen, Marit Nilsen-Hamilton

377 The folding of 5′-UTR human G-quadruplexes possessing a long central loop
Rachel Jodoin, Lubos Bauer, Jean-Michel Garant, Jean-Pierre Perreault

378 Structural analysis of the 5´-UTR of the HIV-1 genome
Christopher Jones, Adrian Ferré-D'Amaré

379 A Novel Interaction Between the 5´ and 3´ ends of the Pea Enation Mosaic Virus (PEMV) Enhances Translation
Wojciech K. Kasprzak, Anne E. Simon, Bruce A. Shapiro

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Timothy Colussi, David Costantino, John Hammond, Grant Ruehle, Jay Nix, Jeffrey Kieft

381 Are waters around RNA more than just a solvent? - An insight from molecular dynamics simulations
Petra Kuhrova, Michal Otyepka, Jiri Sponer, Pavel Banas

382 High-throughput probing of human lncRNA secondary structure by Mod-seq
Yizhu Lin, Gemma May, Jason Talkish, John Woolford, Joel McManus

383 Structure and dynamics of the HIV-1 frameshift element RNA
Justin Low, Pavel Garcia Miranda, Katie Mouzakis, Robert Gorelick, Samuel Butcher, Kevin Weeks

384 Measuring the Evolution of the Small Subunit of the Ribosome
Joshua Martin, N. Adam Smith

385 Biochemical investigation of RNA thermosensors
Casey Cempre, Hilary Cornell, Jane Frandsen, Kelsey Ulanowicz, Rachel Mitton-Fry

386 Alternate base pairing and conformational changes observed in loop A of the hairpin ribozyme
Patrick Ochieng, Michael Feig, Charles Hoogstraten

387 The ydaO riboswitch forms two symmetry-related pockets for targeting c-di-AMP second messenger
Aiming Ren, Dinshaw Patel

388 Interaction between an octameric RNA structure and different divalent and trivalent metal ions as revealed by x-ray crystallography
Michelle F. Schaffer, Joachim Schnabl, Guanya Peng, Vincent Olieric, Roland K.O. Sigel, Bernhard Spingler

389 Structure prediction benchmarking's not dead
Stefanie Schirmer, Paul Dallaire, François Major

390 RNA structure analysis by SHAPE-MaP
Nathan Siegfried, Steven Busan, Greggory Rice, Julie Nelson, Kevin Weeks

391 Calculation of loop probabilities using an RNA partition function
Michael Sloma, David Mathews

392 The role of a non-coding SNP in COPD predisposition
Amanda Solem, Gabriela Phillips, Katrina Kutchko, Matt Halvorsen, Justin Ritz, Dave Mauger, Alain Laederach

393 Structural Studies of the 5'-Untranslated Region of the HIV-1 Genomic RNA
Andrea Szakal, Amanda Altieri, Robert Brinson, Susan Krueger, John Marino

394 *RNA structural elements and protein interactionsthat regulate HIV genome splicing*
Blanton S. Tolbert, Jeffrey D. Levengood, Niyati Jain, Carrie Rollins

395 Validating the fragment-based drug discovery strategy for targeting biological RNAs: Lead fragments specifically bind and remodel the TPP riboswitch
Katherine Warner, Philip Homan, Kevin Weeks, Alison Smith, Chris Abell, Adrian Ferré-D'Amaré

396 Characterization of Synthetic Regulatory RNA Structures and Interactions in *E. coli* with SHAPE-Seq
Kyle Watters, Timothy Abbott, Julius Lucks

397 The role of RNA structure in the alternative splicing regulation of the apoptotic factor Bcl-X
Carika Weldon, Isabelle Behm-Ansmant, Christiane Branlant, Ian Eperon, Cyril Dominguez

398 Automated Design of RNA 3D Structure
Joseph Yesselman, Rhiju Das

399 Abstract Withdrawn

400 Abstract Withdrawn

RNA System Biology
(401 – 405, 708)

401 Unexpected common developmental programs in *D. melanogaster* and *C. elegans* revealed by RNA-Seq of developmental timecourses
Jingyi Jessica Li, Haiyan Huang, Peter J. Bickel, Steven E. Brenner

402 Dissecting the expression landscape of RNA-binding proteins in human cancers
Bobak Kechavarzi, Sarath Chandra Janga

403 Identification of structural requirements for VapBC toxin-antitoxin interactions
Guangze Jin, J. Scott Butler

404 An RNA-based, Generalizable Synthetic Genetic System for Dynamic Regulation
Yen-Hsiang Wang, Christina Smolke

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Ting Ni, Wenjing Yang, Yanqin Yang, Weiqun Peng, Jun Zhu

708 Global intersection of long non-coding RNA (lncRNA) genes with processed and unprocessed pseudogenes in the human genome
Michael Milligan, Leonard Lipovich

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406 Defining the role and the mechanism of RNA localization to the mitotic apparatus
Julie Bergalet, Félix Legendre, Olivia Zhang, Brandon Vaz, Sulin Oré, Xiaofeng Wang, Eric Lécuyer

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Binkai Chi, Ke Wang, Yanhua Du, Bin Gui, Xingya Chang, Lantian Wang, Jing Fan, She Chen, Hong Cheng

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Hsiao-Yun Huang, Anita Hopper

410 *Drosophila* Imp is required for localization of germ plasm mRNAs
Kelsey Hughes, Danielle Snowflack, Elizabeth Gavis

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Benoit Laffont, Aurélie Corduan, Matthieu Rousseau, Anne-Claire Duchez, Eric Boilard, Patrick Provost

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Shuai Hou, Hui Li, Tian Hao, Mei Lu, Nianzhu Zhang, Lei Shi, Marlene Winkelbauer-Hurt, Robin Reed, Haixin Lei

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Ida Rishal, Rotem Ben-Tov Perry, Katalin F. Medzihradszky, Ashley Kalinski, Albina Lin, Alma L. Burlingame, Jeffery L. Twiss, Mike Fainzilber

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Simone Carla Rufener, Marc David Ruepp, Oliver Mühlemann

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Erica Hutchins, Ben Szaro

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Ryo Tanaka, Saeka Nishino, Henry Krause, Masamitsu Yamaguchi, Hideki Yoshida

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Takanari Umegawachi, Hiromu Koshida, Momoko Yamada, Kazuma Usui, Tetsuya Sato, Mikita Suyama, Megumi Ito, Yasuyuki Ohkawa, Masamitsu Yamaguchi, Henry M. Krause, Hideki Yoshida

418 Luzp4 Defines a New mRNA Export Pathway In Cancer Cells
Nicolas Viphakone, Michaela Livingstone, Marcus Cumberbatch, James Catto, Paul Heath, Mark Dickman, Stuart Wilson

RNA Turnover
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419 Hrp1p and Vts1p contribute to targeted degradation of nitrogen specific mRNAs in dynamic environments
Rodoniki Athanasiadou, Nathan Brandt, David Gresham

420 Mechanistic insights of SIDER2 retroposon-mediated mRNA decay in *Leishmania*
Hiva Azizi, Tatiany Patrícia Romão Pompílio de Melo, Karen Santos Charret, Barbara Papadopoulou

421 Splicing-factor SRSF1's dual function in nonsense-mediated mRNA decay: NMD fast-forward and UPF1 dephosphorylation
Isabel Aznarez, Oliver Fregoso, Jaclyn Tetenbaum-Novatt, Holly Rees, Zuo Zhang, Adrian Krainer

422 Identification and characterization of novel factors that act in the NMD pathway in nematodes, flies and mammals
Angela Casadio, Dasa Longman, Raul Vallejos-Baier, Claudio R. Alonso, Javier F. Caceres

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Stephen Coleman, Ashton Herrington, Hend Ibrahim, Alexa Dickson, Jeffrey Wilusz, Carol Wilusz

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Edward Darzynkiewicz, Marta Radziak, Zbigniew M. Darzynkiewicz, Aleksandra Scibor, Maciej Lukaszewicz, Janusz Stepinski, Elzbieta Bojarska

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Clementine Delan-Forino, Stepanka Vanacova, Elena Conti, David Tollervey

426 A genome-wide comparative study identifies novel *cis*-regulatory elements involved in nuclear mRNA decay
Rene Geissler, Elizabeth Fogarty, Andrew Grimson

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Liang Guo, Daniel Beisang, Mai Lee Moua, Irina Vlasova-St. Louis, Paul Bohjanen

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Hana Cho, Sisu Han, Ok Hyun Park, Joori Park, Jeonghan Kim, Sang Ho Ahn, Jesang Ko, Yoon Ki Kim

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Zhiyun Ge, J. Robert Hogg

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Bhaskar Anand Jha, Zhiquan Lu, Chun Wai Yip, Vaibhav Mehta, Hanrong Wu, Vahid H Gazestani, Reza Salavati

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Katla Kristjansdottir, Elizabeth Fogarty, Andrew Grimson

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Vladyslava Liudkovska, Seweryn Mroczek, Aleksandra Czarnocka-Cieciura, Joanna Kufel

433 Investigating an RNA element that inhibits mRNA nuclear export
Eliza S. Lee

434 Functional analysis of IMP3, a RNA-binding protein
Rena Mizutani, Naoto Imamachi, Yoshio Suzuki, Hiroshi Yoshida, Naobumi Tochigi, Tadahiro Ohnishi, Yutaka Suzuki, Nobuyoshi Akimitsu

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Elisha Mugo, Esteban Erben, Christine Clayton

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Pamela Nicholson, Christoph Josi, Hitomi Kurosawa, Akio Yamashita, Oliver Muhlemann

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Mikael Nissbeck, Niklas Henriksson, Jens Berndtsson, Gergana Papova, Per Nilsson, Magnus Lindell, Anders Virtanen

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Anton Paier, Margus Leppik, Aksel Soosaar, Tanel Tenson, Ülo Maiväli

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Jana Wolf, Eugene Valkov, Mark D. Allen, Birthe Meineke, Mark Bycroft, Murray Stewart, Lori A. Passmore

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Megan Peccarelli, Megan Steele, Bessie Kebaara

441 Pumilio proteins bind and regulate mRNAs associated with progression of Parkinson's disease in humans
Joseph Russo, Wendy Olivas

442 Probing interactions of NMD factors in a distance-dependent manner by BioID
Christoph Schweingruber, Oliver Mühlemann

443 The human DIS3 nuclease clears the cell of pervasive transcription products
Teresa Szczepinska, Rafal Tomecki, Katarzyna Kalisiak, Dorota Adamska, Andrzej Dziembowski

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Woan-Yuh Tarn, Tzu-Wei Chuang, Chia-Chen Lu, Yuan-Chao Lou, Chinpan Chen

445 The Mtr4 Ratchet Helix and Arch Domain Act in Concert to Promote RNA Unwinding
Lacy Taylor, Ryan Jackson, Alejandra Klauer King, Megi Rexhepaj, Lindsey Lott, Ambro van Hoof, Sean Johnson

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Ashis Chowdhury, Swathi Kalurupalle, Sundaresan Tharun

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Dmytro Ustianenko, Lukas Bednarik, Biter Bilen, Mihaela Zavolan, Stepanka Vanacova

448 The mechanism of RNA decay by an Rrp6-associated RNA exosome
Elizabeth Wasmuth, Christopher Lima

RNA-protein Interactions
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449 Asp/Glu residues interacting with RNA hydrogen bond acceptors - What sorcery is that?
Pascal Auffinger, Luigi D'Ascenzo

450 Effects of G-Quadruplex Formation on Biophysical Properties of QRRM1-RNA complex of hnRNP H1
Saba Barezi, Theresa Ramelot, Blanton Tolbert

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Deivid Carvalho Rodrigues, Wesley Lai, Joel Ross, Wei Wei, Alina Piekna, Peter Pasceri, James Ellis

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Michael C. Chen, Pierre Murat, Adrian Ferré-D'Amaré, Shankar Balasubramanian

454 Defining the Role of Staufen1 in Developing and Mature Skeletal Muscle *In Vivo*
Tara Crawford, Aymeric Ravel-Chapuis, Guy Bélanger, Bernard Jasmin

455 Climbing the vertebrate branch of U1A/U2B″ protein evolution
Kimberly Delaney, Sandra Williams, Mariah Lawler, Kathleen Hall

456 Interaction of OAS1 with the 5′ and 3′ terminal region RNAs of West Nile virus
Soumya Deo, Trushar Patel, Edis Dzananovic, Evan Booy, Kevin McEleney, Sean McKenna

457 Structural and Dynamic Investigation on ETR-3 RRM3 and its Interaction with AU-rich RNAs
Nana Diarra dit Konte, Frédéric Allain

458 Double stranded RNA sensing by 2′-5′ oligoadenylate synthetases
Jesse Donovan, Gena Whitney, Alexei Korennykh

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Malgorzata Duszczyk, Frédéric Allain

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Edis Dzananovic, Trushar Patel, Grzegorz Chojnowski, Michal Boniecki, Soumya Deo, Kevin McEleney, Janusz Bujnicki, Sean McKenna

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Hayriye Verda Erkizan, Jeffrey Schneider, Kamal Sajwan, Garrett Graham, Brittany Griffin, Sergey Chasovskikh, Sarah Gamble-Youbi, Maksymilian Chruszcz, Radhakrishnan Padmanabhan, John Casey, Aykut Uren, Jeffrey Toretsky

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Erika Bell, Haley Englert, Ivelitza Garcia

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Brittany L. Griffin, Sergey Chasovskikh, Anatoly Dritschilo, John L. Casey

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Gayathri Gurunathan, Yan Coulombe, Jean-Yves Masson, Stephane Richard

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Christian Hauer, Tomaž Curk, Simon Anders, Jernej Ule, Wolfgang Huber, Matthias W. Hentze, Andreas E. Kulozik

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Gitta A. Heinz, Katharina U. Vogel, Stephanie L. Edelmann, Katharina M. Jeltsch, Vigo Heissmeyer

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Olivier Joassard, Guy Belanger, John Lunde, Claire Legay, Bernard Jasmin

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Kass Jobst, Katalin Hudak

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Lars Joenson, Jan Christiansen, Thomas Hansen, Jonas Vikesaa, Yohei Yamamoto, Finn Cilius Nielsen

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Julia Kenyon, Liam Prestwood, Sarah Mercier, Andrew Lever

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Emily G. Kuiper, Graeme L. Conn

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Alexandre Desjardins, Jonathan Bouvette, Pascale Legault

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Samir Amrane, Karine Rebora, Ilyass Zniber, Denis Dupuy, Cameron Mackereth

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Evan Booy, Ewan McRae, Oksana Marushchak, Emmanuel Ariyo, Trushar Patel, Markus Meier, Jorg Stetefeld, Sean McKenna

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Rogerio Lourenco, Fernando Gonzales, Carla Oliveira

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Franziska Pinker, Anthony Gobert, Olivier Fuchsbauer, Pierre Roblin, Claude Sauter, Philipper Giege

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Anton Polyansky, Mario Hlevnjak, Bojan Zagrovic

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Andrea Putnam, Huijue Jia, Fei Liu, Zhaofeng Gao, Quansheng Yang, Eckhard Jankowsky

479 Exon Junction Complex (EJC) protein components are recruited at transcription sites independently of splicing in Drosophila melanogaster
Subhendu Roy Choudhury, Marco Blanchette, Paul Badenhorst, Saverio Brogna

480 Investigating the structural basis for Roquin-mediated post-transcriptional gene regulation
Andreas Schlundt, Gitta Heinz, Arie Gerloof, Ralf Stehle, Michael Sattler, Vigo Heissmeyer

481 The Nucleic Acid Binding Mechanism of the PWI Motif
Hamideh Keshavarz-Mohammadian, Ibtesam Alja'afreh, Ramakrishna Guda, Blair Szymczyna

482 Constitutive patterns of gene expression regulated by RNA-binding proteins
Gian Gaetano Tartaglia

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Sandrine Coquille, Aleksandra Filipovska, Oliver Rackham, Stéphane Thore

484 Remodeling of U2 snRNA/Cus2 Complexes by the DEAD-box ATPase Prp5
Sandy Tretbar, Aaron A. Hoskins

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Xueyin Wang, Jacob Schwartz, Thomas Cech

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Jianming Wang, Saverio Brogna

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Callie Wigington, Paula Vertino, Anita Corbett

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Huey Nan Wu

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Jingwei Xie, Kalle Gehring

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Chang Yu-Wei, Huang Yi-Shuian

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Ruth Zearfoss, Carina Clingman, Sean Ryder

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Parameet Kumar, Sharmistha Bhattacharyya, Kathi Peters, Raymond Frizzell, Aditya Sen, Rachel Cox, William Guggino, Hung Caohuy, Harvey Pollard, Roopa Biswas

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Durga Rao C., Poonam Dillon

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Danae Campos-Melo, Kathryn Volkening, Michael J. Strong

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Ahmed Moursy, Frédéric H.-T. Allain, Antoine Clery

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Anna Malinova, Daniel Mateju, David Stanek, Zuzana Cvackova

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Ruben de Bruin, Martijn Dane, Dae Hyun Lee, Eric van der Veer, Marko Roeten, Iris Schmidt, Janani Sundararajah, Roel Bijkerk, Hetty de Boer, Ton Rabelink, Anton Jan van Zonneveld, Janine van Gils

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Yoandris Del Toro Duany, Gillian Rice, Yanick Crow, Sun Hur

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Ye Ding, Chaochun Liu, William Rennie, C. Steven Carmack, Jun Lu, Shaveta Kanoria

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Cristian Droppelmann, Kathryn Volkening, Michael J. Strong

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Dairong Feng, Ruey Su, Yi Cheng, Vincent Lobo, Yan Meng, Liping Zou, Barbara Triggs-Raine, Shangzhi Huang, Jiuyong Xie

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Jonathan Hall, Martina Roos, Ugo Pradere

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Fengyang Zong, Xing Fu, Feng Wang, Wenjuan Wei, Lijuan Cao, Hongbin Ji, Jingyi Hui

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Sara W. Leung, Seth M. Kelly, ChangHui Pak, Ayan Banerjee, Kenneth H. Moberg, Anita H. Corbett

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Faiz Nasim, Samina Ejaz, Muhammad Ashraf, Gulzar Ahmad

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Chantana Polprasert, Isabell Schultze, Mikkael Sekeres, Carsten Müller-Tidow, Jaroslaw Maciejewski, Richard Padgett

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Natoya Peart, Eric Wagner

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Christine Péladeau, Adel Amirouche, Bernard Jasmin

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Alexandra Porlier, Annie Dubé, Dany Patoine, Richard Debigaré, François Maltais

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Aymeric Ravel-Chapuis, Tara Crawford, Marie-Laure Blais-Crépeau, Guy Bélanger, Chase Richer, Bernard Jasmin

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Sarah A. Safran, Osama A. Youssef, Takahisa Nakamura, Gökhan S. Hotamisligil, Brenda L. Bass

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Asaf Shilo, Vered Ben Hur, Polina Denichenco, Ilan Stein, Eli Pikarsky, Jens Rouch, Walter Kolch, Lars Zender, Rotem Karni

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Hitomi Tsuiji, Asako Furuya, Ikuyo Inoue, Koji Yamanaka

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Eric van der Veer, Ruben de Bruin, Lily Shiue, Hetty de Boer, Janine van Gils, Jacques Duijs, Marko Roeten, Annemarie van Oeveren-Rietdijk, Bernadetta Bandyga, Hilde van Esch, Ton Rabelink, Erik Biessen, Manuel Ares Jr., Anton Jan van Zonneveld

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Pegine Walrad, Richard Burchmore, Keith R. Matthews

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Fernando Martinez, Stephanie Huelga, Katannya Kapeli, Seung Chun, Chelsea Gelboin-Burkhart, Eric Van Nostrand, Balaji Sundararaman, Frank Bennett, Paul Taylor, Frank Rigo, Gene Yeo

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Yong Yu, Wei Xia, Tomohiro Yamazaki, Robin Reed

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520 Uncovering parallel ribosome biogenesis pathways during pre-60S subunit maturation
Lisbeth C Aguilar, Marlene Oeffinger

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Kelsey Anthony, Erin Garside, Andrew MacMillan, Daniel Pomeranz Krummel

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Veronika Bacikova, Josef Pasulka, Karel Kubicek, Richard Stefl

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Mihwa Lee, Archa Fox, Charlie Bond

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Etienne Antoine, Khalil Rihan, Kristin Patrick, Kelly Nissen, Florence Rage, Johann Soret, Nevan Krogan, Christine Guthrie, Remy Bordonne

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Mateusz Dobrychlop, Joanna Kasprzak, Janusz Bujnicki

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Georg Kempf, Klemens Wild, Irmgard Sinning

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Hemant Kini, Xinjun Ji, Ian Silverman, Brian Gregory, Stephen Liebhaber

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Mathew Kramar, Mark-Albert Saroufim, Celia Jeronimo, Francoise Stutz, Francois Robert, Daniel Zenklusen, Marlene Oeffinger

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Tatsuaki Kurosaki**,** Wencheng Li, Mainul Hoque, Maximilian W.-L. Popp, Dmitri Ermolenko, Bin Tian, Lynne E. Maquat

531 A Conserved RNA Motif Required for 30S Ribosome Assembly
Mollie Rappe, Sarah Woodson

532 Structural and functional analysis of the spliceosomal RNP remodeling enzyme, Brr2
Karine Santos, Matthias Theuser, Dmitry Burakovskiy, Pohl Milon, Sina Mozaffari-Jovin, Reinhard Lührmann, Marina Rodnina, Markus Wahl

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Matthew Seetin, Rhiju Das

534 Surveillance of spliceosomal snRNP assembly by sequestration of incomplete particles in Cajal bodies
Ivan Novotny, Anna Malinova, Eva Stejskalova, Dan Mateju, Klara Klimesova, Martin Sveda, Zdenek Knejzlik, David Stanek

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Hendrik Täuber, Marcel Köhn, Stefan Hüttelmaier

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Jesús Valdés, Tomoyoshi Nozaki, Emi Sato, Yoko Chiba, Kumiko Nakada-Tsukui, María Saraí Mendoza-Figueroa, Nicolás Villegas-Sepúlveda, Natsuki Watanabe, Herbert J. Santos, Yumiko Saito-Nakano, Elisa Azuara-Liceaga, José M. Galindo-Rosales

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537 eIF4A1 modulates the slicing activity of AGO1 in plant RNA silencing
Clément Chevalier, Jacinthe Azevedo-Favory, Grégory Schott, Olivier Voinnet

538 Understanding microRNA function in animal germline
Alexandra Dallaire, Syed Irfan Ahmad Bukhari, Martin Simard

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Felipe de Felippes, Olivier Voinnet

540 A Novel Role for Symplekin in the Biogenesis of Endogenous Small Interfering RNAs
Andrew Harrington, Mindy Steiniger

541 MicroRNAs are essential for sex chromosome stability during mammalian spermatogenesis
Stephanie Hilz, Andrew Modzelewski, Paula Cohen, Andrew Grimson

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Kahori Ikeda-Takane, Yuka Hirose, Kiriko Hiraoka, Emiko Noro, Kosuke Fujishima, Masaru Tomita, Akio Kanai

543 Characterization of *NEFM* mRNA targeting microRNAs in Amyotrophic Lateral Sclerosis
Muhammad Ishtiaq, Kathryn Volkening, Michael Strong

544 Functional characterization of the PIWI domain of microRNA-specific Argonaute in Caenorhabditis elegans
Guillaume Jannot, James A. Brackbill, Kotaro Nakanishi, Martin Simard

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Daniel Jasinski, Emil Khisamutdinov, Peixuan Guo

546 RNA detection by ligating two DNA oligos annealed to a complementary RNA splint using Chlorella virus DNA ligase
Jingmin Jin, Sophie Vaud, Alexander Zhelkovsky, Larry McReynolds

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Alexander Klenov, Lydia Burns, Gabriela Krivdova, Kira Neller, Katalin Hudak

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Shiping Liu, Wei Wang, Lanting Zhou, Yaxi Huang, Xiaoyan Wu, Qingyou Xia, Zhonghuai Xiang

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Gakuto Makino, Yoshiki Ikeda, Motomu Matsui, Masaru Tomita, Akio Kanai

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Jakub Nowak, Nila Roy Choudhury, Flavia de Lima Alvez, Juri Rappsilber, Gracjan Michlewski

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Lian Mignacca, Emmanuelle Saint-Germain, Gerardo Ferbeyre

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Subrata Panja, Andrew Santiago-Frangos, Sarah A. Woodson

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Yuta Sakai, Koichi Abe, Saki Nakashima, Koji Sode, Kazunori Ikebukuro

554 Transcriptome and transposable elements dynamics during PIWI-mediated regulation in Drosophila ovarian cell cultures
Yuliya Sytnikova, Reazur Rahman, Gung-wei Chirn, Josef Clark, Christina Post, Nelson Lau

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Juan Pablo Tosar, Julia Sanguinetti, Fabiana Gambaro, Braulio Bonilla, Alfonso Cayota

556 NUFIP/R2TP and ZNHIT3 interact with the SMN complex to promote assembly of U4-specific proteins
Jonathan Bizarro, Maxime Dodré, Alexandra Huttin, Bruno Charpentier, Florence Schlotter, Christiane Branlant, Séverine Massenet, Céline Verheggen, Edouard Bertrand

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Hsaio-Lin V. Wang, Junhye Shin, Brandon Dinwiddie, Julia Chekanova

558 The *C. elegans* Argonaute CSR-1 promotes germline gene expression
Christopher J. Wedeles, Monica Z. Wu, Julie M. Claycomb

559 MicroRNA regulation of neonatal immunity
Erin Wissink, Norah Smith, Roman Spektor, Brian Rudd, Andrew Grimson

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561 RNase L is involved in some microRNAs biogenesis
Guixiang Lv, Hanjiang Fu, Xu Gao, Xiaofei Zheng

562 MicroRNA inhibitors specifically associate with target microRNAs in the context of Argonaute displacing the target-miRNA regulated mRNA transcript
DJ Hogan, T Vincent, SK Fish, E Marcusson, B Bhat, BN Chau, DG Zisoulis

Splicing Mechanisms
(563 – 587)

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Sandya Ajith, Michael Mallory, Kristen Lynch

564 Genome-wide intron mapping by spliceosome footprinting
Weijun Chen, Hennady Shulha, Nick Rhind, Zhiping Weng, Melissa Moore

565 The Interactions of NTC and Cwc2 with the 5′ Splice Site in Formation of the Active Spliceosome
Che-Sheng Chung, Yen-Chi Liu, Chi-Kang Tseng, Soo-Chen Cheng

566 The Ll.LtrB group II intron from the gram-positive bacterium Lactococcus lactis excisses as circles and generates double-stranded head to tail DNA junctions in vivo
Caroline Monat, Cecilia Quiroga, Benoit Cousineau

567 Analysis of the interaction of splicing factor SF1 with U2 snRNP components
Angela Crisci, Flore Raleff, Ivona Bagdiul, Angela Krämer

568 Mechanistic and structural studies of U4/U6 annealing by Prp24
Allison Didychuk, Eric Montemayor, David Brow, Samuel Butcher

569 Splicing Inhibitors - Tools to dissect the Spliceosome
Kerstin Effenberger, Robert James, Walter Bray, Veronica Urabe, Arun Ghosh, Roger Linington, Melissa Jurica

570 The Roles of Intrinsic and Extrinsic Factors on the Interactions between snRNPs and RNAs
Sarah Hansen, Joshua Larson, Aaron Hoskins

571 SRSF3 promotes Exon 9 skipping of pro-apoptotic caspase-2 through exon 8
Ha Na Jang, Minho Lee, Tiing Jen Loh, Seung-Woo Choi, Hyun Kyung Oh, Heegyum Moon, Sunghee Cho, Seong-Eui Hong, Do Han Kim, Zhi Sheng, Michael R. Green, Daeho Park, Xuexiu Zheng, Haihong Shen

572 Genetic screen to identify factors that modulate function of C-rich exon motifs in yeast
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573 A hierarchical clustering approach to single molecule FRET analysis: dissecting pre-mRNA dynamics during spliceosome assembly and catalysis
Matthew Kahlscheuer, Mario Blanco, Joshua Martin, Alain Laederach, Nils Walter

574 Brr2 Retinitis Pigmentosa Mutations Reduce Helicase Processivity
Sarah Ledoux, Christine Guthrie

575 Elucidation of hnRNP A1 binding to ssA7 of HIV-1
Jeffrey Levengood, Jennifer Meagher, Carrie Rollins, Jeanne Stuckey, Blanton Tolbert

576 Choosing between Human or yeast? Co- and post-transcriptionally, *Ustilago maydis* has it all!
Rebeca Martinez-Contreras, Nancy Martinez-Montiel, Xadeni Burgos-Gamez, Julio M. Hernández-Pérez

577 Intron Circles Are The Main Splicing Products IN *Entamoeba histolytica*
Maria Sarai Mendoza Figueroa, Elisa Azuara Liceaga, Jesus Valdes Flores

578 Brr2-facilitated unwinding of U4/U6 is promoted by a mutually exclusive stem loop in U4
Klaus Nielsen, Amartya Das, Deepti Bellur, Jonathan Staley

579 High-throughput sequencing of *SMN1* exon 7 reveals low intrinsic splicing error rate and patterns of splicing errors
Derrick Reynolds, William Mueller, Klemens Hertel

580 Structural studies of the core spliceosomal component Prp19
Tales Rocha de Moura, Reinhard Lührmann, Vladimir Pena

581 Genetic screen for factors modulating branch site selectivity during spliceosome assembly
Andrea Yuste Rivero, Susana Rodríguez Santiago, Varun Gupta, Alberto Moldón, Charles Query

582 The N-terminal domain of the unusual SR protein, hPrp38, is a protein-protein interaction hub in the spliceosome
Tonio Schütze, Luise Apelt, Gert Weber, Alexander Ulrich, Ulrich Stelzl, Markus C. Wahl

583 Stemloop 4 of U1 snRNA is essential for splicing and interacts with the U2 snRNP specific SF3A1 protein
Shalini Sharma, Somsakul Wongpalee, Ajay Vashisht, James Wohlshlegel, Douglas Black

584 Single Molecule Dynamics Analysis of Prespliceosome Assembly and Stability
Inna Shcherbakova, Larry J. Friedman, Victor Serebrov, Jeff Gelles, Melissa J. Moore

585 The 3′-end of U6 snRNA is required for spliceosome disassembly and fidelity
Rebecca Toroney, Jonathan Staley

586 A structural framework for the association of U1 snRNP with alternative splicing factors
Clarisse van der Feltz, Melissa Trieu, Zhi Yang, Nikolaus Grigorieff, Daniel Pomeranz Krummel

587 FUS mediates an interaction between U1 snRNP and RNAP II and functions in coupling transcription to pre-mRNA splicing
Yong Yu, Robin Reed

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588 Structural and functional analysis of the N-terminal helicase-associated region of the spliceosomal Brr2 protein
Eva Absmeier, Christian Becke, Karine Santos, Markus Wahl

589 Identification of Novel Type of Splicing Induced by CLK Inhibitor Using Second and Third Generation Sequencer
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590 Genome scale analyses of pre-mRNA alternative splicing in human cells submitted to heat shock or oxidative stress reveals how splicing participates to adaptation to stresses
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591 Functional analysis of the RNA-binding protein ZRANB2
Katerina Bendak, Paula Vaz, Joel Mackay

592 PTB regulates the alternative splicing of the apoptotic gene *BCL-X*
Pamela Bielli, Matteo Bordi, Claudio Sette

593 The polypirimidine tract-binding protein contributes to drug-resistance in pancreatic cancer cells by regulating the alternative splicing of pyruvate kinase
Sara Calabretta, Ilaria Passacantilli, Pamela Bielli, Emanuela Pilozzi, Volker Fendrich, Gabriele Capurso, Gianfranco Delle Fave, Claudio Sette

594 PSF promotes exon7 inclusion of SMN2 pre-mRNA by contacting exon7
Sunghee Cho, Heegyum Moon, Tiing Jen Loh, Hyun Kyung Oh, Darren Reese Williams, D. Joshua Liao, Jianhua Zhou, Michael R Green, Xuexiu Zheng, Haihong Shen

595 Esrp1 regulates pre-mRNA alternative splicing in pluripotent stem cells and enhances reprogramming
Benjamin Cieply, Russ Carstens

596 A comprehensive screen of RNA binding proteins to identify novel splicing factors
Benjamin Cieply, Russ Carstens

597 Permanent alterations of alternative splicing by transiently depleting RNPS1
Alexandre Cloutier, Jérôme Barbier, Johanne Toutant, Benoit Chabot

598 Identification of Quaking-regulated Alternative Splicing events in Glia cells
Lama Darbelli, Stephane Richard

599 Identifying Regulators of Alternative Splicing in *Schizosaccharomyces pombe*
Benjamin Fair, Ali Awan, Jeff Pleiss

600 Truncation of NTC related factor Prp45 delays co-transcriptional spliceosome assembly
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601 Analysis of co-regulated alternative exons using AVISPA's splicing codes
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602 EJC components regulate alternative splicing and pervasive transcription but not NMD in *Cryptococcus neoformans*
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603 High-Throughput Analysis of Alternative Splicing Regulatory Networks in Embryonic Stem Cells
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604 Inhibition of HIV replication by RNA *trans*-splicing technology
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605 Protein Arginine Methylation Affects pre-mRNA Splicing in *Saccharomyces cerevisiae*
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606 When splicing goes wrong – a mechanism for spermatid differentiation
Duangporn Jamsai, Brett Clark, Eileen McLaughlin, Moira O'Bryan

607 Functional study of the Ser/Arg-rich splicing factor SRSF5a during zebrafish embryonic development
Marine Joris, Marc Muller, Patrick Motte

608 The B52/SRp55 splicing factor modulates growth and cell competition
Céline Fernando, Agnès Audibert, Françoise Simon, Jamal Tazi, François Juge

609 A conserved and cell-type specific program of regulated mRNA splicing supports postnatal liver development
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610 Capturing transcriptome-wide sites of spliceosome assembly and action using RIPiT-Seq
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611 Splicing regulation of RBM4 in neuronal differentiation
Hung-Che Kuo, Hsin-I Yu, Woan-Yuh Tarn

612 Concerted alterations in non-productive alternative splicing of core spliceosome components and splicing factors during smooth muscle cell phenotypic modulation
Miriam Llorian, Adrian Buckroyd, Martina Hallegger, Clare Gooding, Selina Wang, Rajgor Dipen, Melis Kayikci, Nicolas Bellora, Eduardo Eyras, Jernej Ule, Catherine Shanahan, Chris Smith

613 Identification of Phosphorylation Sites in Spliceosome Components Reveals Roles in Removal of Suboptimal Introns in Fission Yeast
Michael C. Marvin, Jesse Lipp, Kevan M. Shokat, Christine Guthrie

614 Abstract Withdrawn

615 Transcriptome analysis of Drosophila sex-specific, neuronal alternative splicing and in vivo analysis of target genes
Carmen Mohr, Marco Blanchette, Britta Hartmann

616 Coupling between alternative polyadenylation and alternative splicing is limited to terminal introns
Maliheh Movassat, Tara Crabb, Anke Busch, Chengguo Yao, Yongsheng Shi, Klemens Hertel

617 Shaping of a Genetic Mutation Phenotype by Tissue Specific Alternative Splicing
Daniel Murphy, Peter Stoilov, Saravanan Kolandaivelu, Visvanathan Ramamurthy

618 Alternative Splicing in Neuronal Differentiation Identified by Novel Transcriptome Array Analysis
Yusuke Nakayama, Shinsuke Araki, Kazunori Yamanaka, Momoko Ohori, Shouichi Nakao, Asano Asami-Odaka, Tokuyuki Shinohara, Masanobu Shouji, Ryujiro Hara, Osamu Nakanishi, Atsushi Nakanishi, Hiroyoshi Toyoshiba, Samuel Aparicio

619 A Role for the Histone Variant H2A.Z and the SWR1 Nucleosome Remodeling Complex in Pre-mRNA Splicing in Fission Yeast
Kelly Nissen, Kristin Patrick, Christine Guthrie

620 Modulation of synaptic transmission, behavior, and alternative splicing in distinct neuron classes by a pair of RNA binding proteins
Adam Norris, John Calarco

621 Genetic interaction mapping reveals a role for the SWI/SNF nucleosome remodeler in early steps of splicing in fission yeast
Kristin Patrick, Colm Ryan, Jesse Lipp, Kelly Nissen, Nevan Krogan, Christine Guthrie

622 DDD00944892 – A new splicing modifier
Andrea Pawellek, Ursula Ryder, Andrew Woodland, David Gray, Stuart McElroy, Timur Samatov, Reinhard Luehrmann, Angus Lamond

623 An Ultraconserved Element Controls Alternative Splicing of Differentially Localizing ARGLU1 mRNA
Stephan Pirnie, Gordon Carmichael

624 Dissecting the in vivo functions of the neural splicing regulator nSR100/SRRM4
Mathieu Quesnel-Vallières, Manuel Irimia, Sabine Cordes, Benjamin Blencowe

625 Actinomycin D modulates splicing decisions, CUG RNA and splicing factor levels across various DM1 in vivo models
Ruth Siboni, Matt Tanner, Leslie Coonrod, Masayuki Nakamori, John A. Berglund

626 Functional Switch of a Human Protein through an Evolutionary G Tract "Invasion" into a 3'SS
Muhammad Sohail, Wenguang Cao, Say-Pham Hong, Manli Zhang, Sam Kung, Jiuyong Xie

627 Identify genes that are alternatively spliced by Sam68 in mTOR signaling pathway
Jingwen Song, Stephane Richard

628 SRPK2 possesses distinct mechanisms for the phosphorylation of different SR proteins
Weng Hong Sou, Jacky Chi Ki Ngo

629 Molecular study of the MEC-8 splicing factor from *C. elegans*
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630 Deep-sequencing of RNA lariats from the fission yeast *Schizosaccharomyces pombe*
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631 Splicing regulation of BRCA1 exon 11
Claudia Tammaro, Michela Raponi, David Wilson, Diana Baralle

632 Unraveling the role of the MAPK pathway in alternative splicing regulation through Cdk12 phosphorylation
Eric Vaillancourt-Jean, Sylvain Meloche

633 Identification of minimal motifs required for circular RNA production
Jeremy Wilusz, Phillip Sharp

634 Exploring the complex post-transcriptional roles of the ESRPs in genome-wide regulatory programs of EMT
Yueqin Yang, Thomas Bebeet, Juw Won Park, Shihao Shen, Yi Xing, Russ Carstens

635 Dissecting protein-protein interactions that regulate the RNA-binding capacity of PSF
Christopher Yarosh, James Lipchock, Kristen Lynch

636 Nuclear Export of Discarded Splicing Intermediates
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637 An mRNA delivery approach for HIV gene therapy
Maggie Bobbin, Anton McCaffrey, John Burnett, John Rossi

638 Antisense Oligonucleotides for the Treatment of Batten Disease
Francine Jodelka, Anthony Hinrich, Maria Ruiz, Mallory Havens, Frank Rigo, Dominik Duelli, Michelle Hastings

639 Antisense oligonucleotide-mediated splicing modulation for the treatment of Alzheimer's disease
Anthony Hinrich, Francine Jodelka, Rida Khan, Daniella Brutmann, Angela Bruno, Jeffrey Huang, Grace Stutzmann, David Bennett, Frank Rigo, Robert Marr, Michelle Hastings

640 A combinitorial microRNA therapeutc approach to suppressing non-small cell lung cancer
Andrea Kasinski, Andreas Bader, Frank Slack

641 mRNA-ITGA4 transfection of mesenchymal stem cells to improve diapedesis for efficient cell delivery to the brain via a minimally invasive intravascular route
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642 In Silico design, in vitro characterization and in vivo delivery of multifunctional RNA-based nanoparticles
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643 Formation of stress granules by anticancer drugs
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644 Control of Stress Granules formation during caliciviruses infection
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645 MicroRNA Regulation of Apolipoprotein B-100 mRNA Stability and Translational Control via 5′ and 3′ Untranslated Regions
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646 Structural studies of the CCR4-NOT complex: CNOT1 recruitment and activation of the DDX6 ATPase
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647 Structural studies of the CCR4-NOT complex: the CNOT1-CNOT9 interaction and its tryptophan-binding pockets
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648 Control of mRNA fate through dynamic regulation of DEAD-box helicases
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649 Identification of inhibitory codon pairs that modulate translation in yeast
Christina Brule, Caitlin Gamble, Kimberly Dean, Stanley Fields, Elizabeth Grayhack

650 DDX3 Promotes Cancer Cell Metastasis Through Activating Rac1 Translation
Hung-Hsi Chen, Hsin-I Yu, Wei-Chih Cho, Woan-Yuh Tarn

651 Ribosome footprinting demonstrates an important role for translational control in the orchestration of skeletal muscle differentiation
Eleonora de Klerk, Ivo F.A.C. Fokkema, Klaske A.M.H. Thiadens, Marieke von Lindern, Johan T. den Dunnen, Peter A.C. ‘t Hoen

652 NMR-structure of PTBP1-RRM2 in complex with EMCV-IRES-domain F
Georg Dorn, Christophe Maris, Frédéric H.-T. Allain

653 Embryonic stem cells control translation initiation to bypass mRNA upstream open-reading frames
Kyle Friend, Hunter Brooks, Nick Propson, James Thomson, Judith Kimble

654 Rapid Kinetics of Iron Responsive Element (IRE) RNA/Iron Regulatory Protein1 and IRE-RNA/eIF4F Complexes Respond Differently to Metal Ions
Dixie Goss, Mateen Khan, Jia Ma, William Walden, Elizabeth Theil, William Merrick

655 Elongation Factor 1A binds the 3′ Translation Element of the Barley Yellow Dwarf Virus
Estella Gustilo, Dixie Goss

656 *Toxoplasma gondii* encodes two eIF4E isoforms that differ in stress response
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657 Analysis of the 5′ untranslated region of human *UPF1* mRNA indicates both cryptic promoter and internal ribosome entry site activity
Rafaela Lacerda, Ana Marques-Ramos, Alexandre Teixeira, Luísa Romão

658 Hypoxia induces autophagy through translational up-regulation of lysosomal proteins in human colon cancer cells
Ming-Chih Lai, Shaw-Jenq Tsai, H. Sunny Sun

659 Determining the Role of Pea Enation Mosaic Virus (PEMV) mRNA Untranslated Region (UTR) in Translation Initiation
Qiao Liu, Dixie Goss

660 Global translational control and regulation of eIF4E activity during norovirus infection
Elizabeth Royall, Nicole Doyle, Majid NH Al-Sailawi, Ian Goodfellow, Lisa O. Roberts, Nicolas Locker

661 Identification of an IRES element in the human mTOR transcript: its structural and functional features
Ana Marques-Ramos, Juliane Menezes, Rafaela Lacerda, Alexandre Teixeira, Luísa Romão

662 Regulation of mRNAs stability and translation upon heat shock in *Trypanosoma brucei*
Igor Minia, Christine Clayton

663 Defining the neuronal function of the polyadenosine RNA-binding protein ZC3H14
Kevin Morris, Jennifer Rha, Sara Leung, Christina Gross, Gary Bassell, Kenneth Moberg, Anita Corbett

664 Translational control of the human erythropoietin *via* an upstream open reading frame in cardiac tissue
Cláudia Onofre, Cristina Barbosa, Luìsa Romão

665 HuD accelerates cap-dependent translation in a distinct way from PABP mediated translation stimulation
Hiroshi Otsuka, Akira Fukao, Toshinobu Fujiwara

666 Translational control of specific mRNAs is important for cellular survival and the anti-inflammatory feedback during macrophage activation
Sonja Reitter, Johanna Schott, Janine Philipp, Heiner Schäfer, Georg Stoecklin

667 Human *Argonaute 1* 5′ untranslated region can mediate cap-independent translation initiation *via* an internal ribosome entry site
Rafaela Lacerda, Ana Marques-Ramos, Alexandre Teixeira, Luísa Romão

668 Sequence Specific Modulation of G-Quadruplex Folding
Samuel Rouleau, Martin Bisaillon, Jean-Pierre Perreault

669 Elimination of translational repressor ELAVL2 accompanies acquisition of developmental competence of mouse oocytes
Katerina Chalupnikova, Petr Solc, Vadym Sulimenko, Radislav Sedlacek, Petr Svoboda

670 An oxygen-regulated switch in the cap-dependent translation machinery is required for the adaptation to hypoxia and the tumor microenvironment
Jim Uniacke, J. Kishan Perera, Gabriel Lachance, Camille Francisco, Stephen Lee

671 Fail-safe Mechanism of GCN4 Translation Control – uORF2 Promotes Reinitiation by Analogous Mechanism to uORF1 and thus Secures its Key Role in GCN4 expression
Stanislava Gunisova, Leos Valasek

672 A blood pact: significance and implications of eIF4E in hematological diseases
Veronica Venturi, Martin Pospisek

673 Hypoxia-Activated HnRNP L Induces Mir-574-3p Dissociation from Polysomes and Promotes Oncogenesis
Peng Yao, Alka Potdar, Paul Fox

674 DDX3 modulates neurite outgrowth via Rac1-mediated signaling pathway DDX3 modulates neurite outgrowth via Rac1-mediated signaling pathway
Hsin-I Yu, Hung-Hsi Chen, Wei-Chih Cho, Woan-Yuh Tarn

675 Triticum Mosaic Virus 5′ leader acts as a bona fide internal ribosome entry site for translation
Jincan Zhang, Robyn Roberts, Karen Browning, Satyanarayana Tatineni, Aurélie Rakotondrafara

676 The RNA affinity landscape of the core eukaryotic translation factor eIF4G
Boris Zinshteyn, Wendy Gilbert

tRNA, snRNA, snoRNA, rRNA
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677 Determining the mechanism of action of the tumor suppressor snoRNA U50
Kristen Bartoli, Wendy Gilbert

678 Analysis of RNA interactome of human RNA methylase ABH8
Marek Bartosovic, Stepanka Vanacova

679 The identity of the discriminator base has an impact on CCA-addition
Sandra Wende, Heike Betat, Mario Mörl

680 Role of wobble uridine tRNA modifications in translational frame maintenance
Hasan Tukenmez, Hao Xu, Anders Byström

681 An improvement of tRNAscan-SE: revised analysis of tRNA genes in the human genome more than doubles the complete set
Patricia Chan, Aaron Cozen, Peter Ryabinin, Todd Lowe

682 Alternative pre-mRNA splicing patterns are refractory to disruptions in ongoing Sm-snRNP supply in *Drosophila* larvae
Eric Garcia, A. Gregory Matera

683 Determinants of tRNA function and rapid tRNA decay in yeast
Michael P. Guy, David L. Young, Matthew J. Payea, XiaoJu Zhang, Yoshiko Kon, Kimberly M. Dean, Elizabeth J. Grayhack, David H. Mathews, Stanley Fields, Eric M. Phizicky

684 Characterization of nematode-specific tRNAs (nev-tRNAs) that can decode an alternative genetic code
Kiyofumi Hamashima, Yoshiki Andachi, Masaru Mori, Masaru Tomita, Yuji Kohara, Akio Kanai

685 Conditional Mouse Models in the Study of the Regulation of RNA Polymerase I Transcription
Chelsea Herdman, Nourdine Hamdane, Victor Stefanovsky, Michel Tremblay, Tom Moss

686 An archaeal RNA binding protein, FAU-1, is a novel ribonuclease involved in the processing of rRNAs
Yoshiki Ikeda, Yasuhiro Okada, Asako Sato, Tamotsu Kanai, Masaru Tomita, Haruyuki Atomi, Akio Kanai

687 Precise mapping of mitochondrial and genomic tRNA-derived fragments (tRFs) in the development of *Triops cancriformis* (Tadpole shrimp)
Yuka Hirose, Kahori Ikeda-Takane, Emiko Noro, Kiriko Hiraoka, Masaru Tomita, Akio Kanai

688 Computational analysis of ribosomal RNA gene organization and their evolutionary divergence in Archaea
Asaki Kobayashi, Kiyofumi Hamashima, Masaru Tomita, Akio Kanai

689 Phenotypes and translational deficiencies of tRNA-i6A37 anticodon loop modification in yeast and human patient cells
Tek Lamichhane, John Yarham, Sandy Mattijssen, Robert Taylor, Rich Maraia

690 Identification of Novel Determinants for the Rapid tRNA Decay Pathway in Yeast
Matthew J. Payea, David L. Young, Michael P. Guy, XiaoJu Zhang, Yoshiko Kon, Kimberly M. Dean, Elizabeth J. Grayhack, David H. Mathews, Stanley Fields, Eric M. Phizicky

691 Empirical fitness landscapes of the U3 snoRNA
Puchta Puchta, Grzegorz Kudla, David Tollervey, Guido Sanguinetti

692 Structure and function effects of *E. coli* $tRNA^{Arg4}_{UCU}$ anticodon domain modifications
Kathryn L. Sarachan, William A. Cantara, Kun Lu, Grazyna Leszczynska, Robert J. Kaiser, Minhal Makshood, Erick Harr, Andrzej Malkiewicz, Paul F. Agris

693 Identification of discrete classes of small nucleolar RNA featuring different ends and RNA binding protein dependency
Gabrielle Deschamps-Francoeur, Daniel Garneau, Fabien Dupuis-Sandoval, Marie Frappier, Audrey Roy, Mélissa Barbe-Marcoux, Sherif Abou-Elela, Michelle Scott

694 Archaeal Elp3 Catalyzes tRNA Wobble Uridine Modification at C5 via a Radical Mechanism
Kiruthika Selvadurai, Pei Wang, Joseph Seimetz, Raven Huang

695 The exosome and its cofactors contribute to multiple steps of 18S rRNA maturation in *Arabidopsis thaliana*
Pawel J. Sikorski, Dominique Gagliardi, Joanna Kufel, Heike Lange

696 Mitochondria Outer Membrane Proteins Are Required For The Proper Function And Localization Of tRNA Splicing Endonucleases
Yao Wan, Jingyan Wu, Anita Hopper

Viral RNAs
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697 Elimination of a persistent endosymbiont virus in Leishmania by RNAi
Erin Brettmann, F. Matthew Kuhlmann, Haroun Zangger, Katherine Owens, Suzanne Hickerson, Nicolas Fasel, Stephen M. Beverley

698 Harmine Inhibits HIV-1 Gene Expression Independent of Its Effects on DYRK1a Function
Tyler Cabral, Chathura Wijewardena, Alex Chen, Alan Cochrane

699 Widespread Alternative Transcription Start Site Usage Expands the Human Cytomegalovirus Proteome
Prakash Koodathingal, Meredith Corley, Kyle Arend, Erik Lenarcic, Jason Upton, Alain Laederach, Nathaniel J. Moorman

700 Interplays between RNA binding proteins determine viral infection outcomes
Chun Kew, Kam-Leung Siu, Man-Lung Yeung, Chi-Ping Chan, Dong-Yan Jin, Kin-Hang Kok

701 Identification of RNA partners of viral proteins in infected cells
Anastassia Komarova, Chantal Combredet, Odile Sismeiro, Marie-Agnès Dillies, Bernd Jagla, Raul Y. Sanchez David, Jean-Yves Coppée, Frédéric Tangy

702 Quantitative assessment of influenza's cap-snatching repertoire by RNA sequencing
David Koppstein, Joseph Ashour, Joanna Stefano, Hidde Ploegh, David Bartel

703 Conserved features of an RNA promoter for RNA Polymerase II determined from high-throughput sequencing of a population of hepatitis *delta* virus
Yasnee Beeharry, Lynda Rocheleau, Martin Pelchat

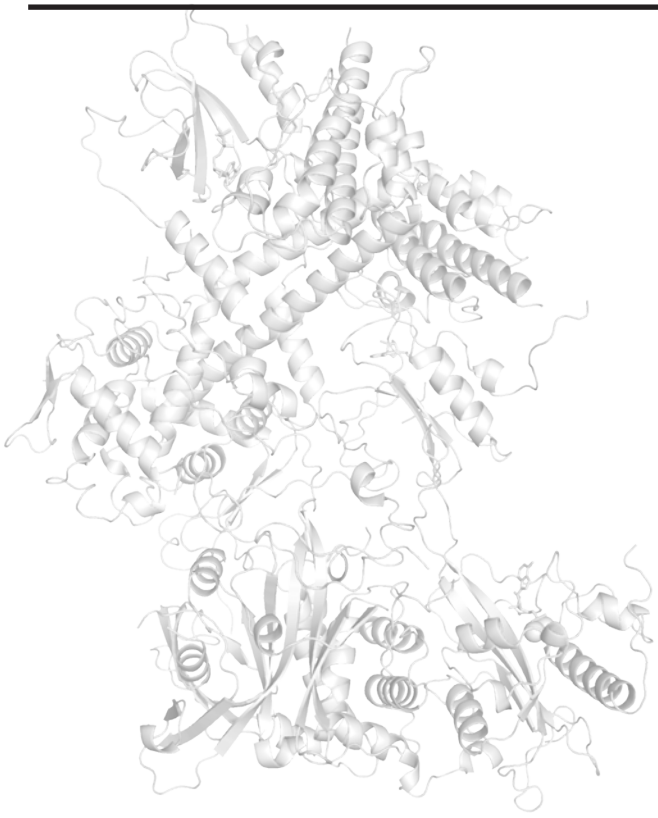
704 Selection of an Antiviral RNA Aptamer against Ebola Virus Glycoprotein
Shambhavi Shubham, Marit Nilsen-Hamilton

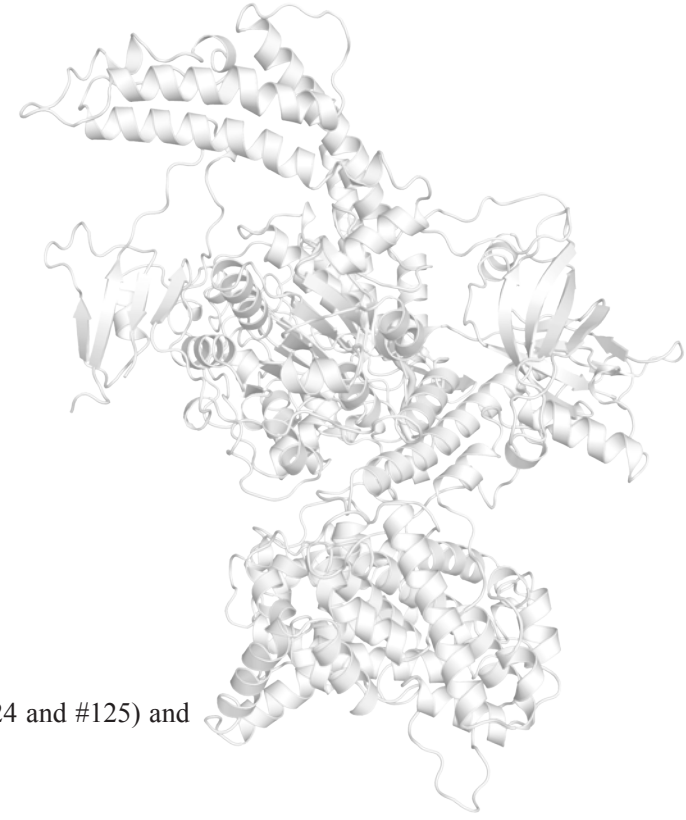
705 Real time analysis of HIV-1 transcription with single polymerase sensitivity
Katjana Tantale, Florian Mueller, Xavier Darzacq, Christophe Zimmer, Eugenia Basyuk, Edouard Bertrand

706 The Lsm1-7-Pat1 complex promotes viral RNA translation and replication by differential mechanisms
Jennifer Jungfleisch, Ashis Chowdhury, Isabel Alves-Rodrigues, Sundaresan Tharun, Juana Díez

707 Investigating Nuclear Envelope Budding of Lytic Viral Transcripts During Kaposi's Sarcoma-Associated Herpesvirus Infection
Tenaya Vallery, Joan Steitz

NOTES





Structures of a yeast Pan2-Pan3 core complex (see abstracts #124 and #125) and the spliceosomal helicase Acquarius (see abstract #11).

1 Recognition of budding yeast telomerase RNA by TERT and its role in template boundary definition

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Telomerase, a ribonucleoprotein (RNP) enzyme, serves the essential role in maintaining chromosome integrity by replenishing eroded telomere. The RNA subunit of telomerase, which is named TLC1 in *Saccharomyces cerevisiae*, not only provides the template during reverse transcription, but also has the scaffold role for the assemblies of Est1p, TERT (called Est2p in *Saccharomyces cerevisiae*), Ku, and Sm proteins with TLC forming the telomerase RNP. Although RNA elements of TLC1 that are responsible for mediating interactions with Est1p and with Ku have been identified and characterized, features of the core region of TLC1 that enable specific recognition of TLC1 by Est2p is much less known. To address this question, we probed Est2p-binding sites in TLC1 with single-nucleotide resolution using phosphorothioate footprinting. We find that the Est2p-binding sites lie in both sides of the template region. To our surprise, the secondary structure of the RNA elements, but not the sequence *per se*, is important for Est2p binding. Mutations introduced to disrupt the stem-loop secondary structure lead to shortened telomeres, whereas compensatory mutations that restore the secondary structure rescue telomeres to the wild-type length. Interestingly, we find that the distance between the two Est2p-binding sites in TLC1 is important for defining template boundary for reverse transcription; shortening of such distance by nucleotide deletion causes read-through of the template. Our study reveals an active role of specific RNA-protein interactions in ribonucleoprotein enzyme function.

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2 A self-regulating template in human telomerase

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Telomerase is a specialized reverse transcriptase (RT) containing an intrinsic telomerase RNA (TR). It synthesizes telomeric DNA repeats, (GGTTAG)n in humans, by reiteratively copying a precisely defined, short template sequence from the integral TR component. The specific mechanism of how the telomerase active site utilizes this short template region accurately and efficiently during processive DNA repeat synthesis has remained elusive. Here we report that the human TR template, in addition to specifying the repeat sequence, is embedded with a single-nucleotide signal to pause DNA synthesis. After the addition of a dT residue to the DNA primer, specified by the signaling residue 49 rA in the template, telomerase extends the DNA strand with three additional nucleotides and then pauses DNA synthesis. This sequence-defined pause site coincides precisely with the structurally defined template boundary, and cooperatively precludes incorporation of non-telomeric nucleotides from residues outside the template region. Additionally, this sequence-defined pausing mechanism prevents premature arrest of nucleotide synthesis and is the predominate mechanism for generating the characteristic 6-nt ladder banding-pattern of telomeric DNA products *in vitro*. In the absence of the pausing signal, telomerase stalls nucleotide addition at multiple sites along the template, generating DNA products with diverse repeat registers at the termini. Our findings demonstrate a unique self-regulating mechanism of the human TR template for high fidelity synthesis of DNA repeats.

3 NMR and X-Ray structure-function analyses of box C/D ribonucleoprotein particles assembly complexes at atomic level

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Eukaryotic Box C/D RNPs play key roles in rRNA processing and UsnRNP biogenesis. Box C/D RNAs contain C/D motifs folded into K-turn structures that recruit 4 core proteins: Snu13p(yeast)/15.5kD(human), Nop58p/NOP58, Nop56p/NOP56, Nop1p/fibrillarin to form C/D RNPs. Core protein assembly is assisted by a set of conserved cellular factors. We and others previously identified protein Rsa1p(yeast)/NUFIP(human) as a platform protein scaffolding C/D RNP assembly, together with the AAA+ helicases Rvb1p/TIP49 and Rvb2p/TIP48 of the R2TP complex, that associate with two other R2TP components, Pih1p/PIH1 and Tah1p/SPAGH, the adaptor of HSP90. The idea is that Rsa1p/NUFIP together with R2TP favor formation of a pre-RNP complex containing the RNA and core proteins and that, activities of the AAA+ helicases and HSP90 are required for remodeling pre-RNPs into mature stable C/D RNPs.

By proteomic, genetic and biochemical assays, we identified a new player in C/D RNP assembly, protein Hit1p/TRIP3. We showed that Hit1p/TRIP3 is required to stabilize Rsa1p/NUFIP. Simultaneous deletions of the *HIT1* and *PIH1*genes or of the *RSA1* and *PIH1* genes are synthetic lethal, which can be explained by the decreased stabilities of C/D RNPs and pre-rRNA processing defects observed upon single deletions of these genes. To further understand the roles of C/D RNP assembly factors, we started an exhaustive structural analysis of protein-protein interactions among yeast C/D RNP assembly factors and core proteins. We showed that the $Rsa1p_{230–381}$ region contains binding sites for two core proteins, Snu13p and Nop58p, and for Hit1p. We determined the crystal structure of the Snu13p-$Rsa1p_{230–261}$ interaction. It defines a complex network of electrostatic and hydrophobic interactions established through a movement of one Snu13p helix. We also determine the NMR 3D structure of the $Rsa1p_{317–352}$-$Hit1p_{70–164}$ interaction. Through a previously uncharacterized mode of protein-protein interaction, Hit1p acts as a jaw enclosing $Rsa1p_{317–352}$. Our NMR structure of the Hsp90-Tah1p interaction explains Tah1p specificity for Hsp90 versus Hsp70. We are presently establishing the NMR structure of the Tah1p-Pih1p interaction. The structural and functional knowledge gained on protein-protein interactions, and our study of multi-protein complexes formed by co-expression in *E. coli,* bring new insights on orchestration of C/D box assembly in eukaryotes

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4 Roles of Eukaryote-Specific rRNA Expansion Segments in Ribosome Biogenesis

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A universal core secondary structure for ribosomal RNA (rRNA) has been identified across kingdoms of life. However eukaryotic rRNA has extra blocks of sequences relative to that of prokaryotic rRNA called “expansion segments” (ES). These expansion segments vary in their length and sequence, both within and among different organisms. This variability seems to preclude expansion segments from playing crucial roles in the function of the ribosome.

There have been isolated attempts to understand the roles of expansion segments. It was apparent that some, but not all expansion segments can tolerate insertions. Also, deletion mutational analysis of two expansion segments has been shown to affect ribosome biogenesis. Despite these reports, a thorough study of the precise roles of these expansion segments remains to be done, possibly because of the limitations in the availability of convenient systems to study rRNA mutants.

We set out to systematically investigate the potential functions played by the 25S rRNA expansion segments in *Saccharomyces cerevisiae* ribosome biogenesis. We used a temperature sensitive PolI mutant yeast strain (Nomura) in which transcription of rDNA is driven from a plasmid-borne rDNA copy with a GAL promoter. We deleted eukaryote-specific ES in yeast 25S rRNA using this rDNA mutagenesis system. The phenotype of the mutants was first assayed by studying their growth. Following this, northern blotting, primer extension assays and affinity purifications were used to zoom in on the precise ribosome assembly phenotype that these mutants exhibit. Preliminary results show that deletion of most of the expansion segments of the large subunit rRNA results in a growth defect and inability of the cells to make mature 25S rRNA. Further, we have shown that deletion of some expansion segments including ES5 and ES7 results in a 27SA3 processing defect in yeast.

This ongoing study is the first of its kind in systematically identifying the precise functions of eukaryote-specific expansion segments in ribosome biogenesis. This study will help us unravel the yet unexplored functions of these eukaryote-specific expansion segments and pave the way for a deeper understanding of the mechanisms of ribosome biogenesis in general.

5 The DEAH-box helicase Ecm16 Dissociates U3 snoRNA from the pre-rRNA to promote rRNA folding

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A central question in ribosome biogenesis is how RNA-RNA and RNA-protein structural rearrangements are coordinated. In eukaryotes, the highly conserved U3 snoRNA base-pairs with the pre rRNA to coordinate early cleavage and folding events within pre-40S particles. U3 interactions with the 5′ ETS region of the pre-rRNA are essential for cleavage at sites A0, A1 and A2, which generate the 20S, an 18S precursor, and separate the large and small subunit precursors. U3 binds multiple sites in the pre-rRNA, bringing distant elements together. In particular, U3 promotes a long-range interaction termed the central pseudoknot (CPK), a universally conserved architectural feature of the small ribosomal subunit. However, binding of U3 is also mutually exclusive with folding of the mature CPK structure, necessitating its removal during pre-40S maturation. This was presumed to require a helicase activity, and we provide genetic and physical evidence that Ecm16 (Dhr1) is the helicase responsible for U3 displacement. Active site mutations in Ecm16 trapped a novel ~50S particle containing U3 snoRNA, the core SSU Processome factors Mpp10 and Imp4 and 20S and 21S pre-rRNA. These RNAs have undergone U3-dependent cleavage at sites A0 and A1 but are only partially cleaved at site A2. Chemical probing was consistent with U3 snoRNA remaining base-paired with pre-rRNA. UV crosslinking identified Ecm16 binding sites in the 5′ region of U3 snoRNA. Positions U29, C39, G47 and A48 were frequently mutated in the sequencing analysis, identifying these nucleotides as direct contacts for Ecm16. These residues are adjacent to U3 box A, which is believed to bind across the CPK, and to U3 5′-hinge, a sequence that base-pairs to the 5′ ETS. Separately, we identified mutations in U3 snoRNA that suppressed the growth defect of an *ecm16* cold-sensitive mutant. The suppressing mutations in U3 mapped to residues 1 through 28, immediately upstream of the Ecm16 binding. Finally, we show that purified Ecm16 is an active RNA helicase that can displace a model U3 snoRNA-pre-rRNA duplex. Together, these data strongly suggest that Ecm16 promotes the release of U3 snoRNA to promote the formation of the CPK and separation of pre-40S from the early pre-ribosome.

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6 Enzymatic Regulation of a DEAD-box RNA Helicase Promotes Efficient mRNP Assembly During Transcription

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Proper RNA structure and ribonucleoprotein (RNP) complex formation are critical for gene expression. A class of RNA helicases called DEAD-box proteins play fundamental roles in RNA and RNP structure in every aspect of RNA metabolism. DEAD-box proteins contain a highly conserved helicase core and recognize the phosphate backbone of RNA substrates in a sequence-independent manner. Studies have shown that many DEAD-box proteins utilize protein co-factors to provide substrate specificity and regulate intrinsic ATPase and/or duplex unwinding activity. Our laboratory recently demonstrated that the *S. cerevisiae* DEAD-box protein Dbp2 is an active, double-stranded RNA-directed helicase *in vitro*. Furthermore, we have found that Dbp2 is associated with actively transcribing genes and is required for efficient assembly of mRNA-binding proteins Yra1, Nab2 and Mex67 with mRNA. We now provide evidence that the recruitment of Dbp2 to actively transcribing genes is RNA-dependent. Moreover, we show that Dbp2 interacts directly with Yra1 and that this interaction is necessary for removal of Dbp2 from mRNA *in vivo*. Finally we show that Yra1 regulates the helicase activity of Dbp2 by promoting ATP hydrolysis and release of Dbp2 from single-stranded RNA. We present a model whereby Dbp2 is recruited to nascent RNA during transcription, unwinds secondary structure in the RNA to facilitate RNA-binding protein assembly, and is then released from the single-stranded RNA product by Yra1. Thus, a sequential order of events involving regulation of a DEAD-box RNA helicase is required for efficient mRNP assembly in the nucleus.

7 Mixed Doubles: The structural basis for dsRNA recognition by NF90/ILF3

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Nuclear factor 90 (NF90, also known as ILF3 and DRBP76), is a protein involved in gene expression at transcriptional and post-transcriptional levels. In complex with its partner, NF45, NF90 has been proposed to interact with the 3′UTR regions of several mRNAs and to affect their stability and/or translation. However, a coherent molecular model of how NF90 functions in the cell is currently lacking. Recently, Rigo et al (2012) showed that NF90 binds preferentially to dsRNA duplexes containing a 2′fluoro (2′F) modification on the ribose moiety. This interaction was exploited to direct therapeutic changes in splicing by targeting NF90 to intronic sequences. Using 2′F modified oligonucleotides, we have co-crystallised the tandem dsRNA binding domains of NF90 with dsRNA and show that each domain has a distinct binding preference. We further integrate our structural data with crosslinking and cDNA analysis (CRAC), derived from UV-crosslinking NF90 in 293 cells. By combining *in vitro* and *in vivo* data we present a model for mRNA recognition by NF90.

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8 Comprehensive Analysis of RNA-Protein Interactions by High Throughput Sequencing-RNA Affinity Profiling

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RNA-protein interactions have critical roles in gene regulation. However, a high-throughput method for their quantitative analysis is lacking. Existing technologies for characterization of the RNA interaction profile of a protein are either limited in scale, allowing only a few pairs of RNA-protein interactions to be evaluated, or non-quantitative, yielding only relative binding affinities at best. We adapted an Illumina GAIIx sequencer in a novel technique that overcomes both of these limitations to make several million quantitative measurements of affinity at once, while also yielding the sequence of the RNA. We have named this assay High-Throughput Sequencing - RNA Affinity Profiling (HiTS-RAP). Millions of cDNAs are sequenced, made double stranded, bound by a sequence specific DNA binding protein, and transcribed in situ. The DNA binding protein halts transcription by T7 RNA polymerase, leaving RNA stably attached to its template DNA through the polymerase. The binding of fluorescently-labeled protein to this RNA is then quantified in the sequencer. We have used HiTS-RAP to measure the affinity of mutagenized libraries of GFP and NELF-E binding aptamers to their respective targets and thereby identified regions in both aptamers that are critical for their RNA-protein interaction. We show that mutations additively affect the binding affinity of a NELF-E binding aptamer, whose interaction occurs mainly through a small single-stranded RNA motif, but not that of the GFP aptamer, whose interaction depends primarily upon the secondary structure of the RNA. Our results demonstrate that HiTS-RAP is robust method, capable of massively parallel quantitative characterization of RNA-protein interactions.

9 Mechanism of U4/U6 di-snRNA recognition by a novel RNA-binding domain in spliceosomal protein Prp3 and its role in U4/U6•U5 tri-snRNP assembly

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Pre-mRNA splicing is catalyzed by a multi-subunit RNA-protein enzyme, the spliceosome. For each round of splicing, a spliceosome facilitates two successive transesterification reactions (steps 1 and 2) that lead to removal of an intron and ligation of its flanking exons. Spliceosomes are formed via the stepwise recruitment of small nuclear ribonucleoprotein particles (snRNPs) and numerous non-snRNP proteins to a pre-mRNA substrate (1). Spliceosomal protein Prp3 is a component of the U4/U6 di-snRNP and of the U4/U6•U5 tri-snRNP(2,3). The protein is required before the first step of splicing (4) and for U4/U6•U5 tri-snRNP stability (2) but the molecular mechanisms underlying its activities are presently unknown. Here we show by crystal structure analyses that Prp3 contains an unconventional RNA-binding domain and clarify how this domain together with neighboring regions of the protein specifically recognizes the stem II duplex and the U6 3′-overhang in U4/U6 di-snRNAs. The unconventional RNA-binding domain is conserved in all Prp3 orthologs but is not found in other known RNA-binding proteins. We also show that mutations in Prp3, which were designed based on the structural results to interfere with U4/U6 di-snRNA binding, lead to growth and snRNP assembly defects in yeast. Our results reveal an example of a novel RNA-binding domain that evolved to serve spliceosome-specific tasks, acting as a crucial element that allows Prp3 to bridge U4/U6 and U5 in the tri-snRNP.

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10 An in vitro peptide complementation assay for CYT-18-dependent group I intron splicing reveals a new role for the N-terminus

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The mitochondrial tyrosyl tRNA synthetase from *Neurospora crassa* (CYT-18 protein) is a bifunctional group I intron splicing co-factor. CYT-18 is capable of splicing multiple group I introns from a wide variety of sources by stabilizing the catalytically active intron structures. CYT-18, and mt TyrRSs from related fungal species, have evolved to assist in group I intron splicing in part by the accumulation of three N-terminal domain insertions. Biochemical and structural analysis indicate that the N-terminal insertions serve primarily to create a structure-stabilizing scaffold for critical tertiary interactions between the two major RNA domains of group I introns. Previous studies concluded that the primarily α-helical N-terminal insertion, H0, contributes to protein stability and is necessary for splicing the *N. crassa* ND1 intron, but is dispensable for splicing the *N. crassa* mitochondrial LSU intron. We have shown that CYT-18 with a complete H0 deletion retains residual ND1 intron splicing activity and that addition of the missing N-terminus *in trans* is capable of restoring a significant portion of its splicing activity. The development of this peptide complementation assay has allowed us to explore important characteristics of the CYT-18/group I intron interaction including the stoichiometry of H0 in intron splicing and the importance of specific H0 residues. Evaluation of truncated H0 peptides in this assay and a re-examination of the CYT-18 crystal structure suggest a previously unknown structural role of the first 5 N-terminal residues of CYT-18. These residues interact directly with another splicing insertion, making H0 a central structural element responsible for connecting all three N-terminal splicing insertions.

11 Structural investigation of the spliceosomal helicase Aquarius

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Beyond participating in splicing *per se*, some spliceosomal components from higher eukaryotes play pivotal roles in coupling splicing to other RNA-processing events. The massive spliceosomal helicase Aquarius (172 kDa) is loaded at a specific location on the intron, where it acts as a key molecular linker between splicing, the deposition of the exon-junction complex on the mRNA and the formation of snoRNPs from intronic segments.

We succeeded to determine two crystal structures of the full-length human Aquarius in complex with a non-hydrolysable ATP analog and in complex with a single-stranded RNA, providing snapshots of different steps of the catalytic pathway. The structures revealed the presence of several specific accessory domains and evidence a novel mode of RNA binding previously not observed in other helicases. Strikingly, we found that Aquarius exhibits opposite unwinding polarity in comparison to the structurally similar helicase Upf1, a key player in the non-sense mediated RNA decay. The Aquarius-RNA structure unraveled architectural adaptations that support the reversal of polarity and brought insightful implications into the structural and functional divergence of the two helicases from a common ancestor.

Additionally, we identified an accessory domain from the armadillo-repeat (ARM) protein family. This domain functions as a scaffold for a heteropentameric complex that acts as a vehicle for the recruitment of Aquarius to the spliceosome and guides its positioning by sensing the branch-site-associated microenvironment. Finally, for the first time, we gained insight into a role of this enzyme in pre-mRNA splicing.

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12 A New Molecular Signature for Activation of Human 2′-5′ Oligoadenylate synthetase-1 (hOAS1)

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The human 2′-5′ oligoadenylate synthetase-1 (hOAS1) is an important part of innate cellular immunity. Upon binding viral double-stranded (ds)RNA, hOAS1 synthesizes 2′-5′ linked oligoadenylate second messengers that activate RNAse L, thereby halting replication. Adenoviruses produce an RNA Polymerase III (Pol III) non-coding (nc)RNA transcript, VA RNA_I, that accumulates to high levels in later stages of infection, and is critical for efficient replication. VA RNA_I is well recognized for its inhibition of dsRNA-activated protein kinase (PKR)-mediated shut-down of general translation. Counterintuitively, however, VA RNA_I *activates* rather than inhibits OAS1. While activation consensus sequences have been reported for OAS1, and a recent crystal structure revealed many of the RNA-protein contacts that drive OAS1 activity, we found that the 3′-poly U tail of VA RNA_I, which is present as a consequence of Pol III termination, is critical for optimal activity against hOAS1. Additionally, this effect is general, and occurs in other ncRNA Pol III transcripts such as the Epstein-Barr virus EBER and the cellular nc886. The activity of an 18 base pair (bp) model duplex RNA used in the hOAS1-dsRNA structural studies is also augmented by addition of a 3′-poly U tail. This potentiation of hOAS1 activation is dependent upon the 3′ region being single-stranded and has a strong preference for pyrimidines over purines. Using the 18 bp model system, we further show that a single U is sufficient for maximal enhancement. Based upon these findings, we present here a new molecular signature for activation of hOAS1, the **3′ s**ingle-**s**tranded **py**rimidine (3′-ssPy) motif. Work is currently underway to determine the structural and mechanistic bases for the activity of 3′-ssPy. These studies and the implications of this novel molecular signature will be discussed.

13 RNA regulation by the *Caenorhabditis elegans* oocyte maturation determinant, OMA-1

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Maternally supplied mRNAs encode for necessary developmental regulators that pattern early embryos in many species until zygotic transcription is activated. In *Caenorhabditis elegans*, post-transcriptional regulatory mechanisms guide early development during embryogenesis. Maternal transcripts remain in a translationally silenced state until fertilization and a suite of RNA-binding proteins regulate these maternally supplied mRNAs during oogenesis, the oocyte to embryo transition, and early embryogenesis. Identifying the target specificity of these RNA-binding proteins will reveal their contribution to patterning of the embryo. We are studying post-transcriptional regulation of maternal mRNAs during oocyte maturation. Maturation is an essential part of meiosis that prepares oocytes for fertilization. Although the physiological events taking place during oocyte maturation have been well studied, the molecular mechanisms that regulate oocyte maturation are not well understood.

OMA-1 and OMA-2 are essential proteins that function redundantly during oocyte maturation. Both OMA1/2 have CCCH-type tandem zinc finger (TZF) RNA-binding domains suggesting that they may be post-transcriptional regulators of oocyte maturation. We defined the RNA-binding specificity of OMA-1, and demonstrated that OMA-1/2 are required to repress the expression of a *glp-1* 3′-UTR reporter in developing oocytes. To determine the RNA-binding specificity of OMA-1 we performed *in vitro* selection. The selected sequences demonstrate that OMA-1 binds UAA and UAU repeats in a cooperative fashion. Interestingly, OMA-1 binds with high affinity to a conserved region of the *glp-1* 3′-UTR. Multiple RNA-binding proteins regulate translation of GLP-1 protein, a homolog of Notch receptor. OMA-1 interacts with the conserved region of the *glp-1* 3′-UTR that has been previously shown to interact with POS-1 and GLD-1, RNA-binding proteins required for *glp-1* reporter repression in the posterior of fertilized embryos. Additionally, OMA-1 and OMA-2 repress *glp-1* reporter expression in *C. elegans* oocytes. In summary, we show that OMA-1 is a sequence specific RNA binding protein that regulates expression of the *glp-1* mRNA in oocytes. Mapping the OMA-1 dependent regulatory sites in the *glp-1* mRNA and characterizing the interplay between OMA-1 and other factors will help reveal how multiple regulatory signals coordinate the transition from oocyte to embryo.

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14 A network of prion-like domains in RNA-binding proteins underpins paraspeckles: subnuclear RNP granules

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Paraspeckles are stress-induced subnuclear bodies that form around a long noncoding RNA (lncRNA) NEAT1. Paraspeckles affect gene expression, at least in part, by the sub-nuclear sequestration of specific transcription factors. But what are the molecular mechanisms driving proteins into paraspeckles?

NEAT1 ‘seeds’ paraspeckle formation by acting as a scaffold for specific proteins to bind. These proteins then take part in multiple protein-protein interactions to cooperatively build up a micron-scale RNP particle. To reveal the network of protein-protein interactions in paraspeckles we carried out a combinatorial yeast-two-hybrid screen of the forty known paraspeckle proteins. Surprisingly, only a few important hub proteins seem to link the paraspeckle interactome together. Accordingly, the three main hub proteins, HNRNPK, FUS and RBM14, are also essential for paraspeckle formation: knocking them down results in loss of paraspeckles. Interestingly, the hub proteins are also enriched in low complexity prion-like domains.

We have focused our downstream analysis on one key hub protein, RBM14 (RNA Binding Motif Protein 14; also known as CoAA/PSP2/SIP). Crucially, RBM14 is the only protein linking the essential DBHS proteins (NONO and SFPQ) to the rest of the network. Super-resolution microscopy shows a distinct spatial arrangement for RBM14, NONO and NEAT1 in different, yet partially co-localising, zones of paraspeckles, implying a hierarchical structural organization by each component. We found that the RBM14 prion-like domain is responsible for mediating the interaction with DBHS proteins and is also required for targeting RBM14 to paraspeckles. As with another hub protein, FUS, the prion-like domain of RBM14 forms a hydrogel when expressed in vitro. Mutations in critical tyrosines in the FUS and RBM14 prion-like domains disrupt both hydrogel formation and paraspeckle targeting by these proteins. Together these data indicate that specific prion-like domains in RNA binding proteins are intimately involved in the formation of paraspeckles.

15 Defining the protein-protein interactions of the polyadenylate-binding protein nuclear 1 (PABPN1), the protein mutated in Oculopharyngeal Muscular Dystrophy

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Mutation of the polyalanine binding protein nuclear 1 (*PABPN1*) gene causes Oculopharyngeal Muscular Dystrophy (OPMD), a late onset, primarily autosomal dominant disease presenting with weakness of the eyelids, pharynx and proximal limbs. The causative mutation is an expansion of a short (GCN) trinucleotide repeat in the coding region of *PABPN1* (GCG_6 to $GCG_{8\text{-}13}$), which results in a modest expansion of an alanine stretch at the N-terminus of PABPN1 (10 to 12-17 alanines). PABPN1 is a multifunctional RNA-binding protein vital for efficient polyadenylation as well as processing of coding and non-coding RNA. The basis for the muscle-specificity of OPMD despite the ubiquitous expression of the PABPN1 is not known. Identifying PABPN1 interacting partners that show differential interactions with wildtype and alanine-expanded PABPN1 in muscle will provide insight into both PABPN1 function and the mechanisms underlying muscle pathology in OPMD. Thus, we are conducting a comprehensive analysis of the protein interactions of PABPN1 in muscle using a global proteomics approach.

To define the spectrum of PABPN1-interacting proteins, we immunoprecipitated PABPN1 from muscles of mice that transgenically express wildtype or alanine-expanded PABPN1 specifically in skeletal muscles. Mass spectrometry was employed to identify co-immunoprecipitated proteins. One of the proteins identified as a novel binding partner of wildtype PABPN1 but not alanine-expanded PABPN1 is Matrin3. Matrin3 is a nuclear RNA-binding protein involved in stabilizing both coding and non-coding RNA. Mutations in Matrin3 are linked to an adult-onset myopathy that presents with weakness of limb and pharyngeal muscles. We confirmed the interaction of PABPN1 with Matrin3 in cultured cells and determined that this interaction is RNA-dependent. RNA immunoprecipitation assays confirm that PABPN1 is bound to specific transcripts *in vivo* that are previously identified targets of Matrin3. In addition to the shared target transcripts, PABPN1 and Matrin3 also share common protein binding partners, PABPC1 and hnRNPK. Together these data suggest that PABPN1 and Matrin3 could work together in muscle cells to determine the fate of transcripts critical for maintaining muscle homeostasis. Characterizing protein interactions of PABPN1 will further our understanding of its function in muscles and help us better understand the pathogenesis of OPMD.

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16 Alternative Splicing of MKK7 forms a Novel Feed-Forward Loop to Promote T cell Activity

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Alternative splicing is an important mechanism of gene regulation that expands the genome's coding capacity. In particular, the ability to regulate alternative splicing in response to foreign antigens is critical for immune responses. We have shown that during T cell activation alternative splicing is widespread and enriched for signaling pathways important for T cell activity. One such signaling pathway that is regulated at the level of splicing is the JNK pathway. Specifically, we find that in response to T cell activation the JNK kinase MKK7 is alternatively spliced to favor an isoform that lacks exon 2. Repression of exon 2 is predicted to promote MKK7's interaction with JNK and promote JNK signaling.

We have now undertaken studies to define both the mechanism and functional consequence of MKK7 alternative splicing. Through minigene based analysis we have identified sequences within both the upstream and downstream introns of MKK7 exon 2 that are necessary and sufficient to promote activation-induced skipping. Either intron in isolation is not sufficient for this activity, suggesting that these act cooperatively to repress exon 2. Analysis of RNA-protein interactions has identified several RNA binding proteins, including CELF1/2, hnRNPC and HuR that bind to these regions in a signal-dependent manner. The binding of at least HuR and CELF2 is required for splicing regulation as knockdown of these factors alters splicing outcome. Interestingly, we further demonstrate that inhibition of JNK blocks the activation-induced skipping of MKK7 exon 2, while JNK activity is sufficient for its repression in unstimulated cells. Moreover, to test the functional consequences of MKK7 splicing we used an antisense morpholino oligo to force skipping of exon 2. Strikingly, forced exclusion of this exon results in a marked increase in expression of JNK-target gene TNF-alpha, confirming that exon repression enhances the ability of MKK7 to interact with and promote JNK activity.

Together, these data demonstrate positive reciprocal regulation between MKK7 splicing and JNK signaling. We conclude that exclusion of MKK7 exon 2 is utilized by T cells to sustain JNK signaling and effector functions after antigen withdrawl, and may additionally impact JNK-dependent pathways in other cells types.

17 Regulation of cell-type specific and activation-dependent Traf3 alternative splicing

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Upon activation, T cells undergo robust alterations in gene expression to allow profound functional changes. Recent RNA-Seq analyses in resting and activated T cells have suggested that regulated transcription and alternative splicing are independent, equally important mechanisms to control protein expression and potentially function upon T cell activation. However, two key questions remain unanswered for the vast majority of splicing changes: how is activation-dependent splicing achieved and what is the contribution of alternative splicing to changed functionality upon activation?

Here we have performed a detailed analysis of activation-induced Traf3 exon 8 exclusion. Regarding functionality, we show that expression of Traf3 lacking exon 8 allows activation of the non-canonical (nc) NFkB pathway in activated T cells, which is in stark contrast to the negative regulatory function of full length Traf3. Activation of the ncNFkB pathway induces expression of several chemokines, suggesting an important role of Traf3 alternative splicing in mediating T cell function.

To define the mechanism of Traf3 exon 8 skipping, we have performed an siRNA screen and identified Celf2 as the required trans-acting factor. In addition, we have used minigenes and mapped an intronic cis-regulatory element necessary and sufficient for splicing regulation. Using X-link analyses we show that this RNA-element is bound by Celf2 preferentially in stimulated T cells. Mutations that interfere with Celf2 binding also interfere with splicing regulation pointing to a critical role of Celf2 in regulating activation-induced Traf3 alternative splicing. The Traf3 splicing switch is also cell-type specific, as it is not observed in other cell lines, human B cell lines amongst others. In agreement with earlier studies we find Celf2 expression increased in activated T cells; however, B cell activation does not increase Celf2 expression providing a possible explanation for cell-type specificity.

Interestingly, mouse T cell lines do not show activation-induced Traf3 alternative splicing which is consistent with data from primary mouse T cells. Having defined a mechanism for activation-dependent and cell-type specific Traf3 alternative splicing, we are now using this system to start investigating the basis for species specific alternative splicing regulation.

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18 Mechanistic insights into alternative splicing regulation by the neural-specific SR-related protein nSR100/SRRM4

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Alternative splicing (AS) is a widespread process that greatly increases the functional and regulatory capacity of metazoan genomes. Previously, we identified and characterized the vertebrate- and neural-specific Ser/Arg-repeat-related protein of 100 kDa (nSR100/SRRM4), a splicing regulator required for nervous system development that promotes the inclusion of a large network of brain-enriched alternative exons (Calarco et al., Cell, 2009) (see abstract by Quesnel-Vallieres et al.). In addition to activating neural gene expression by regulating a switch exon that silences the activity of REST, a transcriptional repressor of neurogenesis genes (Raj et al., Mol Cell, 2011), nSR100 modulates a multitude of AS events that shape neural-specific protein interaction networks (Ellis et al., Mol Cell, 2012). However, the molecular mechanisms by which nSR100 promotes neural-specific AS is poorly understood.

We use RNA-Seq profiling to identify a greatly expanded network of nSR100-regulated AS events in human and mouse cells. Interestingly, this expanded network includes additional conserved AS events in the Mef2 family of transcriptional activators, which play important roles in neuronal survival and plasticity. Exons in the nSR100 network are associated with suboptimal 3´ splice sites. Through co-immunoprecipitation and mass spectrometry, we identify U2af65/U2af2 and Ccar1, a previously identified U2af65-interacting protein (Hegele et al., Mol Cell, 2012) with an unknown role in AS, as prominent nSR100-interactors. Knockdown experiments confirm U2af65 and Ccar1 as co-factors of nSR100-regulated AS. Consistent with nSR100 functioning via interactions involving U2af65 and Ccar1, using CLIP-Seq, we observe that nSR100 directly binds intronic UGC motifs adjacent to polypyrimidine tracts upstream of the 3´ splice sites of regulated target exons. Mutation of the UGC motifs abolishes nSR100-dependent AS. Through RNA-Seq and CLIP-Seq experiments, we further show that PTBP1 directly binds and suppresses the inclusion of ~30% of nSR100-dependent exons, whose inclusion levels correlate with the expression of both proteins during in vivo neural development. In splicing reporter assays performed in vitro, nSR100 directly counteracts PTBP1-mediated inhibition of AS.

Collectively, our results provide evidence that nSR100 interacts with U2af65/Ccar1 near suboptimal, UGC-containing 3′ splice sites and outcompetes negative regulation mediated by PTBP1 to promote inclusion of brain-enriched exons during neurogenesis.

19 Functional characterization of PTBP1 regulated alternative splicing events during neuronal differentiation

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Alternative pre-messenger RNA splicing guides the proper expression of proteins during development. The RNA binding proteins PTBP1 and PTBP2 regulate many alternative splicing events during neuronal differentiation and maturation. However, the cellular functions of PTBP1 and PTBP2 regulated isoforms remain largely unknown. We modeled *in vitro* neuronal development using embryonic stem cells (ESCs). As ESCs differentiate into neuronal progenitors (NPC) and then into motor neurons, PTBP1 expression declines and disappears, while PTBP2 expression peaks with differentiation and neurite outgrowth. These results recapitulate observations in neurons from embryonic brain. We hypothesized that when PTBP1 is depleted, new alternatively spliced isoforms will be induced that promote the neuronal phenotype. Using RNA-sequencing, we profiled alternative splicing events in populations of human and mouse ESCs, NPCs, and mature neurons. To identify PTBP1 and PTBP2 regulated exons, we have knocked down these proteins in ESCs and NPCs. In parallel, we are identifying all the PTBP1 and PTBP2 binding sites across the expressed RNA during the differentiation process using the crosslinking-immunoprecipitation (iCLIP-seq) method. Comparing our database of the observed splicing changes with sites of PTBP1 and PTBP2 binding, we have identified hundreds of splicing events directly regulated by PTBP1 and PTBP2. We are interested in alternative exons in the MED23 and PBX1 transcripts. MED23 is a component of the transcriptional Mediator complex and PBX1 is a homeobox transcription factor. Both genes contain PTBP1 regulated exons that are induced during neuronal differentiation. These exons, which are conserved across mammalian species, presumably alter transcriptional programs during neuronal development. We are currently working to assess the cellular function of the neuronal MED23 and PBX1 isoforms, and how gene expression might be changed by these neuronal isoforms. Through these studies, we hope to understand how the PTBP1 alternative splicing program contributes to neuronal development.

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20 The splicing factor Rbfox2 coordinated splicing is required for myoblast fusion

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Collective alternative splicing of several transcripts ensues in distinct temporal clusters during myoblast differentiation in culture. However, the factors that coordinate these splicing transitions during myogenic differentiation are largely unknown. It is also not known whether the resultant muscle-specific protein isoforms cooperate to control specific aspect of the myogenic program. The Rbfox family of RNA binding proteins are highly conserved and have been shown to regulate muscle-specific splicing in C.elegans and zebrafish. Of the three mammalian Rbfox paralogs, Rbfox1 and Rbfox2 are expressed during myogenic differentiation. However, their role in mammalian myogenesis has not been investigated.

Using RNAi-mediated knockdown of Rbfox2 and Rbfox1 during myoblast differentiation combined with transcriptome profiling by RNA sequencing (RNA-Seq), we demonstrated that Rbfox2 but not Rbfox1 affects 30% (182 of 609) of the splicing transitions during myogenesis. Phenotypic and biochemical characterization of myoblast differentiation in absence of Rbfox2 identified a defect in myoblast fusion without affecting expression of myogenic differentiation markers. We used integration of Rbfox2-dependent splicing outcomes from RNA-seq with Rbfox2 iCLIP data, gene ontology analysis, and a candidate gene approach to identify Mef2d and Rock2 as Rbfox2 splicing targets relevant to the myoblast fusion defect. Interestingly, the Rbfox2 mediated splicing transition leads to a gain of function of Mef2d and a loss of function for Rock2, illustrating an elegant utilization of coordinated splicing regulation during myogenesis. Restored activities of Mef2d and Rock2 rescued myoblast fusion in cultures lacking Rbfox2 demonstrating functional cooperation of protein isoforms generated by coordinated alterative splicing. The results demonstrate that coordination of alternative splicing by a single RNA binding protein modulates transcriptional (Mef2d) and cell signaling (Rock2) programs to drive tissue-specific functions (cell fusion) during a developmental transition.

21 Independent regulation of transcription and alternative pre-mRNA splicing by a histone methyltransferase

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Splicing of precursor mRNA (pre-mRNA) is an important regulatory step in gene expression. Alternative splicing (AS) allows the production of multiple protein isoforms from one pre-mRNA molecule, thereby contributing to proteomic diversity. Recent evidence points to a regulatory role of epigenetic marks and higher order chromatin structure in AS regulation. In order to identify novel chromatin regulators of AS, we performed an RNAi screen using a cell-based in-vivo assay for high-throughput screening. We identified 10 chromatin proteins that regulate AS of a TAU reporter-gene. 8 of the 10 identified proteins have also been implicated in transcription. For mechanistic characterization, we focused on EHMT2, a H3K9 methyltransferase with a known role in transcription silencing. Genome-wide analysis of transcription and AS regulation revealed very limited overlap in affected genes indicating distinct roles for EHMT2 in these two processes. One of the most prominent AS targets of EHMT2 was VEGF. Silencing of EHMT2, as well as its heterodimer partner EHMT1, reduced $VEGF_{189}$ isoform formation but did not affect VEGF total mRNA. The epigenetic regulatory mechanisms of AS involves an adaptor system consisting of the chromatin modulator HP1γ known to bind H3K9Me1/2 and SRSF1, a splicing regulator of VEGF, which we found to bind HP1γ. Functionality of this system in AS regulation is demonstrated by complementary silencing and tethering experiments and biochemical characterization of H3K9me1/2, HP1γ and SRSF1. The epigenetic regulation of VEGF is physiologically relevant since hypoxia induces EHMT2 and changes splicing of VEGF. These results characterize a novel epigenetic regulatory mechanism of AS and they demonstrate separate roles of epigenetic modifiers in transcription and alternative splicing.

21A RBFox2 Provides Essential Cardiac Function by Regulating Alternative Splicing, Modulating MicroRNA Function, and Transcriptional Repression

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RBFox2 is a well-studied RNA binding protein predominantly expressed in stem cells, brain, and heart. At the biochemical level, it has been shown to regulate alternative splicing via binding to the GUAUG motif in a position-dependent manner, activating exon inclusion when binding on a downstream intronic element whereas inducing exon skipping when acting on a upstream intornic element. RBFox2 has been shown to be essential for viability of ES cells, play a critical in brain development and function, and participate in regulated splicing in various cancer cells. To understand the biological function of RBFox2 in the heart, we ablated the RBFox2 gene in cardiac muscle, demonstrating that it is critical for muscle contraction and excitation-contraction (EC) coupling.

Through characterizing the functional defects coupled with biochemical and genomics analysis, we showed that RBFox2 is widely participated in tissue-specific alternative splicing events, including its prevalent role in regulating alternative splicing of the MEF family of transcription factors vital for cardiac functions. Interestingly, we also found that the RNA binding protein showed extensive binding at 3′UTR, consistent with the prediction for RBFox2 in the regulation of microRNA function. We have substantiated this hypothesis that showing that RBFox2 modulates the targeting efficiency of multiple cardiomyocyte-specific microRNAs and disruption of RBFox2 caused disorganized T-tubules, a structure critical for EC coupling.

Strikingly, our functional genomics study showed that RBFox2 is also widely involved in regulated gene expression at the transcription level. RBFox2 ablation induced transcriptional de-repression of many genes, including a set of microRNAs critical for cardiac functions. This investigation eventually led to the discovery that RBFox2 plays a central role in the recruitment of the Polycomb complex 2 (PRC2) to nascent RNA to mediate transcriptional repression. These findings have thus unveiled the enigmatic association of PRC2 with active genes, highlighted the importance of sequence information in the gene body to gauge transcriptional output, and demonstrated nascent RNA as a critical signal for transcriptional feedback control to maintain cell type-specific gene expression programs.

22 Drosha Promotes Splicing of a Pre-microRNA-like Alternative Exon

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The ribonuclease III enzyme Drosha has a central role in the biogenesis of microRNA (miRNA) by binding and cleaving hairpin structures in primary RNA transcripts into a precursor miRNA (pre-miRNA). Many miRNA genes are located within protein-coding host genes. These intragenic miRNAs are cleaved by Drosha in a manner that is coincident with splicing of the introns by the spliceosome. The close proximity of splicing and pre-miRNA biogenesis suggests a potential for co-regulation of miRNA and host gene expression. Here, we describe a cleavage-independent role for Drosha in the splicing of an exon that has a predicted hairpin structure resembling a Drosha substrate. We find that Drosha can cleave the alternatively spliced exon 5 of the *eIF4H* gene into a pre-miRNA both in vitro and in cells. However, the primary effect of Drosha on *eIF4H* gene expression is to promote the splicing of alternative exon 5. Drosha binding and splicing stimulation depends on RNA structure but not on cleavage by Drosha. We conclude that Drosha can function like a splicing enhancer and promote exon inclusion. Our results reveal a new mechanism of alternative splicing regulation based on a previously unappreciated role for Drosha in splicing.

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23 SRSF2 and ZRSR2: Spliceosomal RNA-binding Proteins and Myelodysplastic Syndrome Factors

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SRSF2 (originally named SC35*) and ZRSR2 (originally named Urp^) are RNA-binding proteins involved in pre-mRNA splicing. Both interact with U2AF35/U2AF65. Mutations in these 4 (and a few others) spliceosomal proteins are frequently found -mutually exclusively- in myelodysplastic syndrome (MDS) patients**. However, the role of spliceosomal proteins in MDS pathophysiology is not clear.

SRSF2 has an N-terminal RRM and a C-terminal RS domain. The lone MDS mutation is at proline 95 located near the tail of the RRM. We developed stable cell lines in TF-1 erythroleukemia cells that express either wildtype SRSF2 or SRSF2 with mutations of P95H, P95L, or P95R. The mutant cells exhibited increased alternative splicing of CDC25C to a short transcript missing exons 2-5 found in patients with MDS. The mutant cells also exhibited higher proliferation and apoptosis rates than the parental or wildtype-expressing cells.

ZRSR2 has two zinc-finger motifs flanking a U2AF homology motif (UHM), and a C-terminal RS domain. MDS mutations of missense, nonsense, or frameshift are found throughout the protein; thus, it is postulated that ZRSR2 mutations are loss of function (ZRSR2 is on the X chromosome and many MDS patients are male), while SRSF2 mutations are likely gain of function. We made sequence-specific TALE-nucleases to mutagenize ZRSR2 (at exon 2 or exon 7) in TF-1 erythroleukemia cells and in HT1080 fibrosarcoma cells, both are male. In HT1080, sequencing analysis of relevant genomic DNA and messenger RNA fragments showed that the exon 2 of ZRSR2 was disrupted in two isolated clones. RNA-Seq results indicated U12-type introns were significantly retained in the ZRSR2-disrupted clones. Expression of ZRSR2 by transfection reduced U12-type intron retention, indicating that the intron retention phenotype in ZRSR2 mutant lines is likely due to the loss of ZRSR2 function in HT1080. This is consistent with a previous report that ZRSR2 is involved in the first step of U12-type splicing^^.

The relevance of splicing alteration or defect associated with SRSF2 or ZRSR2 mutations to cell pathophysiology will be discussed.

*Fu and Maniatis (1992) Science; ^Tronchere et al. (1997) Nature; **Yoshida et al. (2011) Nature; ^^Shen et al. (2010) G&D

24 Exitron Splicing, a New Type of Alternative Splicing Event

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Alternative splicing (AS) is a key regulatory mechanism involved in increasing transcriptome and proteome complexity as well as controlling important biological processes such as development and response to the environment. In this work, we identify exitron splicing as a novel type of alternative splicing event present in plants and humans. Exitrons (**ex**onic **i**n**trons**) are internal regions of coding exons that possess all the canonical core splicing signals (acceptor and donor splice sites and branch point sequences) and are clearly distinguishable from both retained and constitutive introns. Their particular exonic nature and the fact of being surrounded by exonic sequences suggest that specific factors are involved in the regulation of exitron splicing. Intriguingly, intronless genes can be also alternatively spliced *via* exitron usage, providing the first evidence of splicing in these genes. We show that exitron splicing is regulated in a tissue-specific manner, in response to stress and by splicing factors. Exitron-encoded sequences contain protein domains interacting with different molecules and are enriched in disordered regions and post-translational modifications; consequently their splicing impacts proteome dynamics and remodelling. We propose a "splicing memory" mechanism for the origin of exitrons whereby exitrons are derived from ancestral coding exons through a history of intron loss and maintenance of vestigial splicing regulatory elements that drives exitron evolution.

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25 Evidence for regulatory splicing in nature's smallest spliceosome

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Transcription and pre-mRNA splicing involve a large number of proteins that are conserved across eukaryotes. The proteins involved in these key steps of RNA-processing play significant roles in regulating gene expression, and can be influenced by additional factors. Pre-mRNA splicing is mediated by the spliceosome, which is made up of five small nuclear RNAs (snRNAs) and over 100 proteins in yeast. Although well characterized in some systems, much can be learned about the functional flexibility and evolution of these large complexes by examining their components and targets in reduced systems.

Cyanidioschzyon merolae is a unicellular red alga that lives at high temperatures and low pH. This model photosynthetic eukaryote has simple cellular architecture, comprised of a single nucleus, mitochondrion, and plastid, and lacks a cell wall. At 16.5Mb and encoding just five thousand genes, the *C. merolae* genome is compact and reduced. It retains just 27 annotated introns in 26 genes, requiring a functioning spliceosome to produce viable mRNA for these genes. Upon investigation, we find the spliceosome of *C. merolae* to be highly reduced. Fewer than fifteen annotated spliceosomal protein homologs are present. Surprisingly, U1 snRNA has not been found, even after thorough biochemical and bioinformatic searches. How this extensive reduction in protein components and putative lack of U1 snRNA affect splicing is unknown.

We have performed high-throughput transcriptome sequencing to examine the effects of reduction on pre-mRNA splicing and RNA-processing in general. We find instances of overlapping sense-antisense transcript pairs that could be playing a role in regulating expression in a manner previously not predicted to be found in *C. merolae*. Also, splice junctions have very low levels of splicing, all with more than 40% of transcripts retaining an intron. Approximately half of the junctions show significant differences in splicing levels between day and night, suggesting regulation of splicing. These findings indicate regulation of splicing in a system with the smallest predicted spliceosome; a function we propose could be preventing intron loss under reductive pressure.

26 The Spinach RNA aptamer contains a G-quadruplex and activates fluorescence in a GFP-like fluorophore

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The demand for RNA imaging techniques has sparked the recent development of fluorescent RNA modules mimicking the Green Fluorescent Protein (GFP). Spinach is an in vitro selected RNA aptamer that binds a GFP-like ligand and activates its green fluorescence. It is thus an RNA analog of GFP, and has potentially widespread applications for in vivo labeling and imaging, such as genetically encoded RNA sensors for metabolite imaging as well as tools for synthetic biology applications. We used antibody-assisted crystallography to determine the structures of Spinach both with and without bound fluorophore at 2.2 and 2.4 Å resolution, respectively. Spinach RNA has an elongated structure containing two helical domains separated by an internal bulge that folds into a G-quadruplex motif of unusual topology. The G-quadruplex motif and adjacent nucleotides comprise a partially pre-formed binding site for the fluorophore. The fluorophore binds in a planar conformation and makes extensive aromatic stacking and hydrogen bond interactions with the RNA, similar to the manner in which GFP surrounds its chromophore. Biochemical and biophysical analysis support the functional relevance of the global structure and the significance of the G-quadruplex motif for fluorophore binding and activation. Overall, our findings provide a foundation for structure-based engineering of new fluorophore-binding RNA aptamers, and serve as a guide to design constructs for in vivo studies that fuse Spinach with RNAs of interest.

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27 RNA Mango: a tool for real-time visualization of RNA in living cells

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The discovery of green fluorescent protein has revolutionized the study of proteins by allowing their sensitive and real-time detection both *in vitro* and *in vivo*. Since no natural and genetically-encodable nucleotide sequence is known to generate strong fluorescence by itself, it has proven to be a considerable challenge to effectively track RNA molecules in living cells and in real time. To address this problem, we have selected a small 39-nt G-quadruplex type RNA aptamer, called RNA Mango, that tightly binds to two thiazole orange derivative series with nanomolar affinity (RNA Mango:TO1 series K_D = 3.4 nM, RNA Mango:TO3-Biotin K_D = 8 nM). Upon binding these dyes become highly fluorescent (TO1 series: excitation 260, 510 nm and emission 535 nm, 1,100 fold fluorescent enhancement. TO3-Biotin: excitation 260, 637 nm and emission 658 nm, 400 fold fluorescent enhancement).

Phylogenetic information from a reselection together with circular dichroism analysis and enzymatic protection data suggests that the dye binds to the aptamer on one face of a parallel stranded G-quadruplex, which has highly conserved 3-nt propeller arms. The aptamer-dye complex is very stable and can be visualized by single molecule fluorescence microscopy and native gel shift experiments. Injection of TO1-Biotin into *C. elegans* gonads yields negligible fluorescence, while injection of RNA Mango:TO1-Biotin yields a bright fluorescent signal that is stable at least 2.5 hours post injection, suggesting that RNA Mango will be useful for tracking RNA *in vivo*. We hope that our aptamer-dye system provides a viable solution to the problem of RNA tracking in living cells and offers distinct advantages over existing methodologies that suffer either from high intrinsic backgrounds, or that have much weaker dye-aptamer binding affinities that to date have made cellular RNA imaging challenging.

28 RNA Structure-Function Relationships in a Dengue Virus Genome

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As the most rapidly spreading mosquito-borne virus worldwide, the dengue virus (DENV) is an increasing threat to global public health. Despite this, there is currently no available vaccine or drug treatment against DENV infection. Design of a DENV vaccine that targets viral proteins has proven particularly difficult because the vaccine must protect against infection by each of the four DENV serotypes. Alternatively, conserved DENV RNA targets could prove useful in therapeutic development. However, we have only a basic understanding of the role of RNA in the viral replication cycle. Current knowledge of the structure and function of DENV RNA genome is largely limited to the 5′ and 3′ untranslated regions. Thus, the role of RNA structure in many steps of DENV replication has remained unclear. To discover functional roles of DENV RNA structure during replication we used SHAPE chemical probing technology, read out by massively-parallel sequencing, to experimentally characterize the secondary structure of an authentic Dengue-2 (DENV2) viral RNA genome at nucleotide resolution in the virus (*in virio*) and in the absence of viral proteins (*ex virio*). SHAPE reactivities were used as pseudo-free energy constraints in a thermodynamics-based folding algorithm to develop a secondary structure. The resulting SHAPE-derived structure includes previously proposed elements located in the 5′ and 3′ UTR, and is the first time many of these structures have been experimentally confirmed. SHAPE reactivities and Shannon entropy, a measure of the heterogeneity in structure prediction, were used to identify a number of key structural elements to test for biological functions. The identified elements include five new pseudoknots located throughout the genome. So far, one of these pseudoknots, located in the capsid-coding region, has been verified to be important in regulating genome circularization. By comparing the *in virio* and *ex virio* SHAPE reactivities, we identified regions that are less flexible *in virio*, likely due to capsid protein binding or to localized RNA refolding, and reveal a candidate capsid binding consensus sequence. This work illustrates how a structure-first approach can reveal novel regulatory elements in large RNAs.

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29 Molecular basis for discrimination in RNA 3′-termini binding

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RNA 2′,3′-cyclic phosphate termini play a significant role in RNA metabolism as intermediates in the chemical or enzyme-catalyzed hydrolysis of the RNA phosphodiester backbone, as substrates for the tRNA ligase RtcB, and as recognition elements present on U6 snRNA. RNA 2′,3′-cyclic phosphate termini can be synthesized from 3′-phosphate termini in an ATP-dependent reaction catalyzed by RNA 3′-phosphate cyclase (RtcA), an enzyme conserved in bacteria, archaea, and eukarya. RtcA and the tRNA ligase RtcB are the only known enzymes that activate RNA 3′-phosphate termini, with each enzyme proceeding through two similar reaction steps. The mechanism of nucleotidylation of histidine residues in RtcA and RtcB has been elucidated; however, the location of the RNA binding sites and the mechanism of RNA 3′-phosphate nucleotidylation remains unknown for each enzyme. Moreover, how RtcA and RtcB avoid binding to the abundant RNA 3′-OH termini *in cellulo* has remained an important unanswered question. Here we present a crystal structure of RtcA in complex with a 3′-phosphate terminated RNA and adenosine in the AMP-binding pocket. Our studies reveal that RtcA discriminates against 3′-hydroxyl termini by ensuring that a terminal 3′-phosphate makes a large contribution to RNA binding. Furthermore, our work elucidates the mechanism of AMP transfer to an RNA 3′-p terminus and reveals significant conformational changes upon RNA binding.

30 RNA crosslinking using Pt(II) complexes to probe the tertiary structure of the HDV Ribozyme

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The HDV Ribozyme is a catalytic RNA found in numerous organisms that self-cleaves to give a 5′-hydroxyl and a 2′,3′-cyclic phosphate. It is known to coordinate a magnesium ion important for catalysis in its active site. Current models suggest that a hydrated magnesium ion occupies a metal ion binding pocket at the active site of the HDV ribozyme, held in place through inner sphere coordination to a non-bridging oxygen at the scissile phosphate and outer sphere coordination to the N7 position of G25 in the active site of the ribozyme (Chen et al. 2010). Inner sphere coordination to the scissile phosphate is derived from an incomplete crystal structure however, so further biochemical evidence is needed to validate this model (Chen et al. 2010, Golden 2011). *Cis*-dichlorodiammine platinum(II) (cisplatin) is a widely used anticancer compound, known to coordinate at the N7 position on purine residues in both DNA and RNA (Kozelka 1999). It also has a strong kinetic preference for thiols, a fact we take advantage of to preferentially recruit cisplatin to phosphorothioate-substituted positions within a structured RNA (Chapman and DeRose 2011). We use phosphorothioate-directed Pt(II) crosslinking to preferentially recruit cisplatin-derived $Pt(NH_3)_2$ to the active site within an inhibited and folded HDV ribozyme enzyme-substrate complex. Crosslinked RNA complexes are isolated and mapped using primer extension and hydrolysis, and compared to a current crystal structure. We show that cisplatin crosslinking in the HDV ribozyme reaches efficiency as high as ~40%, and demonstrate that cisplatin and a functionalized cisplatin derivative, 2-azido 1,3 diaminopropane Pt(II) (2-ADAP Pt), result in similar crosslinks. 2-ADAP Pt is an azide-functionalized platinum(II) compound capable of participating in high-yielding click reactions with alkyne-functionalized molecules. We are working toward using this functionality as an affinity tag for easy isolation of platinated and crosslinked RNAs, with the goal of developing a reagent for crosslinking and subsequent affinity purification for high-throughput structure analysis. This study demonstrates the usefulness of kinetically inert Pt(II) complexes as both general and site-specific crosslinking reagents, and shows promise for future tertiary structure determination using Pt(II)- derived crosslinking tools.

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31 *In vitro* evolution of self-cleaving ribozymes in the presence of iron - implications for RNA function on the early earth

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In vitro evolution of RNA offers insight into the potential roles of RNA in the very early evolution of life. However, ribozymes generated by this method are usually evolved in the presence of millimolar concentrations of magnesium and at neutral pH, which likely is not representative of many aspects of the environment of the early Archean eon during which life emerged. Fe^{2+}, which is not included in standard *in vitro* evolution experiments, was abundant on the early earth and likely used by RNA for folding and catalysis. It has also been suggested that magnesium took the place of iron in supporting RNA function with the rise of oxygen and subsequent drop of soluble iron. Here we explore the impact of Fe^{2+} on the fitness landscape (functional capacity of all nucleotide sequences within a given selective environment) of RNA by evolving self-cleaving ribozymes in an anoxic atmosphere and varying pH and metal ion composition. We show that Fe^{2+} substitutes for Mg^{2+} in self-cleaving reactions in a pH-dependent manner. The activity of ribozyme populations evolved in the presence of Mg^{2+} at pH 5 is low in the presence of Fe^{2+}. Analogously, the activity of iron-dependent ribozyme populations evolved at pH 5 is low in the presence of Mg^{2+}. Surprisingly, however, ribozyme populations evolved at pH 7 are active in the presence of both Fe^{2+} and Mg^{2+}, regardless of whether they evolved in the presence of Fe^{2+} or Mg^{2+}. These findings show that pH modulates the ability of one ion to substitute for another in supporting RNA function. The shape of RNA fitness landscapes can therefore be identified comprehensively only in the context of a variety of metal ions, ligands, and specific chemical environments. Our results suggest the possibility of new capabilities for the RNA catalyst under non-standard conditions with implications for the role of RNA in the earliest life.

32 Networks of post-transcriptional control in trypanosomes

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African trypanosomes rely on post-transcriptional mechanisms to control their gene expression. Transcription is polycistronic, without any apparent control of initiation. Individual mRNAs are excised by *trans* splicing and polyadenylation. We have measured the rates of *trans* splicing and mRNA decay in two life cycle stages of trypanosomes, the bloodstream and the procyclic form, by transcription inhibition and RNASeq. Many trypanosome mRNAs are spliced within a few minutes of synthesis. Some mRNAs show simple exponential decay, but mRNAs with short half-lives tend to show initial fast degradation, followed by a slower phase. These mRNAs are often degraded by a deadenylation-independent pathway that depends on the 5′-3′ exoribonuclease XRNA. Another set of longer-lived mRNAs shows initial slow degradation followed by rapid destruction: the slow phase most likely reflects gradual deadenylation. Developmentally regulated mRNAs usually show regulated decay rates, and may also switch their decay pattern.

Rates of mRNA decay are quite good predictors of steady state levels for short trypanosome mRNAs. In contrast, mRNAs longer than 3 kb are generally less abundant than shorter ones and their decay rates predict their steady state levels poorly. Mathematical modelling results indicate that co-transciptional degradation plays a major role in determining mRNA levels, and can explain the loss of long mRNAs. Moreover, differences in processing kinetics can only affect the mRNA level if processsing is competing with degradation.

To find proteins that are responsible for regulating mRNA translation and decay, we conducted a high-throughput screen that relied on artificial attachment of proteins to mRNAs within the parasite. We identified over 200 proteins that were capable of either increasing or decreasing expression. In addition to known regulators and components of the degradation machinery, we found translation factors, proteins with RNA-binding domains, and numerous proteins that had not previously been linked to mRNA metabolism, including proteins of unknown function and metabolic enzymes. A quarter of these novel regulators were found to have RNA-binding ability using a targeted proteome array.

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33 The spliced leader RNA of *Trypanosoma brucei* determines the life and death and social motility of the parasites

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In trypanosomes, the causative agent of sleeping sickness, all mRNAs undergo *trans*-splicing. In *trans*-splicing an exon, the spliced leader (SL) is derived from a small RNA, the SL RNA. These parasites lack transcription regulation of protein coding genes and lack a conventional unfolded protein response. Under ER stress induced by silencing of factors involved in ER translocation or chemical-induced ER stress, the parasite induces the spliced leader silencing mechanism SLS, whereby a signal is transmitted from the ER to the nucleus to shut-off SL RNA transcription leading to complete inhibition of *trans*-splicing and eventually to programmed cell death (PCD) (Lustig et al., EMBO rep 2007; Goldshmidt *et al.,* PLoS pathogens, 2010). We will present data demonstrating the function of key regulator in SLS pathway, a serine-threonine kinase (PK3), which under ER stress translocates from the ER to the nucleus and phosphorylates a serine residue on the TATA-binding protein (TBP), leading to dissociation of the entire SL RNA transcription complex for the promoter and its spreading in the entire nucleus. PK3 silencing compromised the PCD induced by SLS. In contrast to SLS, perturbation in SL RNA biogenesis by silencing Sm core proteins, leads to transport of SL RNA to the cytoplasm in a form of an RNP complex which is eventually secreted via exosomes from the parasite. We propose that RNA excretion has implications on the mechanism of quorum sensing of the parasite within its insect host.

34 Cross-linking Mass Spectrometry and Random Mutagenesis Approaches Reveal Detailed Insights into the Functional Architecture of *Trypanosoma brucei* Editosomes

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RNA editing in the mitochondrion of kinetoplastid pathogens is an essential process that entails uridine insertion and deletion to create functional mRNAs. Editosomes are multiprotein complexes that provide the enzymatic activities of RNA editing, but our current understanding of the structural organization of proteins within editosomes is extremely limited. *Trypanosoma brucei* has three distinct editosomes that are typified by mutually exclusive RNase III endonucleases (KREN1, KREN2, or KREN3) with distinct cleavage specificities. How these editosomes discriminate amongst thousands of editing sites *in vivo* may be key to understanding differential regulation of RNA editing between life cycles. In an effort to better understand the functional architecture of editosomes, we have used chemical cross-linking/mass-spectrometry (XL/MS) and random mutagenesis approaches to obtain both structural and functional data with amino acid resolution.

XL/MS was used to identify the spatial organization of proteins within KREN1 editosomes (~1.6 MDa). In this approach, proximal amino acids are cross-linked and these peptides are subsequently identified by mass spectrometry. Using this technique we obtained 97 high confidence inter-peptide crosslinks between 33 editosome protein pairs. Cross-links obtained using this method have provided the first evidence of binary interactions between the editing endonucleases and other editosome proteins, and have allowed us to create a detailed structural map of editosome proteins.

In parallel, we have also developed a random mutagenesis screen to study the sequence-function relationship of essential editosome proteins, initially focusing on KREPB5. This approach screened 77% of amino acids in a 384 amino acid protein, identifying 20 essential residues. These essential residues occurred within the predicted RNase III and PUF protein domains of KREPB5, as well as within regions that have no detectable homology to known motifs. Interestingly, several residues required for bloodform survival are not essential in procyclic cells, revealing the first functional differences of an editosome protein between *T. brucei* life cycle stages. Application of this approach is novel in trypanosomes, and widely applicable to other areas of research in these parasites.

Together, our data reveal key insights into the functional architecture of editosomes, and describe novel approaches that are immediately transferable to other experimental questions.

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35 Biology of small RNAs in trypanosome mitochondria

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The U-insertion/deletion mRNA editing in mitochondria of trypanosomes is directed by guide RNAs (gRNAs). Mature 50-60 nt gRNAs are generated from ~800 nt precursors by 3′ end nucleolytic processing and subsequent TbRET1-catalyzed uridylation. We previously demonstrated that TbRET1 repression leads to a loss of 3′ oligo(U) tails and accumulation of gRNA precursors. Conversely, mature gRNAs are stabilized by association with the gRNA binding complex, GRBC. In this work, we defined essential factors that are responsible for the entire gRNA processing pathway and gRNA delivery into the RNA editing cascade. We find that gRNA precursors are targeted by a complex composed of TbRET1, 3′-5′ exonuclease TbDSS1 and five proteins without discernible motifs, which we termed TbRDS. In addition to TUTase and exonuclease activities, the TbRDS complex displays an RNA unwinding activity. The TbRDS complex also targets messenger and ribosomal RNA precursors and therefore fulfills the role of a mitochondrial processome. We find that gRNAs, as defined by complementarity to edited mRNAs, represent only a subset of small mitochondrial RNAs that otherwise are processed in the same pathway. However, gRNAs are significantly enriched in a tripartite ribonucleoprotein assembly, termed the RNA editing substrate binding complex (RESC), of which GRBC is a part. The RESC particle is also responsible for mRNA recruitment and interactions with the core editing and polyadenylation complexes. Although both gRNAs and mRNAs are associated with RESC, their post-editing metabolic fates are distinct: gRNAs are degraded whereas edited mRNAs undergo 3′ adenylation / uridylation. Our results indicate that core editing and RESC complexes typify enzymatic and substrate binding constituents of the RNA editing holoenzyme enzyme. Although parallels have been drawn between mRNA cleavage during editing and RNAi-induced mRNA degradation, our results support the model in which both mRNA and guide RNAs are held together by an RNA binding platform (RESC) while the enzymatic core complex binds transiently and targets individual editing sites.

36 New and old players in the translation apparatus of Leishmania

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Exposure of eukaryotes to extreme conditions causes a global arrest of cap-dependent translation. However, translation of specific proteins continues under such conditions using cap-independent mechanisms. We aim to understand how the translation apparatus of digenetic parasites that cycle between invertebrate vectors and mammalian hosts, adapts to the changing environments. Leishmanias and trypanosomes encode multiple paralogs of eIF4E, the cap-binding protein, as well as of other components of the translation initiation complex. We show that each eIF4E paralog in *Leishmania* comes into play in response to different stresses. The canonical translation initiation complex, anchored via LIF4E-4, is active in promastigotes at ambient temperatures, but its complex disintegrates at mammalian-like temperatures. Under these conditions an alternative cap-binding complex, LIF4E-1, comes into play. It assembles a complex with a multitude of initiation factors, but is devoid of any eIF4G ortholog. A novel and non-conserved 4E-Interacting protein (L4E-IP, 85 kDa) that binds only to LIF4E-1 appears to regulate its function in a manner that is not fully understood. It is still unclear how the parasite LIF3 along with its associated small subunit of the ribosome, are recruited to the different complexes. We identified 11 subunits that comprise the IF3 complex of *Leishmania*, and our preliminary findings suggest that unusual interactions are involved in the recruitment of this important factor to the initiation complex.

Leishmania parasites experience another type of stress within sand flies, once the mammalian blood drop is exploited, since plant juices taken in by the vector do not provide for all the required nutrients. This starvation induces metacyclogenesis and increases parasite virulence. Another paralog of eIF4E, LIF4E-3, has been assigned a function during starvation. Its cap-binding activity is reduced due to a mutation in the cap-binding pocket, preventing its ability to compete with the canonical factors. Instead, it enters into granules that are induced during starvation, most probably to accompany and protect inactive RNAs. We highlight how orthologs of conserved factors have been recruited during evolution to acquire novel functions that promote survival under harsh conditions.

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37 A *Leishmania* DEAD-box RNA helicase homolog plays a key role in translational control under stress and during the intracellular parasite development

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DEAD-box proteins form the largest family of RNA helicases present in all eukaryotes and also in bacteria and archaea that utilize ATP to bind or to remodel RNA and RNA-protein complexes. Nearly all steps of RNA biogenesis involve DEAD-box RNA helicases, including ribosome biogenesis, pre-mRNA splicing, mRNA export, mRNA turnover and translation. *Leishmania* and other trypanosomatid protozoa encode a large number of the DEAD-box RNA helicase SF2 superfamily that employ similar functions to those of other eukaryotes. We have shown previously that the *Leishmania* DEAD-box ATP-dependent RNA helicase HEL67, the ortholog of the *S. cerevisiae* Ded1p and the *Drosophila* Belle and Vasa proteins, interacts with ribosomal RNA (rRNA) and protects the parasite from rRNA degradation and translation inhibition upon conditions of stress and apoptosis-like programmed cell death through a novel mechanism that prevents antisense rRNA fragmentation[1]. Our more recent studies show that genomic inactivation of HEL67 leads to a decrease in global translation and that both ATPase and helicase activities are required for HEL67 function in translation regulation. Moreover, the *Leishmania* HEL67 null mutant is unable to undergo differentiation into its amastigote intracellular form and to survive inside macrophages. The increased sensitivity of HEL67-depleted Leishmania to various intracellular stresses, such as heat shock, acidic pH, and reactive oxygen species may explain the mutant's impaired growth. Also, several developmentally regulated transcripts were downregulated in parasites lacking HEL67, as revealed by RNA-seq analysis, suggesting a role of HEL67 in the preferential translation of these mRNAs under stress. Specific interactions of HEL67 with other ribosome-associated RNA-binding proteins seem to control rates of translation under conditions of stress or not by a mechanism that needs to be explored further. Overall, these data underscore the central role that DEAD-box RNA helicases play in the *Leishmania* intracellular development and define this class of proteins as major regulators in the parasite response to environmental insults.

[1]Padmanabhan PK, Samant M, Cloutier S, Simard MJ and Papadopoulou B. Cell Death Differ. 2012, Dec:19 (12): 1972-82.

38 Development and Applications of CRISPR-Cas9 for Genome Editing

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The Cas9 endonuclease from the microbial adaptive immune system CRISPR can be easily programmed to bind or cleave specific DNA sequence using a short RNA guide. Cas9 is enabling the generation of more realistic disease models and is broadening the number of genetically-tractable organisms that can be used to study a variety of biological processes. The Cas9 nuclease can also be modified to modulate transcription, alter epigenetic states, and track the dynamics of chromatin in living cells. In this presentation we will look at the latest developments and applications of the Cas9 nuclease for understanding the function of the mammalian genome. We will also look at the on-going challenges as well as future prospects of the technology.

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39 Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients

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Single murine and human intestinal stem cells can be expanded in culture over long time periods as genetically and phenotypically stable epithelial organoids. Increased cAMP levels induce rapid swelling of such organoids by opening the cystic fibrosis transmembrane conductor receptor (CFTR). This response is lost in organoids derived from cystic fibrosis (CF) patients. Here we use the CRISPR/Cas9 genome editing system to correct the CFTR locus by homologous recombination in cultured intestinal stem cells of CF patients. The corrected allele is expressed and fully functional as measured in clonally expanded organoids. This study provides proof of concept for gene correction by homologous recombination in primary adult stem cells derived from patients with a single-gene hereditary defect.

40 Repurposing Cas9 as a Multifunctional DNA binding protein

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The genetic interrogation of human cells requires methods for robust and specific activation and repression of endogenous genes. We show the CRISPR-associated catalytically inactive dCas9 protein can be repurposed as a multifunctional RNA-guided DNA binding platform in human cells. dCas9 can be fused to multiple effector domains with distinct regulatory functions enabling stable and efficient transcriptional repression or activation of endogenous genes with the site of delivery determined solely by a co-expressed short guide (sg)RNA. Synthetic dCas9-effector transcription factors can be multiplexed to robustly silence or activate expression of multiple endogenous genes. RNA-seq analysis indicates that CRISPR interference (CRISPRi)-mediated transcriptional repression is highly specific. Additionally, we show that a modified dCas9-EGFP fusion protein can be used to image endogenous DNA in living human cells. This imaging method has the potential to visualize chromosome organization and movement providing a new method for understanding how genome organization regulates genome function in live human cells. Our results establish that a modified CRISPR system can be used as a modular and flexible DNA-binding platform for the recruitment of proteins to target DNA sequences and reveals the potential of dCas9 as a general tool for regulating gene expression or for imaging DNA in living human cells.

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41 An mRNA delivery approach for HIV gene therapy

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RNA-based therapeutics are more suitable than other gene-based therapies for HIV as targeted delivery with non-viral carriers, transient expression, or absence of gene disruption/integration into the genome is necessary to treat HIV host cells. Many small RNA-based therapeutics, including siRNAs and aptamers, have reached clinical and preclinical testing, illustrating practicability for using RNA. Delivery of protein encoding mRNA presents new challenges, including stability, efficient protein translation, and pro-inflammatory response minimization, while providing a strategy for delivery of gene modifying enzymes such as cas9 or zinc fingers. Here, we have investigated effects of various chemical and structural modifications of mRNA to enhance properties required for therapeutic use. Various mRNAs were modified by addition of 5′ or 3′ noncoding sequences to an mRNA via in vitro transcription. Modified mRNAs encoding eGFP and Cre recombinase were successfully translated in vitro after transfection with TransIT-mRNA (Mirus Bio). We found that adding a stick sequence to the 3′ UTR, 5′UTR and/or after the poly(A) tail has minimal effect on translation. We are also currently investigating the effects of the sequence additions on stability and on in vivo inflammatory response. Cre mRNA containing either a poly(A) noncoding sequence complexed with TransIT-mRNA and injected into mTmG mice is translated more efficiently than complexed transcripts without noncoding sequence. We hypothesized that this was due to reduced mRNA degradation by exonucleases and measured the stability of the mRNA with PCR and by translation duration of mRNA in cells. Based upon our in vitro results, we have begun testing the in vivo potential for these modifications using our Cre mRNA in a Cre-LoxP mouse model. In addition, we have analyzed the in vivo biodistribution of the mRNAs labeled with infrared dye 800CW upon delivery with TransIT-mRNA in our mouse model. Chemical and structural modifications of mRNA are necessary for improving its properties for therapeutic use. Our study has revealed that such modifications may enhance the mRNA stability and non-immunogenicity without compromising the mRNA translational capacity. Moreover, our study continues to evaluate better, more targeted delivery systems relative to the conventional delivery modes, including lipid-based transfection, PAMAM dendrimer, or electroporation approaches.

42 Next-Generation CRISPR-Cas Nucleases with Improved Specificities

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Engineered CRISPR-Cas nucleases have become highly popular and broadly used tools for performing targeted genome engineering in a wide range of cell types and organisms. However, the Joung lab and others have previously shown that high frequency indel mutations can be induced at off-target sites harboring up to five mismatched nucleotides and that these effects can be site-dependent and challenging to predict. Therefore, improving the specificities of CRISPR-Cas nucleases is of utmost importance if these reagents are to be used for human therapeutic applications. In this talk, I will describe a modified CRISPR-Cas nuclease architecture that substantially improves the specificities of these reagents. We demonstrate that our modified nucleases can reduce mutagenesis rates by up to 5000-fold or more at previously defined off-target sites in human cells. These next-generation CRISPR-Cas nucleases represent an important improvement to the platform that will help to further advance these reagents as potential therapeutics for genetic-based diseases.

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43 Systems Level Analysis of Alternative Pre-mRNA Splicing: The roles of RNA structure and RNA Chaperone Proteins

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Alternative pre-mRNA splicing is one of the major mechanisms utilized by metazoans to regulate gene expression and to increase the functional diversity of the eukaryotic proteomes. In humans, ~95% of multi-exon genes are alternatively spliced and these RNA processing events have implications for health and disease since disease gene mutations that affect the splicing process result in human genetic disorders. Alternative pre-mRNA splicing is regulated both by RNA-binding proteins that interact with pre-mRNAs and by RNA secondary structure. Recent studies have indicated that RNA secondary structure can play an important, and previously underappreciated, role in the regulation of alternative splicing. RNA chaperone proteins are known to alter RNA secondary structures in vitro through RNA-RNA annealing or unwinding and by RNP remodeling. These RNA chaperones aid in RNA folding, but have also been shown to be involved in splicing and transcription in vivo.

Although much progress has been made in understanding different alternative splicing mechanisms at an individual gene level, much remains to be learned including how RNA structure and/or RNA chaperones affects alternative splicing on a global transcriptome-wide level. We aim to systemically link cis-regulatory elements in pre-mRNAs to RNA structural features and protein binding sites that control alternative pre-mRNA splicing in vivo. Using iCLIP and RNA-seq assays, over 175,000 binding sites of hnRNP A1 were mapped and 3600 differential splicing events were detected upon hnRNP A1 RNAi knockdown in K562 cells. The RNA binding and chaperone activities of two RNA chaperone proteins, hnRNP A1 and the p68/DDX5 RNA helicase will be compared to transcriptome-wide changes in RNA structure using chemical probing information and alternative splicing patterns with RNAi-knockdowns of these factors using high-throughput cDNA sequence analyses.

44 High-Throughput Sequencing of Lariat Branch Sites and 5′ Splice Sites Identifies Dozens of Novel Branch Points and Diverse Alternative Splicing in the *Saccharomyces cerevisiae* Genome

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The branch point sequence (BPS) is one of three key nucleotide sequences required for pre-mRNA splicing. However, in metazoans the BPS has been less comprehensively studied than the 5′ splice site (5′SS) and 3′ splice site (3′SS) due to the relative difficulty of identifying each sequence element. Splice sites can be readily identified by aligning spliced cDNAs, ESTs, or RNA-Seq reads to the genome, while lower throughput techniques, such as primer extension, are usually required to map BPS (with some exceptions). To understand how the BPS affects splicing outcomes, we developed a method to locate BPSs on a genome-wide scale. Specifically, we enriched for lariats by isolating RNA from yeast lacking debranching enzyme and used 2D PAGE gels to separate circular RNAs (including lariats) from linear RNAs. Subsequently, we used a custom library preparation to produce inserts in which one end identifies the BPS and the other identifies the 5′SS of a lariat. Applying our method to *S.cer*., one of the only eukaryotes where most BPSs are known, allows us to assess sensitivity and specificity of our method. We obtained reliable BPS information for more than 70% of intron containing genes and discovered dozens of novel BPS both in annotated introns and coding sequences. We verified the coding sequences containing BPSs are novel introns using RNA-seq and a lariat-sequencing method. We observed a considerable amount of alternative splicing (AS) in *S.cer*., which was thought to have only a handful of alternative splice sites and some stress-regulated intron retention events. Additionally, we found several introns with 2 BPSs and one gene with 3 BPSs. Generally, alternative BPS usage is associated with alternative splice site usage where one mRNA isoform produces a truncated open reading frame. We show, using UPF1-null yeast, that these isoforms are often regulated by nonsense-mediated mRNA decay. This suggests AS may control gene expression levels in yeast as is known to be the case in metazoans. Finally, preliminary application of our method to *Drosophila melanogaster* showed that recursive splicing occurs in a 400nt intron, amending previous reports that this phenomenon only occurs in introns larger than 10kb.

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45 Interrogating the contribution of alternative splicing to neuronal physiology through genome-wide analyses of actively translating mRNAs in diverse cell types

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Alternative mRNA splicing contributes to expanded trasncriptomic and proteomic repertoires, and this layer of gene regulation is especially prevalent in the nervous system. Although some examples show that alternative splicing can be modulated by stimuli, it has remained challenging to identify the extent of broader alternative splicing dynamics in individual neuronal cell types or within intact cell circuits *in vivo*. To tackle this problem, we adapted the Translating Ribosome Affinity Purification method (TRAP) (Heiman et al. 2008) in *C. elegans*. In TRAP, cell-type specific promoters drive expression of an eGFP-tagged ribosomal protein of the large subunit to tag sets of genetically defined cell types *in vivo*, which can be subsequently affinity purified to obtain enriched populations of ribosome-associated mRNAs from cells of interest. Here, we describe the coupling of TRAP to deep sequencing and computational analyses to identify nervous system-specific alternative splicing.

By using TRAP, we globally surveyed the repertoire of translated mRNAs from neuronal and muscle cells and measured their relative isoform abundances. Our approach has revealed a diversity of neuron-specific isoforms, and neuronal-enriched evolutionary conserved mRNAs whose functions have not beenof uncharacterized functions. We also used TRAP in animals that have lost the conserved neuronal splicing factor UNC-75/CELF to detect global isoform-level changes in the nervous system.s of animals that have lost the conserved neuronal splicing factor UNC-75/CELF. Our data indicate that in contrast to profiling whole animal populations, the TRAP methodology can be used to more sensitively detect tissue-specific splicing differences than what would be possible from profiling whole animal populations. We will describe follow- up studies from the above analyses, as well as an ongoing efforts using TRAP to obtain repertoires of translated mRNAs from specific neuronal classes, and stimuli. Our results suggest that this powerful approach will allow a better dissection of the regulation and contribution of alternative splicing within the nervous system during metazoan development and in response to dynamically changing environments and stimuli.

46 MOHCA-seq: Nucleotide-precision RNA proximity mapping from single multiplexed experiments

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RNAs perform myriad essential biological functions that require the formation of specific secondary and tertiary structures. Conventional structure determination techniques provide critical insights into RNA behavior but are limited in throughput and challenged by the large sizes and conformational heterogeneity of many RNAs. Three-dimensional structure modeling efforts would benefit from pairwise proximity information for nucleotides that are nearby in space in the 3D fold but not necessarily base-paired. Previously, we reported a technique for discovering tertiary contacts in RNA molecules, termed Multiplexed hydroxyl radical (•OH) Cleavage Analysis (MOHCA), which uses localized generation of hydroxyl radicals from sources that are incorporated at random positions in the RNA backbone. Here, we report that integrating MOHCA with tabletop deep sequencing (MOHCA-seq) achieves nucleotide-resolution proximity maps of RNA structure and nanometer-precision 3D models from a dramatically accelerated and parallelized protocol. After validating MOHCA-seq on RNAs of known structure, we report previously unavailable 3D information for diverse systems, including a complex 16S rRNA domain with controversial structure; ligand-induced conformational changes in the *F. nucleatum* glycine riboswitch; and aiding prediction of the 'RNA puzzle' blind trials, including an adenosylcobalamin riboswitch. We will discuss our efforts to achieve higher resolution data and to expand the method to larger transcripts probed in their cellular and viral milieus, and how collaborative efforts with bioinformatics and experimental labs could significantly accelerate these further advances.

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47 Uridylation pattern of coding and noncoding RNAs in human cells

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The 3′-end RNA modification by uridylation has been generally linked to RNA degradation. In mammals, uridylation plays a role in the turnover of histone mRNAs and precursor and mature forms of at least some miRNAs. The uridylated pre-miRNAs in mammals and uridylated mRNAs in fission yeast are targeted by the DIS3L2 3′ to 5′ exoribonuclease. DIS3L2 has been linked to the Perlman syndrome development and Wilms tumor progression. However, no functional link has been made between uridylation and the involvement of DIS3L2 in these diseases.

Here we report on the RNA binding studies of DIS3L2 that were performed by Crosslinking *in vivo* and immunoprecipitation (CLIP) method. Our study uncovers a broad spectrum of DIS3L2 RNA substrates *in vivo*. A specific bioinformatics approach identifies that many of these targets contain untemplated oligo(U) tail in average length of 8-10 nucleotides. The spectrum of such modified RNAs includes snRNAs, snoRNAs, tRNAs, pre-miRNAs, rRNAs and mRNAs. Furthermore, by using tRNAs as an example, we demonstrate, that the TUT-DIS3L2 pathway targets aberrantly processed molecules that were exported to the cytoplasm. Therefore, the uridylation-DIS3L2 pathway represents the cytoplasmic version of the RNA quality control pathway known from the yeast nucleus where it involves the TRAMP-exosome machines. Moreover, such wide spectrum of RNAs undergoing oligouridylation in human cells points to the essentiality of this modification in the cell metabolism.

48 TORC1 orchestrates ribosome biogenesis and nitrogen catabolism at the transcriptional level to optimize cell growth

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In the model eukaryotic cell, *Saccharomyces cerevisiae* (budding yeast), the molecular form of environmental nitrogen impacts both cell growth rate and mRNA expression. Disentangling causal relationships between nitrogen availability, cell growth rate and differential gene expression poses a considerable challenge. Using experimental control of cell growth rate with chemostats, we studied the effect of environmental nitrogen on differential gene expression. We find that most differential gene expression is explained by nitrogen limitation, not variation in nitrogen source. To study the dynamics of nitrogen-responsive gene expression we perturbed steady-state nitrogen-limited chemostat cultures by transient addition of different nitrogen sources. We find evidence that a transition from nitrogen-limited to nitrogen-replete conditions is accompanied by rapid induction of transcripts required for protein translation. We identified a reciprocal relationship between transcripts required for protein translation (RP and RiBi) and those required for nitrogen assimilation. By means of high resolution time series analysis we find evidence that rapid, and potentially accelerated, mRNA degradation plays an important role in remodeling gene expression programs in response to change in environmental nitrogen. We propose that the evolutionarily conserved TORC1 signaling pathway orchestrates the balance between protein translation and assimilation of nitrogen sources at the transcriptional level to optimize rates of cell proliferation.

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49 Extensive translation of small ORFs revealed by 'Polysomal-RiboSeq'

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Ribosomal profiling has corroborated and expanded proteomes, with thousands of new translated sequences described. However, its application to non-canonical translation events like those on lncRNAs can lead to differing conclusions. We have devised an improvement to ribosomal profiling to purify only those mRNAs, which are undergoing active translation by differentiating between mRNAs that are engaged by polysomes over those bound by sporadic, putatively non-productive single ribosomes or ribosomal subunits. This novel combination of polysome fractionation and ribosome profiling we term 'Polysomal-RiboSeq'.

It is particularly important to discern whether RNAs are undergoing active translation when determining whether RNAs are lncRNAs or they encode small ORFs (smORFs ≤100 aa). Hundreds of thousands of smORFs exist in our genomes and bioinformatic searches have predicted that thousands have the potential to be translated. Examples of functional smORFs have been characterised in a few species but the actual number of translated smORFs, and their molecular, functional and evolutionary features are not known. The key question remains whether predicted smORFs are translated and encode proteins, or act as ncRNAs.

We have performed a genome-wide assessment of smORF translation in *Drosophila melanogaster* S2 tissue culture cells using 'Polysomal-RiboSeq'. To enrich for translated smORF mRNAs, we purify mRNAs bound by 2-6 ribosomes, as we estimate most ORFs ≤ 300nt only have space for maximum of 6 ribosomes. Comparing ribosome footprinting in small and large polysomes reveals that purification of small polysomes does indeed result in enrichment of smORFs over canonical long mRNAs.

Our 'Polysomal-RiboSeq' has revealed that 83% of uncharacterised and minimally annotated smORF mRNAs that are transcribed, are indeed translated. Interestingly, we have also found that 65 putative lncRNAs, 20 previously bioinformatically predicted smORFs and ~2500 upstreamORFs show evidence of active translation. Tagging experiments have confirmed our ribosome footprinting results and preliminary functional analysis of these translated smORFs suggests that they are important for cellular health. To extend the smORF catalogue and deepen our understanding of their biological significance 'Polysomal-RiboSeq' of *Drosophila* tissues is underway.

50 Global Identification of RNA-protein complexes with density gradient centrifugation and SILAC-MS

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RNA-binding proteins regulate post-transcriptional gene expression by interacting with messenger RNA (mRNA) to form ribonucleoprotein complexes (RNPs). These interactions are transient and variable from the time an mRNA is synthesized until its decay. We describe here an approach to the discovery of RNPs using isotopic tagging and RNase treatment followed by density gradient centrifugation and mass spectrometric (MS) analysis. Over 400 RNP–associated proteins were identified, approximately 30% of which were not previously annotated as part of RNPs.

We utilize cesium sulfate-urea density gradient centrifugation and stable isotope labeling of amino acids in cell culture (SILAC) to identify and characterize RNPs in formaldehyde cross-linked yeast lysate. The density gradient separates RNA-protein complexes from free protein and free RNA by virtue of their differing densities. Isotopic labeling provides a tool to aid in distinguishing specific from non-specific RNA-protein interactions. Stable isotope-labeled ("heavy") amino acids and unlabeled ("light") amino acids are incorporated into two otherwise identical cell cultures. The cell cultures are formaldehyde cross-linked, lysed and the cell lysate from the "heavy" cell culture is then RNAse digested. Cell lysates from both cultures are combined and processed in the gradient together. Following fractionation the samples are analyzed by mass spectrometry. Proteins that were part of RNA-protein complexes in the "heavy", RNAse digested lysate become free proteins and migrate to a lower density than the intact RNA-protein complexes from the "light" lysate. The relative intensities of the light and heavy peptides provides a measure of the relative abundance of their parent protein: proteins that have statistically high "light":"heavy" ratios are likely candidates for belonging to RNA-protein complexes.

This technology is applied to analyze differences in RNPs in yeast cultured in both non-stressed (log phase) and stressed (0.7M NaCl added during log phase) conditions. Preliminary results identified over 400 proteins in RNA-protein complexes, 170 of which were observed under both stressed and non-stressed culture conditions. This strategy provides a powerful new tool for the identification of RNPs and how they vary in response to intrinsic (e.g. genetic) or extrinsic (e.g growth conditions) cues.

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51 Quantitative Differential Proteomics Analyses Dissect the Human LINE-1 Retrotransposon Ribonucleoprotein Physical Interactome

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LINE-1 (L1) is a retrotransposable element and a vector for evolution and disease whose sequences comprise a significant proportion of the human genome. Despite its tremendous influence on genome composition, L1 RNAs only encode two proteins. Consequently, L1 particles include 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.

We recently characterized the physical interactome of the human L1 retrotransposon ribonucleoprotein (RNP), as expressed in cell culture, using a combination of techniques including stable isotope labeling and affinity proteomics[1]. To achieve this, we developed methods to separate distinct L1 populations by split tandem affinity capture and to destabilize RNP constituents in the purified complexes through RNase treatment. To build on these analyses, we have developed a novel implementation of stable isotope labeling in conjunction with our previously established workflows. We can now report an unbiased, quantitative survey of the proteins partitioning within distinct L1 populations as well as their relative sensitivity to nuclease treatments. Our observations provide a basis for the classification of L1 interactors with respect to their physical and functional links, facilitating hypotheses to direct *in vivo* experimentation.

[1]Taylor, M. S. and LaCava, J. *et al.* Affinity Proteomics Reveals Human Host Factors Implicated in Discrete Stages of LINE-1 Retrotransposition. *Cell* **155,** 1034-1048 (2013).

52 Massive parallel sequencing based hydroxyl radical probing of RNA accessibility (HRF-Seq) using Fenton chemistry and synchrotron irradiation

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The hydroxyl radical footprinting (HRF) is a well established method for assaying nucleic acid backbone accessibility, which found its applications in studying the tertiary and quaternary structure of RNA and DNA. Traditionally, the signal is detected with either slab-gel or capillary electrophoresis, which considerably limits the throughput. Here we present a method of HRF signal detection utilizing the massive parallel sequencing, called HRF-Seq, which allows for a simultaneous analysis of multiple, long RNA molecules. The HRF-Seq workflow starts with the hydroxyl radical treatment of the RNA ensemble of interest, followed by a randomly primed reverse transcription, adapter ligation, PCR amplification and detection of cDNA 3′ ends and priming sites with Illumina sequencing. We describe a novel computational method of alleviating the PCR bias which uses the random barcodes introduced during the ligation. Moreover, we show the normalization procedure which unifies the signal over the regions of varying coverage and corrects for the background terminations. The HRF-Seq correlates well with the slab-gel electrophoresis and with the RNA backbone accessibility measured from the known crystal structures with the resolution that allows for the observation of differential reactivity of sides of helix. The results indicate that the method can generate useful constraints for the automated three dimensional RNA structure modeling in a high-throughput manner. As a next step we are applying the HRF-Seq method for in vivo RNA probing of mouse liver RNA. Samples were probed with synchrotron generated X-ray beams and the quality of RNA shows clear dose-response relationship with the irradiation time. Further analysis of the probed RNA is ongoing.

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53 mRNAseq procedures for the detection of subtle differences in gene expression

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Current approaches for the analysis of mRNAseq data can reveal large differences in gene expression (for example changes between different tissues) but perform less well in detecting subtle differences, such as those due to behaviour. For example, gene expression changes due to alteration in the social environment in flies are subtle and often difficult to identify at a reasonably high sensitivity (i.e. low false positive rate). To address these problems, we propose a new set of procedures including bootstrap normalization and hierarchical differential expression analysis. The efficiency of the new approaches is discussed using mRNAseq data from an experiment in which D. melanogaster males were exposed to rivals prior to mating. The bootstrap normalization is based on a Monte Carlo sampling without replacement. We show how this can be reliably used to identify true replicates and to align the distributions of expression levels. The resulting, normalized expression levels are then subjected to a two-step differential expression analysis. The genes are first categorised based on a higher-level separation (tissue) and second by a fine-scale classification by presence or absence of rivals. Both steps use an offset fold change for detecting the differential expression.

54 Modeling local splice variations from RNA-Seq data

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Quantifying splicing variations has been the focus of numerous studies since the conception of RNA-Seq. Key RNA-Seq analysis tasks include identifying novel splice variants, quantifying their relative abundance, and assessing splicing changes between different experiments or groups of those. We present a novel computational framework for RNA-Seq analysis named MAJIQ (Model for Alternative Junction Inclusion Quantification), coupled with VOILA, a novel visualization framework. MAJIQ uses a Bayesian framework to tackle all of the aforementioned analysis tasks for local splice variants (LSVs). LSVs include commonly used variants such as cassette exons, alternative 3′/5′ splice sites, mutually exclusive exons, as well as more complex splicing variations. Using efficient coding and multi-core processing for fast execution MAJIQ is able process large amounts of RNA-Seq data, taking into account sequencing biases, variations between read positions, mapper issues, and relations between experiments such as groups and replicates. The visualization package, VOILA, uses efficient HTML5 with vector-based images to allow users to interact with MAJIQ's output. Unlike alternatives such as the genome browser, MISO and MATS, it is able to visualize long lists of LSVs across different conditions, summarizing efficiently both the levels of splicing change and the uncertainty in splicing quantification. In addition, VOILA's components are expected to serve as a general utility for other groups tackling various tasks of transcriptome analysis. To demonstrate the advantages of MAJIQ and VOILA we used mouse and human RNA-Seq datasets from diverse tissues and cell lines, along with over a hundred newly executed RT-PCR experiments. We show MAJIQ significantly outperforms current state of the art in terms of both false positives and false negatives. We also use these datasets for a global transcriptome view, showing how previous analyses of transcriptome variations can be refined. In summary, the combination of MAJIQ and VOILA should thus serve as a significant step forward for the greater scientific community tackling transcriptome variations with RNA-Seq data.

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55 Structure models of bacterial Sm protein Hfq in complex with *rpoS* mRNA

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Bacterial Sm protein Hfq is required for translation regulation by small non-coding RNAs (sRNAs), which play a key role in bacterial stress response and environmental adaptation. Hfq promotes anti-sense base pairing of sRNAs that inhibits or activates translation of the mRNA target. One well-studied example of positive regulation is the stress response regulator *rpoS*, which contains a 576 nt long leader that folds into complex secondary structure. An $(AAN)_4$ motif in the upstream domain of the *rpoS* leader tightly binds the distal face of Hfq. Here, we report that the Hfq lateral rim directly binds a U_5 motif downstream of the sRNA annealing site using SHAPE footprinting and in vivo binding assays. After the sRNA base pairs with *rpoS* mRNA, Hfq remains bound to the $(AAN)_4$ motif but dissociates from the U_5 motif. LacZ reporter assays showed that the U_5 motif is required for sRNA regulation of *rpoS* translation. We next used small angle X ray scattering (SAXS) to show that Hfq folds *rpoS* into a compact tertiary conformation, indicating that Hfq positions and remodels the *rpoS* structure by simultaneously interacting with upstream and downstream motifs. Finally, we generated all-atom structural models of free *rpoS* leader RNA, full-length Hfq protein, and the *rpoS*•Hfq complex by combining MC-Sym structure prediction, rigid body modeling, and Monte Carlo simulation against our SAXS data. We propose that the Hfq distal face anchors the *rpoS* $(AAN)_4$ motif tightly, whereas the lateral rim dynamically interacts with the U_5 motif, moving between closed and open states. In the closed mode, Hfq positions and destabilizes *rpoS* inhibitory stem for efficient sRNA entry and annealing. In the open mode, Hfq disengages from the downstream U5 motif, releasing the sRNA•mRNA duplex and ribosome binding site for translation initiation.

55A Mammalian 5′-capped microRNA precursors that generate a single microRNA

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MicroRNAs (miRNAs) are ubiquitous gene regulators that modulate essential cellular processes at the post-transcriptional level. In metazoans, most miRNAs are produced from pri-miRNA transcripts that are first cleaved by the nuclear Microprocessor, with the resulting pre-miRNAs exported by Exportin-5 and then cleaved by cytoplasmic Dicer. After Dicer action, both strands of the miRNA duplex are candidates for association with Argonaute to form functional microRNPs.

By establishing a small RNA Cap-seq method that employs the cap-binding protein eIF4E, we discovered that ~20 annotated human and mouse miRNAs are derived from m^7G-capped pre-miRNAs whose 5′ ends are generated by RNA polymerase II (Pol II) transcription initiation. Transcription reporter assays indicate that the 3′ end of m^7G-capped pre-miRNAs is generated via Pol II promoter proximal pausing/termination. In this Microprocessor-independent but Dicer-dependent pathway, the presence of the 5′ cap directs pre-miRNAs to the PHAX-Exportin-1 pathway, which functions in the export of small nuclear RNAs (snRNAs). After Dicer cleavage, the 5′-capped 5p-miRNA is unable to associate effectively with Argonaute, resulting in the production of only 3p-microRNPs. We show that in comparison to current strategies for constitutive shRNA expression in RNAi technology, m^7G-capped shRNA constructs can be used to generate a single siRNA (3p-siRNA) in vivo, thereby minimizing off-target effects.

Our results reveal an unusual pathway that is distinct from canonical miRNA biogenesis in pre-miRNA synthesis, nuclear-cytoplasmic transport and guide strand selection. Additional m^7G-capped pre-miRNAs are being predicted using small RNA Cap-seq data based on a conserved downstream adenosine-rich sequence, which promotes miRNA biogenesis presumably by facilitating Pol II termination.

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56 Turning catalytically inactive human Argonaute proteins into active slicer enzymes

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Argonaute (Ago) proteins are central components of small RNA-guided gene silencing. They interact with small RNAs, which guide them to complementary target RNAs leading to inhibition of gene expression. Some but not all Ago proteins are endonucleases, referred to as ‘Slicers’, and can cleave the complementary target RNA. Their endonucleolytic activity was long thought to reside in the PIWI domain which contains four catalytic residues.

We have mutated inactive human Ago1, Ago3 and Ago4 and generated cleavage-competent Ago proteins. We find that additional features within the PIWI domain and within the N domain determine cleavage function, providing important insights into structural requirements that define Ago slicing function. Furthermore, we show that activated Ago1, Ago3 and Ago4 are capable of cleaving the Ago2-processed miR-451, demonstrating that a single Ago-mediated cleavage event and no further Ago2-specific features are required for miR-451 processing. Finally, we complement our findings by generating a phylogenetic tree of human Ago proteins and analyzing this in the context of slicer-defining protein features.

57 A new post-translation modifier implicated in microRNA regulation in *C. elegans*

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In all metazoans, microRNAs play a critical role in the regulation of genes implicated in cell proliferation and differentiation. These small non-coding RNAs form a silencing complex called miRISC and alter protein synthesis upon binding specifically mRNA untranslated regions (UTRs). Recently, miRNA stability and production have been shown to be an important step in this pathway. Several proteins, such as p53, can modulate microRNA biogenesis and many other proteins are implicated in miRNA stabilization and degradation. A tight control of these regulatory RNA is essential since miRNA misregulation is associated with several diseases. Our previous study identified the ortholog of human decapping enzyme DcpS (DCS-1) as an important regulator of miRNA level in *C. elegans* by forming a degradation complex with XRN-1, idependently of its catalytic activity (*Bossé et al, Mol. Cell, 2013*). This work identified the first modulator of microRNA degradation however many questions remain unanswered concerning the release of miRNAs from miRISC and the recruitment of the degradation complex to miRNAs. In order to better understand the regulation of miRNAs, we sought to identify other members of this complex. An initial study of proteins identified by mass spectrometry revealed that the loss-of-function of *dip-1 (**D**CS-1 **I**nteracting **P**rotein)* induces several developmental defects associated with the loss of miRNAs. *Dip-1* genetically interacts with the let-7 microRNA family by enhancing the defects observed for the loss of this miRNA. At the molecular level, mature let-7 is downregulated and the regulation of one of its targets is affected in a *dip-1* mutant. The protein level of key components of the microRNA pathway, such as the microRNA-specific Argonaute (ALG-1), XRN-1 and DCS-1, is not affected by the loss of *dip-1*. However, preliminary results suggest that the interaction between DCS-1 and XRN-1 could be altered in the absence of *dip-1*. As DIP-1 is a post-translational modifier, our results suggest that it could affect the stability of the degradation complex by targeting one of its components. Our data suggest that *dip-1* is the first post-translational modifier implicated in miRNA turnover.

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58 Alternative Slit Promoters Drive Motor Neuron-Specific MicroRNA (miR-218) Necessary for Viability

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Motor neurons are a located in the ventral spinal cord and control all the muscles in the mammalian body. The transcription factor networks that define this neuronal lineage during development have been well characterized. However, features of RNA processing that may contribute to motor neuron identity have been largely unstudied. We performed microRNA sequencing on ES-derived motor neurons and identified miR-218 as the only enriched microRNA. In situ hybridization identify robust miR-218 expression across all known motor neuron subtypes including cranial, pre-ganglionic, medial, and lateral motor neurons, making miR-218 the most specific pan-motor neuron gene product identified. miR-218 is encoded within intron 14 of Slit2 and Slit3 genes; surprisingly, we continued to detect robust miR-218 expression in Slit2 and Slit3 KO mice. We performed polyA+ RNA sequencing and identified motor neuron-specific alternative promoters in both the Slit2 and Slit3 genes located upstream of exon 6, explaining the persistence of miR-218 expression in exon1-targeted animals. Furthermore, we identified novel motor neuron-specific alternative splicing and polyadenylation sites in Slit3 that are not used in other cell types. Strikingly, these alternative splice and polyadenylation sites are located within the same intron as the miR-218 hairpin, suggesting a functional relationship between multiple RNA processing machineries in vivo. To understand this relationship further, we performed RNA-sequencing on motor neurons in which the microprocessing enzyme Drosha was deleted and find evidence suggesting miR-218 is generated from alternatively polyadenylated transcripts and in a co-transcriptional manner. Additionally, the splicing pattern of Slit3 is significantly altered in motor neurons depleted of Drosha, suggesting interaction between the spliceosome machinery and microprocessor. We performed a minigene assay to identify modifiers of Slit3 alternative polyadenylation and find PTBP1 as a likely candidate. Finally, in order to study the functional role of miR-218 in motor neuron development, we have successfully deleted miR-218 in vivo using CRISPR-mediated genome editing. Mice deficient in miR-218 are morphologically similar to wild-type littermates but die within several days of birth from currently unknown causes. We are working to characterize these mice during embryonic and early postnatal stages.

59 Coupled pri-miR-17~92 processing and pre-miRNA stabilization in cancer

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Steady state expression of miRNAs is determined by a series of regulated steps in transcription, maturation and turnover. As miRNAs play important tumor-suppressive and oncogenic functions, biogenesis steps can be impaired or enhanced in tumorigenesis and cancer progression. Several sub-types of cancers select for an accumulation of one or several of the miRNAs derived from the miR-17~92 polycistron. This is often apparent from the genomic amplification of its locus. We precisely mapped the processing steps of the pri-miR-17~92 transcript. Analysis of novel intermediate RNA species revealed that pri-miR-17~92 maturation follows a highly selective series of hierarchic steps. Furthermore, we found that cell lines bearing the miR-17~92 genomic amplicon stabilize pre-miRNAs, possibly by impairing competing turnover outlets to Dicer processing. Our data support a model wherein increased pri-miRNA transcription and maturation are coupled with pre-miRNA stabilization to over-express the miR-17~92 oncogenic miRNAs.

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60 Dissecting the role for microRNA and circular RNA in the development of mammalian brain

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Higher mammals rely heavily on fine-tuned regulation of gene expression, which allows a limited number of genes to account for very high cellular complexity. The extraordinary complexity of the mammalian brain aligns well with a particular rich expression of non-coding RNA including microRNA (miRNA) and circular RNA (circRNA) in this organ. To study the role of miRNA and circRNA in embryonic brain development, expression profiles were investigated in a spatial and temporal fashion. The pig brain closely resembles the human brain in aspects such as development and brain morphology with a similar overall structure including the folding of the cerebral cortex (gyrencephalic brain) in contrast to mice, which have smooth cerebral cortex (lissencephalic brain).

By use of next generation sequencing, the miRNA and mRNA content of multiple embryonic pig brain tissues were sequenced at 6 different time points spanning from early gestation until time of birth. Many miRNAs exhibited a distinct expression profile that, in combination with a genome wide target prediction analysis, was correlated with mRNA expression. A particularly strong correlation pointed to a set of miRNAs as key regulators of DCX and LIS1 mRNA expression, two genes known to be involved in cortical folding in gyrencephalic brains. Among circRNAs, ciRS-7 (circular RNA sponge for miR-7) also exhibited regulated expression during development, suggesting a temporal and spatial control of miR-7 activity. Finally, we also studied ADAR enzyme mediated A-to-I editing of all detected mature miRNAs in different brain tissues. We discovered a number of mature microRNAs that underwent spatio-temporally regulated RNA sequence editing within their seed sequences. We are currently trying to dissect bioinformatically whether these editing events alter the pool of target mRNAs, creating yet another level of complexity in mRNA regulation.

These studies help to further understand the timing and function of miRNA and circRNA expression as well as the interplay with the ADAR editing machinery during the course of embryonic brain development in mammals.

61 Specific Delivery Of Nucleic Acids To Cancer Cells By β-hairpin Forming Cell Penetrating Peptides

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Among non-viral vectors, cell penetrating peptides (CPPs), which generally contain a stretch of cationic and/or amphipathic peptides of 5–30 amino acids, have received considerable attention for their ability to transport nucleic acids into cells both *in vitro* and *in vivo*. Recent reports revealed that cancer cells are anionic due to the overexpression of negatively charged phosphatidylserine (PS) lipid and high levels of sialic acid in comparison to normal cells. These characteristics can be exploited to improve delivery of therapeutic nucleic acid candidates. Herein we describe the rational design and synthesis of cationic/amphiphilic peptides that upon complexing with nucleic acids form β-hairpins and impart amphiphilicity to the peptide/nucleic acid complex. This facilitates the specific traversal of anionic cancer cell membranes and enhances the delivery of a variety of nucleic acid structures that control cellular functions. We have designed a series of peptides containing alternating hydrophobic and polar/positvely charged residues connected by a type II′ turn promoting sequence -V^{D}PPT- and tested them for transfection and for Green Fluorescent Protein (GFP) gene silencing effciency in human breast cancer cells (MDA-MB-231). The ability of these peptides to form β-hairpins upon complexing with nucleic acids such as siRNAs was verified by circular dichrosim spectroscopy. It was observed that the peptides that form β-hairpins showed significant transfection of flurescently labeled RNA/DNA duplexes and were capable of delivering siRNA to efficiently silence GFP expression in the breast cancer cells whereas non β-hairpin forming peptides did not, as revealed by fluorescence activated cell sorting (FACS) analysis. However, the degree of uptake and silencing activity of β-hairpin peptides varied with the peptide sequence. QSAR was able to predict some of the characteristics of these peptides however further experimental investigation is needed to decipher this sequence activity relationship.

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62 Dissecting the molecular mechanisms of small RNA mediated gene activation in *C. elegans*

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It is becoming increasingly evident that small RNA pathways are key players in maintaining a balance between silencing the expression of exogenous (nonself) nucleic acid and licensing the expression of endogenous (self) genes in various species. In *C. elegans*, the piRNA-mediated germline surveillance system encodes more than 30,000 unique 21-nucleotide piRNAs, which silence a variety of foreign nucleic acids, including transposable elements and transgene sequences. If left unchecked, this system would also have the potential to recognize and silence nearly the entire *C. elegans* germline transcriptome. However, my lab recently demonstrated that the Argonaute CSR-1 counteracts the silencing capacity of the piRNA pathway to license germline transcription.

Using an *in vivo* RNA transgene tethering assay, we showed that tethering CSR-1 to a target transcript licensed its expression, and that licensing mainly occurred at the level of transcription. Remarkably, we also observed that over the course of several generations, CSR-1 was capable of trans-activating the expression of a second gene that shared homology with our tethering target, but possessed no tethering sites. More recently, we have also defined a role for CSR-1 in licensing the expression of endogenous genes in the hermaphrodite germline. Together, these results demonstrate a rare positive role for an endogenous Argonaute pathway in heritably licensing germline transcripts. As CSR-1 targets nearly the entire germline transcriptome via its 22G-RNA binding partners, we are now defining the molecular mechanisms by which CSR-1 modulates chromatin to license transcription.

Using candidate approaches and an RNAi screen, we have identified several chromatin modifying factors, including a histone chaperone and a histone methylase that physically interact with CSR-1 and are likely to execute several of the chromatin-directed functions of this pathway. We have also uncovered the complexion of euchromatic histone modifications present at the germline genes targeted by the CSR-1 pathway and demonstrate that these histone modifications are altered in response to loss of CSR-1 or the chromatin modifying factors. Together, these studies advance our understanding of three key emerging areas in small RNA biology: nuclear functions of RNAi pathways, RNAi-mediated gene activation (RNAa), and the mechanisms of transgenerational epigenetic inheritance.

63 Multiple evolutionarily distinct RNA dependent RNA polymerase pathways compensate for the loss of piRNAs in multiple independent nematode lineages

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Small RNAs act at the front line of cellular defenses against transposons across the entire eukaryotic kingdom. The requirement to fight a rapidly mutable target has led to rapid evolution within components of small RNA pathways. Within animals, the piwi interacting small RNAs (piRNAs), which associate with Piwi proteins and target transposons for silencing, are widely conserved and are essential for fertility in fruit-flies, mammals and the nematode *C. elegans*. However, other small RNA pathways appear restricted to specific phyla, meaning that the evolutionary relationship between different small RNA pathways targeting transposons remains mysterious. In order to address this we sequenced small RNAs from multiple evolutionarily distant nematode species, producing the first comprehensive analysis of how small RNAs evolve within a single phylum. Strikingly, despite its apparent importance in maintaining fertility in many animals, piRNAs are absent in all independent nematode lineages with the exception of the clade containing *C. elegans*. This discovery raises the question of how transposons can be controlled in the absence of piRNAs. We found that there are at least two evolutionarily distinct pathways in nematodes that compensate for the absence of piRNAs by attacking transposons. Both pathways involve RNA dependent RNA polymerase (RDRP). Whilst one pathway is unique to nematodes, a second, more ancient pathway is an RNA-directed DNA methylation pathway, hitherto unknown in animals, which bears similarity to transposon-control pathways in fungi and plants. Our results highlight the rapid, context-dependent evolution of the small RNA world and suggest that transposon defense by piRNAs in animals may have replaced an ancient RNA-dependent RNA polymerase pathway ancestral to all eukaryotes.

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64 Multiple pathways for ribosomal small subunit biogenesis *in vivo*

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Ribosome biogenesis in bacteria is a complex yet efficient process that requires synthesis and interaction of more than 5000 nucleotides of ribosomal RNA (rRNA), 50 ribosomal proteins (r-proteins) and several dozen auxiliary factors. Ribosome biogenesis begins during rRNA transcription, is asynchronous and remains poorly understood *in vivo*. We have developed affinity purification techniques to isolate and characterize *in vivo* formed ribosomal small subunit (SSU) assembly intermediates in bacteria. Our approach takes advantage of the regions in precursor 16S rRNA (leader and trailer) that are components of the primary transcript and assembly intermediates but not mature SSU. Pre-16S rRNA is tagged at different positions in between various nucleolytic cleavage sites allowing systematic mapping of the assembly cascade in the wild-type unperturbed cells. Pre-SSUs have been isolated using tags at two different positions in leader sequence and one position in trailer sequence. RNA analysis revealed the isolation of 17S rRNA (16S rRNA with full leader and trailer sequences) independent of position of the tag. Thus, 17S rRNA is the major platform for SSU biogenesis in the cells. Structural probing demonstrates that the 17S rRNA containing intermediates purified with tags at different positions have diverse architectures representing earlier to later stages of the SSU biogenesis. The regions of 16S rRNA involved in the translation show altered structure suggesting these intermediates are functionally inactive. Protein analysis illustrates the stoichiometric differences within ribosomal proteins as well as the presence or absence of auxiliary factors. Several novel, putative auxiliary factors have been identified and substrates for few of the known factors are partially characterized.

Our results indicate that ribosomal assembly occurs largely on 17S rRNA, final RNA processing events happen late in the biogenesis cascade through parallel pathways which can be recapitulated *in vitro* and are coupled with r-protein and biogenesis factors binding. Moreover, there are multiple pathways for r-protein addition and auxiliary factor action for SSU biogenesis *in vivo* in *E.coli*. These findings allow the first integration of rRNA processing events with conformational changes, rRNA modification, r-proteins association and auxiliary factor action during ribosomal small subunit biogenesis in bacteria.

65 Direct binding of Bms1p to the endonuclease Rcl1p is critical for Rcl1p recruitment into pre-ribosomes and early processing of the pre-rRNAs

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A complex series of endonucleolytic cleavages and exonucleolytic degradations is mandatory for proper generation of mature rRNAs. One major challenge in the field of ribosome biogenesis is to identify the different ribonucleases involved and to understand when and how these enzymes are recruited into the pre-ribosomal particles to fulfill their function. The nucleolar Rcl1p protein, related to the 3′ terminal cyclase family, has recently been suggested to catalyze the endonucleolytic cleavage event at site A_2 in the internal transcribed spacer 1 (ITS1) of the pre-rRNA, an important step in the maturation of the 18S rRNA. Rcl1p interacts physically with Bms1p, a GTP-binding protein possessing an intramolecular GTPase-Activating Protein (GAP domain). Here we report the crystal structure of yeast Rcl1p in complex with a minimal fragment of Bms1p and we identified Rcl1p residues establishing direct contacts with Bms1p. Amino acid substitutions of these residues affect the interaction between Rcl1p and Bms1p *in vitro* and result in pre-rRNA processing defects *in vivo*, suggesting that perturbing the interaction between Bms1p and Rcl1p *in vivo* affects cleavage at site A_2. We show that Rcl1p and Bms1p associate in the cytosol, are co-imported into the nucleus, then recruited into the pre-ribosomal particles at a similar stage of the maturation pathway, following incorporation of the UTP-A and UTP-B modules but before Rrp5p recruitment. Following A_2 cleavage, both proteins remain associated with the pre-40S particles, but are not incorporated into nascent pre-60S particles. They are subsequently released from pre-40S particles at an intermediate step of the maturation pathway preceding Rio2p recruitment. Importantly, Rcl1p accumulates in the nucleoli of yeast cells expressing a mutant version of Bms1p defective in GTP-binding, indicating that GTP binding and hydrolysis by Bms1p are not required for Rcl1p nuclear import and its incorporation into the pre-ribosomal particles. Altogether, we demonstrate a systematically intertwined fate of Bms1p and Rcl1p in every tested step of the maturation pathway, suggesting that these proteins function as a heterodimer or remain at least in a close environment within the pre-ribosomal particles.

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66 Rio1 mediates ATP-dependent final maturation of 40S ribosomal subunits

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During ribosome biogenesis, late pre-ribosomal particles are exported to the cytoplasm prior to final maturation into functional 40S subunits. This maturation involves cleavage of the 20S pre-rRNA at site D, which will form the 3′ end of the mature 18S rRNA, by the PIN-domain endonuclease Nob1. We previously showed that site D cleavage can be reproduced in vitro in purified pre-40S particles. Here we report that cleavage occurs only in particles that have largely been stripped of the characterized pre-40S components, but retain the endonuclease Nob1, its binding partner Pno1 (Dim2) and the atypical ATPase Rio1. Within the Rio1-associated particles, pre-rRNA cleavage is strongly stimulated by ATP, and showed lesser stimulation by the non-hydrolysable analog AMP-PNP and GTP. Rio1 particles show more efficient assembly into 80S complexes than do Nob1-associated pre-ribosomes, consistent with more extensive binding of Nob1 with earlier, cleavage incompetent particles. *In vivo* binding sites were mapped for Rio1, Pno1 and Nob1 in actively growing cells. Nob1 and Pno1 bind the same locations over cleavage site D and within the internal transcribed spacer 1 (ITS1) region of the pre-rRNA, consistent with the reported direct interactions between these proteins, but show distinct binding sites in the 18S rRNA region. Binding sites identified for Rio1 were within the core of the 18S rRNA, overlapping key tRNA interaction sites and distinct from the closely related kinase Rio2.

67 Eukaryotic Ribosome Assembly Samples Hybrid and Classical States as a Quality Control Mechanism

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Ribosome assembly in eukaryotes is a complex and highly regulated process with over 200 assembly factors bringing together 79 proteins and 4 ribosomal RNAs. In yeast, one of the key stages during this process is a quality control step where the mature 60S subunit joins the immature pre-40S subunit in an eIF5B-dependent manner to form an 80S-like ribosome. The formation of 80S-like ribosomes acts as a licensing step, where the ability of the pre-mature 40S subunits to bind large subunits and translation factors is tested before their release into the translating pool. However, in addition to binding 60S subunits, 40S must undergo carefully orchestrated conformational rearrangements, which are used for translocating the mRNA and tRNA through the ribosome during each step of protein synthesis. If and how the ability to carry out these changes are tested remains unknown.

Previously, we have demonstrated that depletion of an essential assembly factor Fap7 leads to the accumulation of 80S-like ribosomes. We now show that a mutation in the large ribosomal protein Rpl3, which favors formation of hybrid state (H256A), and addition of sordarin, a drug which binds eEF2 and blocks ribosomes in the hybrid state, both partially rescue the effects of Fap7 depletion and reduce the accumulation of 80S-like ribosomes. In contrast, a mutation in Rpl3 that induces the hybrid state (W255C), and depletion of eEF2, which promotes translocation, both lead to accumulation of 80S-like ribosomes. We are currently carrying out chemical footprinting experiments to probe these different conformational states. Our results suggest that during 40S ribosome maturation the translation factor eEF2 and the assembly factor Fap7 cooperate to promote formation of the hybrid state in a translation-like process, which can serve as a quality control mechanism for nascent 40S subunits.

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68 New structural insights into mechanisms of cap-independent translation initiation

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Conventional initiation of translation of cellular mRNAs depends on the m7G cap located at the 5′ untranslated region (UTR) and on extraribosomal proteins called initiation factors. Translation of many viral mRNAs and some cellular mRNAs can be initiated in a cap-independent manner, with fewer initiation factors, to promote expression under stress conditions when overall cellular translation is downregulated. In these mechanisms, several types of structured RNA at the 5′ UTR are employed, called the internal ribosome entry sites (IRES). The structural basis for how an IRES positions the open reading frame of mRNA on the ribosome is not fully understood. Here, we present molecular structures of the ribosome-IRES complexes, which provide new structural insights into the mechanism of IRES-dependent translation.

69 Structure of the 80S initiation complex bound to histone H4 mRNA

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Translation initiation is a sophisticated process leading to ribosome assembly on the start codon. In eukaryotes, the canonical pathway requires multiple translation initiation factors (eIFs) to drive the assembly of the 40S ribosomal subunit at the 5′ cap (7 methylguanosine) structure of mRNAs, then a scanning step is used to find the correct AUG. Alternative mechanisms includes mRNAs structural elements located in the 5′ UTR of viral mRNA (IRES) that initiate translation with only a partial set of eIFs and without scanning. Recently, our lab showed that translation initiation of histone H4 mRNA is driven by a novel mechanism combining canonical features (cap-dependent translation) with viral strategy (lack of scanning and internal ribosomal entry site)[1]. Two structural RNA elements located in the coding region of H4 mRNA allow ribosome tethering on the AUG codon without any scanning step. In this way histones are massively produced during the S-phase of the cell cycle.

By using a novel pull-down method, we purified H4 mRNA-programmed translation initiation complexes from rabbit reticulocyte lysates[2] and determined their 3D structure by cryo-EM. The structure at ~ 9 Å of the 80S initiation complex of H4 mRNA provides details on its assembly on the AUG and how the scanning is prevented.

1. Martin et al., Mol Cell (2011) 41, 197-209.

2. Prongidi-Fix et al. Biochem J (2013) 449, 719-28.

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70 Redefining the Translational Status of 80S Monosomes

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During translation, mRNAs are decoded by fully assembled 80S ribosomes to produce proteins. Multiple ribosomes can assemble on a given transcript, resulting in polysomes. Such polysomes can be separated from 80S monosomes and free ribosomal subunits by gradient centrifugation. When analyzing these polysome profiles, many researchers commonly assume that only those mRNA molecules associated with polysomes are translationally active, whereas mRNA molecules associated with 80S monosomes are translationally inactive. This assumption may be based on data from rabbit reticulocytes, where incorporation of radiolabelled amino acids was observed to occur only in polysomal fractions and not in 80S monosome fractions (Warner, J.R., et al. *PNAS* 1963). Reticulocytes, however, predominantly synthesize α and β hemoglobin subunits, whose mRNAs likely evolved to be translated with extremely high efficiency in this cell type. In contrast, other cell types must express a much more complex protein repertoire, with the optimal concentration of some proteins being only one or two molecules per cell. Further, the open reading frames (ORFs) of mRNAs encoding very short proteins may only be long enough to accommodate a single ribosome. Finally, it has been proposed that nonsense-mediated decay (NMD) preferentially occurs during the first or pioneer round of translation when an mRNA might be expected to have a single bound ribosome.

We set out to directly determine the translational status of monosome-associated mRNAs. To do so, we isolated the monosome and polysome peaks from polysome profiles and performed ribosome footprinting on each subset. Our data clearly demonstrate that monosome-associated mRNAs are being actively translated. Interestingly, different classes of transcripts exhibit differential distributions of monosomes and polysomes across their ORFs. Finally, mRNAs encoding membrane proteins are significantly enriched in the monosome-associated gene set, suggesting they are preferentially translated by single ribosomes.

71 The β-actin mRNA zipcode controls β-actin translation driving spatially localized actin cytoskeleton remodeling and adherens junction assembly to regulate 2D & 3D epithelial structure and function

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RNA zipcodes are cis-acting elements controlling mRNA stability, transcript trafficking, and translation. Functionally mRNA zipcodes interact with the trans-factor Zipcode Binding Protein 1 (ZBP1) to stabilize their transcript and to inhibit translation during mRNA transport. Transcript targeting is stimulated by active RhoA and translation is initiated by Src phosphorylation of ZBP1. Importantly, epithelial cell-cell contact stimulates both RhoA and Src. Consequently, we observe mRNA zipcode dependent β-actin mRNA targeting and spatially localized transition at de novo epithelial cell-cell contact sites. As expected, perturbing β-actin mRNA zipcode/ZBP1 binding with β-actin mRNA zipcode antisense oligonucleotides delocalizes β-actin translation sites at de novo epithelial cell-cell contact sites. Incredibly, perturbing β-actin mRNA zipcode/ZBP1 binding with β-actin mRNA zipcode antisense oligonucleotides significantly inhibited adherens junction assembly at de novo epithelial cell-cell contact sites. In addition, masking β-actin mRNA zipcodes with antisense oligonucleotide treatment significantly inhibited barrier assembly during in vitro barrier permeability assays. Together these data demonstrate β-actin mRNA zipcodes spatially regulate translation to control actin cytoskeleton remodeling required for epithelial adherens junction assembly. As a consequence of regulating epithelial adherens junction assembly, the β-actin mRNA zipcode controls 2D epithelial tissue structure and barrier integrity. We further demonstrate the β-actin mRNA zipcode regulates 3D epithelial tissue structures. For example, time lapse imaging of 3D MDCK cyst cultures treated with β-actin mRNA zipcode antisense oligonucleotides demonstrate cells spontaneously popping into the organoid lumen. By contrast, time lapse imaging of 3D MDCK cyst cultures treated with β-actin mRNA zipcode sense oligonucleotides does not reveal any cells spontaneously popping into the organoid lumen. In addition, imaging 3D MDCK crypt culture expressing a dominant negative delta zipcode β-actin reporter construct reveals multi-layered organoids compared with single layered organoids observed for normal MDCK cells. In 2D culture, E-cadherin overexpression rescues the dominant negative epithelial adherens junction assembly phenotype cause by our delta zipcode β-actin reporter. Amazingly, β-actin mRNA zipcode antisense oligonucleotides block the E-cadherin overexpression rescue of our dominant phenotype demonstrating the critical role for this genetically encoded regulatory sequence in regulating 2D and 3D epithelial structure and function.

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72 Oxidative and alkylative RNA damage stall the translational machinery and induce No-Go Decay

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Nucleic acids are under constant assault from endogenous and environmental agents. The resulting chemical modifications can lead to mutagenesis and transmission of deleterious genotypes to successive generations. While understanding DNA damage has been an area of intense investigation for many years, it is increasingly appreciated that RNA damage, which is associated with a number of human pathologies including Alzheimer's Disease, also comprises an important component of the cell's response to nucleic acid stress. Moreover, cells possess robust mechanisms to cope with aberrant mRNAs containing features such as premature/missing stop codons (Nonsense/Nonstop Mediated Decay) or significant secondary structure (No-Go Decay). Therefore, we wondered whether chemically damaged mRNA might also have an adverse effect on ribosome function and be under the watch of mRNA surveillance pathways. Using a well-defined in vitro translation system, we systematically evaluated the effects of oxidative and alkylative damaged mRNA on decoding process. We generated ribosomal complexes harboring 8-oxoguanosine (8-oxoG) and O6-methylguanosine (O6-meG) at defined locations and examined their reactivity with aminoacyl-tRNAs (aa-tRNAs) and release factors. Surprisingly, both adducts resulted in dramatic decreases in the rate of peptide bond formation with cognate aa-tRNAs - ~.02 s^{-1} as compared to ~25 s^{-1} measured with control mRNAs - regardless of the position of the modification within the codon triplet. Similar to DNA replication, 8-oxoG and O6-meG adducts had a modest adverse effect on aa-tRNA selection accuracy. We observed a ~10-fold increase in the rate of peptide bond formation for near-cognate aa-tRNAs, but the rates remained more than 100-fold slower than those observed with intact complexes and cognate aa-tRNAs. Our data strongly suggest that 8-oxoG and O6-meG adducts stall the translational machinery. Supporting this, we also found that yeast with defects in mRNA turnover and No-Go Decay accumulated 8-oxoG-containing mRNAs *in vivo*. Together, our data support a mechanism by which chemically damaged mRNAs, like other classes of aberrant mRNAs, are recognized by the ribosome and marked for degradation. This in turn highlights the deleterious effects of mRNA damage on cellular fitness and the evolution of the cell's response to handle it.

73 The crystal structure and a catalytic mechanism of the twister ribozyme

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The twister ribozyme, recently discovered in the Breaker laboratory, is a novel small nucleolytic ribozyme that is widely disseminated in the genomes of bacteria and eukarya. We have solved the crystal structure of this ribozyme from *Oryza sativa* at 2.3 Å resolution.

The RNA adopts a novel compact fold based on a double pseudoknot structure, with the scissile phosphate at its center. All highly-conserved nucleobases form key structural elements including a guanine nucleobase that has its Watson-Crick edge directed towards the scissile phosphate. Mechanistic evidence supports a role for this nucleobase as either general base or acid in a concerted, general acid-base catalyzed reaction. The bell-shaped dependence of cleavage rate on pH is consistent with one of several highly conserved adenine nucleobases also participating in catalysis.

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74 Biological impact of transcripts terminated by HDV-like ribozymes

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The ribozymes first discovered in the human pathogen hepatitis delta virus self-cleave to process concatamers to unit-length RNA genomes during viral replication. The nested double-pseudoknot secondary structure of the ribozyme was previously used in bioinformatic searches to identify this class of self-cleaving ribozyme throughout nature. The distribution of these ribozymes fell to diverse genomic loci and their positions suggested multiple biological functions. Many ribozymes map to the 5′ ends of retrotransposons, leading to a model in which the ribozyme plays several roles in the retrotransposition cycle. Of particular interest is the ribozyme mapping to the R2 retrotransposable element in Drosophila. The predicted coding sequence for the first three amino acids of the R2 protein maps within the ribozyme structure. Previous work showed that these HDV-like ribozymes promote translation initiation both in vitro and in vivo, but the precise location of the first translated codon remained unknown. We are investigating the mechanism of translation initiation of ribozyme-terminated mRNAs. Our results indicate that the correct folding of the ribozyme core and composition of the transcript's 5′ end are important in promoting translation. Translation occurs in the absence of a cap, start codon, and poly-A tail. Taken together our data suggest that this translation is distinct from other cap-independent mechanisms. This example of a structured RNA family that can exist as a ribozyme and code for a protein challenges the delineation of ncRNA and mRNA boundaries.

75 Direct evaluation of tRNA aminoacylation status by the T-box riboswitch using intermolecular stacking and steric readout

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T-boxes riboswitches are gene-regulatory mRNA elements with which many Gram-positive bacteria sense and regulate amino acid availability [1]. T-boxes have two functional domains. Stem I recognizes the overall shape and anticodon of tRNA, while a 3′ domain evaluates its aminoacylation status, overcoming an otherwise stable transcriptional terminator if the bound tRNA is uncharged [2]. EF-Tu is an abundant cellular protein that binds aminoacyl-tRNAs with very high affinity. Thus, EF-Tu might be required for T-boxes to detect tRNA aminoacylation and make the transcriptional decision. Using a novel, facile method to prepare homogeneous aminoacyl-tRNA, we show that the *Bacillus subtilis GlyQS* T-box functions independently of any tRNA-binding protein *in vitro*. Comparison of aminoacyl-tRNA analogs demonstrates that the T-box detects the molecular volume of tRNA 3′-substituents. Calorimetry and fluorescence lifetime analysis of labeled RNAs shows that the tRNA acceptor end co-axially stacks on a helix in the T-box 3′ domain. This intimate intermolecular association, selective for uncharged tRNA, stabilizes the antiterminator conformation of the T-box. Based on these findings and our previously reported co-crystal structure of a T-box Stem I in complex with its cognate tRNA [3], we propose a structural and mechanistic model for the T-box riboswitches.

1. Green et al. 2010 FEBS Lett. 584:318.
2. Grundy et al. 1993 Cell 74:475.
3. Zhang and Ferré-D'Amaré, 2013 Nature 500:363.
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76 Riboswitch and small RNA-induced translation inhibition lead to mRNA degradation using different mechanisms

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To adapt efficiently to physiological changes, bacteria have evolved sophisticated molecular mechanisms to monitor specific cellular signals and to control gene expression accordingly. Riboswitches are molecular sensors regulating gene expression as a function of metabolite binding. The Escherichia coli btuB riboswitch, recognizing the coenzyme B12 metabolite, controls the expression of btuB encoding a cobalamin transporter (1). The riboswitch has been shown to act at the translational level by modulating the access of ribosomes to the ribosome binding site. Interestingly, a recent microarray analysis has suggested that the small RNA OmrB induced under osmotic stress could also be involved in btuB gene expression regulation (2). However, no mechanism has been proposed to explain how OmrB could regulate btuB expression.

In this study, we addressed the role of both the btuB riboswitch and the sRNA OmrB in the regulation of btuB expression and compared regulatory mechanisms employed by both non-coding regulators. By using lacZ fusions and Northern blot experiments, we have shown that both B12 and OmrB effectors negatively regulate btuB translation, leading to mRNA degradation. However, using various sizes of lacZ fusions, we determined that OmrB action relied on genetic elements that are different from those employed by the riboswitch. Moreover, in contrast to the riboswitch, Northern blot analyses performed with mutant strains indicated that OmrB regulation depends on the Hfq chaperone and the RNA degradosome to efficiently repress btuB expression. Surprisingly, in vitro experiments suggested that while OmrB recognizes the riboswitch expression platform, Hfq binds in the vicinity of the AUG start codon. These results put forward a mechanism where the binding of OmrB to the btuB mRNA directs the binding of Hfq nearby the AUG start codon, thus preventing ribosome access and ultimately leading to the inhibition of translation initiation.

This study shows for the first time that riboswitch and small RNA regulators can both be employed to target the same mRNA, thus revealing how RNA-based regulators can be assembled to modulate gene expression using different mechanisms.

(1) Nahvi et al, Chem Biol 2002 9:1043.
(2) Guillier and Gottesman, Nucleic Acids Res 2008 36:6781.

77 Ligand binding by the tandem glycine riboswitch depends on aptamer dimerization but not double ligand occupancy

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The glycine riboswitch predominantly exists as a tandem structure, with two adjacent, homologous ligand-binding domains (aptamers), followed by a single expression platform. The recent identification of a leader helix that eliminates cooperativity between the aptamer domains has reopened the debate over the purpose of the tandem structure of the glycine riboswitch. We sought to directly examine glycine binding in each ligand-binding site independently in order to understand the role of each aptamer in glycine-binding and riboswitch activity. We developed an equilibrium dialysis-based assay that monitors glycine binding in each aptamer with a series of binding-site mutants. We used this assay and a series of mutations that disrupt the dimer interface to probe how aptamer dimerization impacts ligand binding by the tandem glycine riboswitch. We show that, while the wild-type tandem riboswitch binds two glycine equivalents, both individual aptamers are still capable of binding glycine when the other aptamer is inactivated. Intriguingly, glycine binding by aptamer-1 is much more sensitive to dimerization than glycine binding by aptamer-2. However, monomeric aptamer-2 shows dramatically weakened glycine-binding affinity. In addition, dimerization of the two aptamers *in trans* is dependent on glycine binding in at least one aptamer. We propose an updated model for tandem riboswitch function that is consistent with these results, wherein aptamer-1 provides a scaffold for aptamer-2 folding, and ligand binding is linked to aptamer dimerization, which controls the expression platform.

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78 Bacterial riboswitches cooperatively bind Ni^{2+} or Co^{2+} ions and control expression of heavy metal transporters

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Most riboswitches selectively bind small metabolites and control the expression of genes whose protein products function in biochemical pathways related to their ligands. In rare instances, distinct riboswitch classes have been found that respond to changing concentrations of ions such as Mg^{2+} and F^-. Here we report the discovery, validation, and structural details of a novel riboswitch class whose members selectively and tightly bind either Ni^{2+} or Co^{2+}. Cation binding by "NiCo" riboswitches is cooperative, and an RNA construct derived from the bacterium *Clostridium botulinum* exhibits affinities in the low micromolar range for these two heavy metal ions. Moreover, in Bacillus *scindens*, Ni^{2+} is shown to trigger changes in the levels of a NiCo-associated mRNA transcript coding for a putative heavy metal ion transporter. An atomic-resolution structural model of a NiCo riboswitch aptamer bound to four Co^{2+} ions reveals a network of molecular contacts that explain how this riboswitch achieves cooperative binding between adjacent sites. These findings reveal that many bacteria use NiCo riboswitches to detect and respond to toxic levels of specific heavy metal ions.

79 U11 snRNP binding to 3′-UTR leads to nuclear retention

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Eukaryotic cells have developed elaborate mechanisms to prevent the inopportune export of mRNAs into the cytoplasm. The resulting nuclear retention can enable RNA degradation through otherwise kinetically unfavourable nuclear degradation pathways (Doma and Parker, 2007). Alternatively, it offers a mechanism to regulate coordinated release of mRNAs into the cytoplasm (Prasanth et al., 2005). Here, we describe a novel mechanism for mRNA nuclear retention involving components of the minor spliceosome. Previously, we have identified a splicing enhancer (termed USSE) located in the 3′-UTR of the U11/U12-65K gene, which functions as a part of feedback loop regulating the levels of the 65K protein (Verbeeren et al., 2010). This splicing enhancer contains two closely spaced 5′-splice site like binding sites for U11 snRNP. Recognition of the USSE element by U11 (or U11/U12 di-snRNP) does not lead to splicing, but rather activates an upstream U2-type splice site and generates an mRNA isoform with a 2 kb 3′-UTR, which also includes the USSE element. In the absence of U11 binding, a shorter (200 bp) 65K mRNA isoform that excludes the USSE element is formed.

Using single-molecule FISH and cellular fractionation, we show that 65K long mRNA isoforms with USSE element are retained in the nucleus and do not form a clear focus but are rather distributed throughout the nucleoplasm. Replacement of the USSE element with an exonic splicing enhancer leads to export of the 65K long isoform, indicating that U11 binding to the 3′-UTR is necessary for the retention. Through morpholino blocking experiments, RT-PCR and RNase protection, we find that only 50% of the long isoform undergo cleavage/polyadenylation. The defect in 3′-end processing is exacerbated by U1 binding to an evolutionarily conserved binding site upstream of the poly(A) site. Together, our results support a regulatory pathway in which unproductive binding of U11 snRNP prevents mRNA export and, additionally, compromises 3′-end processing to regulate the cytoplasmic levels of U11/U12-65K mRNA and the cellular levels of U11/U12 di-snRNP needed for intron recognition.

References:
Doma and Parker (2007). Cell *131*: 660-668.
Prasanth et al (2005). Cell *123*, 249-263.
Verbeeren et al (2010). Mol Cell *37:* 821-833

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80 Developmentally-Regulated Elimination of Damaged Nuclei Involves a Chk2-Dependent Mechanism of mRNA Nuclear Retention

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The faithful execution of embryogenesis relies on the ability of organisms to respond to genotoxic stress and to eliminate defective cells that could otherwise compromise viability. In syncytial-stage *Drosophila* embryos, nuclei with excessive DNA damage undergo programmed elimination through a yet poorly understood process of nuclear fallout at the midblastula transition. We show that this involves a Chk2-dependent mechanism of mRNA nuclear retention that is induced by DNA damage and prevents the translation of specific zygotic mRNAs encoding key mitotic, cytoskeletal and nuclear proteins required to maintain nuclear viability. For *histone* messages, we show that nuclear retention involves Chk2-mediated inactivation of the *Drosophila* Stem Loop Binding Protein (SLBP), the levels of which are specifically depleted in damaged nuclei following Chk2 phosphorylation, an event that contributes to nuclear fallout. These results reveal a new layer of regulation within the DNA damage surveillance systems that safeguard genome integrity in eukaryotes.

81 Identification of mRNA export targets of SR protein family members reveals variation in cargo and shuttling.

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The SR protein family comprises seven regulatory RNA-binding proteins (SRSF1-SRSF7) that play essential roles in pre-mRNA splicing. Some family members also participate in post-splicing events, such as mRNA export and translation. To systematically investigate role of individual SR proteins in mRNA export *in vivo*, we generated pluripotent murine P19 cells that express GFP-tagged SR proteins from bacterial artificial chromosomes (BACs) at physiological levels. Endogenous expression is crucial, because even a slight over-expression can change pre-mRNA splicing pattern dramatically. To measure the capacity of individual SR proteins to shuttle between nucleus and cytoplasm, a prerequisite for mRNA export function, we established a quantitative shuttling assay. We observed that all SR proteins shuttle in P19 cells, however with different shuttling capacities. This contrasts with HeLa cells, where SRSF2 and SRSF5 do not shuttle, raising the interesting possibility that shuttling behavior reflects cell-type specific functions of SR proteins. The shuttling capacities of individual SR proteins correlate with the length of the phosphorylated RS domain, their interaction with the mRNA export factor NXF1 and their presence in translating polyribosomes.

To identify target mRNAs that depend on individual SR proteins for their export to the cytoplasm, we performed knockdown and cell fractionation of P19 cells, followed by microarrays and/or RNA-Seq. Interestingly, the number and expression level of export targets also correlates with the shuttling capacities of individual SR proteins. Furthermore, the export targets of high shuttling SR proteins are functionally related and enriched in particular splice-isoforms. Our data reveals differences in shuttling capacities of individual SR proteins, showed that individual SR proteins contribute differently to mRNA export in P19 cells and identified mRNAs that depend on individual SR proteins for their export in the cytoplasm. This suggests that high shuttling SR proteins may play an active role in the export and perhaps translation of specific transcript isoforms. We speculate that enhanced shuttling and mRNA export by SR proteins in pluripotent P19 cells thereby regulate different cell fates compared to HeLa cells.

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82 How mRNAs are Localized to the Endoplasmic Reticulum

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Almost all eukaryotic cells use mRNA localization to establish spatial control of protein synthesis. One of the best studied examples is the targeting and anchoring of mRNAs encoding secreted, organellar, and membrane-bound proteins to the surface of the endoplasmic reticulum (ER). We have determined that a large fraction of these mRNAs are targeted and then subsequently anchored to the ER by mRNA receptors, such as p180. We have also identified cis-elements that are required for the p180-dependent anchoring of mRNAs, and have found that in certain cases, these elements map to transmembrane domain coding regions. Moreover, we have found that many mRNAs that were thought to be translated by free ribosomes (i.e., not bound to the ER), are in fact translated on the surface of the ER. One example is the mRNAs that encode tail-anchored ER-resident proteins. These mRNAs are thought to be free-floating, while their encoded polypetides are post-translationaly targeted to the ER by the GET system of chaperones. Interestingly, we show that the initial ER-targeting of this class of mRNAs is dependent on translation and independent on certain components of the GET system. However, the maintenance of these mRNAs on the surface of this membrane is translation-independent. We also found that mRNAs encoding tail-anchored mitochondrial-resident proteins, are free floating in the cytoplasm, but can be redirected to the ER by altering their transmembrane domain coding region. Our data suggests that ER-targeting of these mRNAs acts as an alternative pathway for localizing tail-anchor proteins to the ER. Furthermore, our results explain why the GET system is not essential for viability, despite the fact that some of its target substrates are.

83 Pheromone-induced transport of the yeast MFA2 mRNA to the mating projection is mediatedby specific RNP granules

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Association of mRNAs with large complexes such as P bodies or stress granules plays a pivotal role in regulating their translation and decay. Little is known about other possible functions of these assemblies. Exposure of haploid yeast cells, carrying mating type a, to alfa pheromone stimulates a pheromone synthesis, encoded by MFA2. This treatment also stimulates polarized growth towards the mating partner, resulting in a shmoo projection. Here we show that, in response to pheromone, MFA2 mRNA is transported to the shmoo tip, where it is subsequently translated. Localization and translation of MFA2 mRNA in the shmoo tip depends the assembly of MFA2 mRNA with structural components of P bodies. Interestingly, two kinds of granules are involved. Both contain some canonical P body proteins, yet they differ in size, localization, motility and sensitivity to cycloheximide. MFA2 mRNA found in large, immotile, cycloheximide-sensitive granules is translated locally in the shmoo tip. Local accumulation of these granules in the shmoo tip is likely to play a role in mating, as defects in their assembly result in a sterile phenotype.

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84 In vitro biogenesis of an mRNA-transport complex from yeast

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Messenger RNA localization is a widespread mechanism to temporally and spatially control gene expression. The formation of motor-dependent transport particles is often preceded by nuclear complexes, which are required to assure correct localization within the cytoplasm. One example for a nuclear transit of mRNPs is the *ASH1* mRNA in yeast. Motile *ASH1* mRNPs consist of She2p, and the cytoplasmic proteins She3p and Myo4p. She2p and She3p form a highly specific, synergistic complex with the *ASH1* mRNA. The strictly nucle(ol)ar protein Loc1p is required for efficient cytoplasmic *ASH1* mRNA localization. To date, it is unknown how the Loc1p-containing pre-mRNP is assembled and how the complex is reorganized in the cytoplasm to form motile transport complexes.

Here we show that Loc1p forms a specific and stable ternary complex with She2p and cis-acting localization elements of the *ASH1* mRNA (1). Since Loc1p is restricted to the nucleus, complex reorganization is necessary to remove it from the mRNP and to form mature cytoplasmic transport complexes. Our competition and in vivo experiments demonstrate that the cytoplasmic She3p outcompetes Loc1p from the complex (1). Since She3p constitutively interacts with Myo4p the complex joining of She3p also brings the motor into the mRNP. In reconstitution experiments we determined the mature *ASH1* mRNP to consist of two cis-acting RNA-localization elements, one She2p tetramer, two She3p dimers and two Myo4p motors (2). We further found that the RNA itself is dispensable for motor activation and that the interaction between She2p and She3p induces processivity. Reconstituted complexes show directional movement in single-particle motility assays with properties comparable to in vivo observations (2). For the first time we are able to understand mRNP assembly at the molecular level all the way from the first nuclear events to the processive mRNA transport in the cytoplasm.

(1) Niedner, Müller et al. & Niessing, *Proc. Natl. Acad. Sci USA* 110: E5049-58 (Dec. 9. 2013)

(2) Heym, Zimmermann et al & Niessing, *J. Cell Biol*. 203: 971-84 (Dec. 23. 2013)

85 *In vitro* reconstitution of the mammalian cleavage and polyadenylation specificity factor (CPSF)

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The cleavage and polyadenylation specificity factor (CPSF) is a multi-protein complex crucial for 3′ processing of mammalian pre-mRNA: CPSF contributes to cleavage and polyadenylation site selection by binding to the conserved polyadenylation signal AAUAAA in the 3′ UTR of nascent pre-mRNAs; its 73 kDA subunit is considered the endonuclease responsible for the cleavage reaction; and CPSF mediates efficient poly(A) tail addition by recruiting the poly(A) polymerase to the site of polyadenylation. Mammalian CPSF was initially characterized as a complex of four subunits, CPSF-30k, -73k, -100k and -160k. Later, hFip1 and WDR33 were reported as (putative) subunits of CPSF. However, the actual composition of mammalian CPSF remained elusive, as the factor was not reconstituted from recombinant proteins.

Using the MultiBac baculovirus expression system, we have produced recombinant CPSF active in AAUAAA-specific RNA binding and polyadenylation. A stable complex containing all six subunits was obtained, but activity assays revealed that CPSF-73k and -100k are dispensable for AAUAAA-dependent polyadenylation. Presumably, the function of these two proteins is limited to the cleavage step. CPSF-160k and -30k form a stable, well-behaved subcomplex, and hFip1 and WDR33 also associate with each other.

Dependence of AAUAAA-specific polyadenylation on WDR33 suggests that this polypeptide might recognize the AAUAAA signal. In order to examine a potential role of WDR33 in AAUAAA recognition, we used the photoreactive nucleotide-based CLIP method (PAR-CLIP) to map binding sites of WDR33 *in vivo*: After RNA labeling with 4-thiouridine, WDR33 crosslinked to RNA was immuno-precipitated, RNA fragments were extracted and deep-sequenced, and the dataset was uploaded to the annotation server at www.clipz.unibas.ch and analyzed computationally. A strong enrichment of WDR33 binding near poly(A) signals (A[A/U]UAAA) was obtained with little background over the rest of the transcripts. Analysis of the diagnostic T-to-C mutations introduced when the reverse transcriptase encounters a crosslinked 4-thiouridine indicates binding of WDR33 on and in the very close vicinity of the poly(A) signal. Compared to the CLIP data previously obtained with the other five CPSF subunits, including CPSF-160k, WDR33 has the highest specificity for the hexamer motif.

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86 An mRNA alternative polyadenylation regulation network promotes stem cell self-renewal

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mRNA alternative polyadenylation (APA) is an important mechanism for post-transcriptional for gene regulation and is highly regulated in development and disease. However, the regulatory mechanisms and functional consequences of APA remain poorly understood.

Here we show that an mRNA 3′ processing factor, Fip1, is essential for embryonic stem cell (ESC) self-renewal and somatic cell reprogramming. Fip1 promotes stem cell maintenance, in part, by activating the ESC-specific APA profiles and thereby the optimal expression of a specific set of genes, some of which encode critical self-renewal factors. Fip1 level and the Fip1 APA program change during ESC differentiation and they are restored to an ESC-like state during somatic reprogramming. Mechanistically, we provide evidence that the specificity and the mode of Fip1-mediated APA regulation depend on multiple factors, including Fip1-RNA interactions and the distance between alternative polyadenylation sites. Finally we have identified additional factors as a part of APA regulation network that plays essential roles in ESC functions.

Together, our data reveals a novel post-transcriptional mechanism regulating stem cell self-renewal and pluripotency. Furthermore, these findings provide new mechanistic insights into APA regulation in development and establish an important function for APA in cell fate specification.

87 The mTOR pathway regulates alternative cleavage and polyadenylation for transcriptome-wide control of 3′UTR length

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Mammalian/mechanistic target of rapamycin (mTOR) is crucial for cell proliferation/growth by regulating translation. Here, we investigated the mTOR-activated transcriptome features at a nucleotide resolution to explore the mTOR function beyond translation and discovered widespread 3′untranslated region (3′UTR) shortening in mRNAs. Using genetic or chemical modulations of mTOR activity in cells or terminally differentiated mouse tissues, we provide evidence that mTOR regulates alternative cleavage and polyadenylation (ApA) process and alters the 3′UTR length of mRNAs independent of cell proliferation. Strikingly, mTOR rapidly reprograms multiple ApA factors to promote proximal polyadenylation in 3′UTR. These findings identify ApA as a new targeting mechanism of mTOR, expanding its function beyond translation to post-transcriptional gene regulation. Furthermore, they provide molecular explanation of how proximal polyadenylation signals (PASs) can be preferred over distal PASs by activating mTOR.

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88 Genome-wide Investigation of Poly(A) Tail Length and 3´ End Modifications by TAIL-seq

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Global investigation of the 3´ extremity of mRNA (3´-terminome), despite its importance in gene regulation, has not been feasible due to technical challenges associated with homopolymeric sequences and relative paucity of mRNA. We here develop a method, TAIL-seq, to sequence the very end of mRNA molecules. TAIL-seq allows us to measure poly(A) tail length at the genomic scale. Median poly(A) length is 50-100 nt in HeLa and NIH 3T3 cells. Poly(A) length correlates with mRNA half-life, consistent with the idea that deadenylation is generally a critical step in mRNA decay. Surprisingly, we discover widespread uridylation and guanylation at the downstream of poly(A) tail. The U tails are generally attached to short poly(A) tails (<25 nt), while the G tails are found mainly on longer poly(A) tails (>40 nt), implicating their generic roles in mRNA stability control. TAIL-seq is a potent tool to dissect dynamic control of mRNA turnover and translational control, and to discover unforeseen features of RNA cleavage and tailing. In this presentation, we will show our recent results on the enzymology and regulation of mRNA uridylation.

89 The Histone Locus Body functions to increase the rate of 3'end formation and couple transcription and processing in vivo

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The replication-dependent histone mRNAs are the only known cellular mRNAs that are not polyadenylated, ending instead in a conserved stemloop. The genes for the five histone proteins are clustered in the genome and coordinately regulated. The histone locus body (HLB), a nuclear body containing factors required for histone gene transcription and processing, is localized to the histone gene locus in metazoans. Two HLB components, U7snRNP and FLASH, are required for histone pre-mRNA processing. Histone mRNA processing is efficient; RNA polymerase II pauses just 3′ of the end of the mRNA allowing processing followed by termination. In *Drosophila*, all of the histone genes contain cryptic polyadenylation sites 3′ of the stemloop. Mutants in processing factors, such as FLASH, produce polyadenylated histone mRNAs, which support development until at least 3rd instar larvae. We analyzed a series of FLASH transgenes for their ability to rescue viability and histone pre-mRNA processing in a FLASH mutant. The first 150 aa of FLASH (844 aa) forms a complex with Lsm11, a U7 snRNP specific protein, and together they recruit the histone cleavage complex (HCC) composed of a subset of polyadenylation factors. U7 snRNP and FLASH, but not the HCC, are present in the HLB outside of S-phase, suggesting that recruitment of the HCC to form an active U7 snRNP is cell-cycle regulated. The last 100 aa of FLASH are required for localization to the HLB. A mini-FLASH containing just these three domains rescues the FLASH mutant. A mutation in FLASH (FLASH^{LDIY71}) that disrupts HCC binding has <10% activity in vitro. Surprisingly expression of FLASH^{LDIY71} protein restored viability and the majority of the mRNA was properly processed. When the FLASH^{LDIY71} protein was mislocalized there was no processing. Hence this defective protein must be concentrated in the HLB to function *in vivo*. Mislocalizing wild-type FLASH or U7snRNP results in the same phenotype as the FLASH^{LDIY71} mutant. Transcription continues into the 3′ intergenic region, as monitored by in situ hybridization. There is accumulation of some polyadenylated histone mRNA and unprocessed pre-mRNA, but the mRNA was primarily properly processed. We conclude that the HLB coordinates processing and transcription termination.

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90 Changing nature of the CTD structure

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RNA polymerase II associates with a large number of enzymes and protein/RNA-binding factors through its C-terminal domain (CTD). The CTD consists of multiple tandem repeats of the heptapeptide consensus $Y_1S_2P_3T_4S_5P_6S_7$ which is specifically phosphorylated depending on the transcription cycle event. The CTD does not possess any rigid structure, it is represented by an ensemble of multiple conformations. Equilibrium between different conformations can be shifted by reversible post-translational modifications and the subsequent binding with protein factors recognizing modification.

Flexibility and repetitive nature of the CTD makes structural studies a challenging task. Current structural information is limited to proteins bound to a few CTD repeats but the full-length CTD structure and the requirement for the repetitiveness of the CTD are poorly understood.

In order to describe overall structure of the CTD and transitions between differently bound states, we have created model system mimicking the full-length CTD with specific phosphorylation pattern. By combining this system with advanced nuclear magnetic resonance (NMR) methods (PRE, spin relaxation, NOEs) and small angle X-ray scattering (SAXS) we follow changes in structural behavior of both the CTD and respective binding factor at atomic level resolution.

Using this approach we visualized for the first time how the structure of full-length CTD is modulated upon binding with multiple copies of CTD-interacting domain (CID) of Rtt103, a subunit of yeast Rat1 exonuclease complex. Our study shows that one CID is accommodated on 2 repeats of the CTD heptadepeptide and the strength of CID-CTD interaction is affected by the CTD sequence adjacent to its minimal binding site. Resulted ensemble of structures indicates that CTD retains its highly flexible character upon binding, no fixed contact between CIDs is formed.

92 Processing of HIV-1 short transcripts into miRNA

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Retroviruses, like the human immunodeficiency virus type 1 (HIV-1), are RNA viruses that replicate using a DNA intermediate. A growing body of evidence is accumulating that retroviruses are able to produce microRNAs (miRNAs). To do this, retroviruses have to use alternative sources for their miRNA precursors so that the primary RNA transcript is not cleaved and replication is not inhibited by this processing.

The HIV-1 transacting responsive (TAR) RNA element has been suggested as a possible source for miRNAs. TAR is present as a hairpin at the 5′ and 3′ end of the RNA genome. Recruitment of the viral transcription activator protein Tat and the cellular pTEFb complex to the 5′ TAR hairpin is needed to activate transcription elongation.

We characterized the TAR derived miRNAs in detail by using the SOLiD ultra-deep sequencing technique. Approximately 1% of the HIV-1 sequences were derived from the 3′ side (miR-TAR-3p) and 0.03% from the 5′ side (miR-TAR-5p) of the TAR stem. This indicates that miR-TAR-3p is the functional miRNA, which is loaded into the RISC complex and miR-TAR-5p is the passenger strand.

The existence of these TAR miRNAs was confirmed by northern blot analysis. Furthermore, the presence of short TAR RNAs (~58 nt) was revealed. Using a Dicer knockdown cell line, we demonstrated that this short TAR transcript is being processed by Dicer into miRNA. Analysis of 5′ or 3′ TAR deleted HIV-1 variants revealed that these TAR RNAs and miRNAs are produced exclusively from the TAR element present at the 5′ end of the viral transcripts. Furthermore we also could show that non-processive transcription, yielding short transcripts, is sufficient to produce these TAR products.

Our results demonstrate that HIV-1 can prevent the cleavage of its full length RNA genome, by using the short transcripts as source for miRNA.

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93 Xrn1-resistant RNA structures present in the 3′ untranslated region of Flaviviruses

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During infections caused by arthropod-borne Flaviviruses, high levels of 300-500nt long fragments of the viral 3′UTR accumulate within infected cells. Through still incompletely understood mechanisms these subgenomic flaviviral RNAs (sfRNAs) play a direct and decisive role in disease progression. sfRNAs are formed due to the presence of conserved Xrn1-resistant RNA structures (xrRNAs) located at the 5′ end of the viral 3′UTR. These structures blockade 5′->3′ degradation by the cellular exonuclease Xrn1, protecting downstream (3′) RNA elements from further decay. The truncated sfRNA products that remain determine the virulence of tick and mosquito-borne flaviviruses.

To further understand the operation of xrRNA structures we have pursued experimentation geared toward determining the biochemical and biophysical properties that impart Xrn1-resistance. These studies have revealed Xrn1-resistance is conferred entirely by a structured RNA element, is not the result of chemical ‘mistep’ of the exonuclease and is demonstrated by discrete and portable RNA structures of as little ~60nt’s. Together these findings guided determination of the three-dimensional structure of a fully-operational xrRNA from Murray Valley Encephalitis. The x-ray crystal structure of this RNA reveals that it adopts a previously unobserved three-dimensional topology that includes threading of the 5′-end of the RNA through a ring structure formed by a conserved three-way junction. The unique architecture of this RNA rationalizes the unidirectional-resistance it demonstrates against the otherwise efficient 5′->3′ helicase activity of Xrn1, presenting the exonuclease with problem similar to that of untying a slipknot. Mutation of conserved nucleotides within difefrent xrRNA structures disrupts their operationin vitro and mutation of xrRNAs within the 3′UTRs of mutant West Nile viruses leads to a loss of sfRNA production in human cells. Together these discoveries bring focus upon an important class of RNA structures at the center of threatening disease.

94 Mechanism of regulated RNA cleavage during stress and immune surveillance by kinase-linked receptors RNase L and Ire1

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Mammalian cells respond to homeostatic imbalance and infections by activating two sister receptors, Ire1 and RNase L. They belong to the family of more than 500 protein kinases and contain the typical protein kinase domain. Yet, in contrast to conventional protein kinases that signal by protein phosphorylation, Ire1 and RNase L rely on regulated endonucleolytic RNA cleavage mediated by their unique C-terminal KEN domains. RNA cleavage by Ire1 serves for splicing out an unconventional intron from the transcription factor XBP1-encoding mRNA to activate unfolded protein response (UPR), as well as to decay specific mRNA and micro-RNA molecules in a pathway called regulated Ire1-dependent decay (RIDD). RNase L is a close homologue of Ire1, however it controls an entirely different mammalian program: RNase L cleaves intracellular viral and self-RNA to mediate the interferon (IFN) response.

Our work reveals structures and mechanisms of precise regulation and RNA sequence recognition by these receptors. These findings further our understanding of the IFN response and RIDD, and provide a structural basis for designing small molecules as molecular probes and modulators of the IFN action, and promising adjuvants for treating infections and inflammatory diseases.

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95 The sense and antisense of toxic RNA in neurodegeneration – human iPSC models to study misregulated RNA processing in *C9ORF72* ALS/FTD

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"Toxic" RNA transcribed from expanded tri-, tetra-, penta- or hexanucleotide repeats accumulates as nuclear RNA foci and is implicated in many degenerative disorders of the neuromuscular system. A recent prominent example is the discovery of an expanded GGGGCC repeat in the first intron of the *C9ORF72* locus as the most frequent genetic mutation underlying amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). Mounting evidence suggests an important toxic gain-of-function of the repeat RNA, although the molecular basis is only beginning to emerge. We hypothesize that the accumulation of RNA foci from either the sense strand, the antisense strand, or a combination of both strands directly causes gene expression changes that ultimately lead to the premature death of motor neurons in ALS. *C9ORF72* expansion-specific transcriptome changes have been reported by us and others and treatment with antisense oligonucleotides (ASOs) can reduce nuclear RNA foci and rescue some gene expression changes. Numerous RNA-binding proteins (RBPs) have been suggested to bind *C9ORF72* repeat RNA foci, but direct functional evidence for any of these candidates is lacking. Critically, many current approaches are fundamentally limited by noise in gene expression and the fact that only a fraction of C9-ALS patient cells have RNA foci detectable by fluorescence *in situ* hybridization (FISH). To more definitively answer the question whether accumulating RNA alone is causative of transcriptome changes we have generated novel cellular model systems and analysis methods. To eliminate the influence of inter-individual genetic variation, we have generated stable iPSC lines expressing $(GGGGCC)_{80}$ constructs, resulting in nuclear RNA foci. This allows us to compare cells harboring the repeat expansion to otherwise isogenic control cells. We further combined RNA FISH with fluorescence activated cell sorting (FACS) to separate C9-ALS patient cells into fractions containing sense and/or antisense RNA foci or are foci-negative. RNAseq of these subpopulations alongside single-cell RNAseq of iPSCs and iPSC-derived motor neurons reveals specific gene expression changes, which will be critical in the design of therapeutic strategies as well as for the development of disease biomarkers.

96 Identification of genes in trinucleotide repeat RNA toxicity pathways in *C. elegans*

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Myotonic dystrophy disorders are caused by expanded CUG repeats in non-coding regions. To reveal mechanisms of CUG repeat pathogenesis we used *C. elegans* expressing CUG repeats to identify gene inactivations that modulate CUG repeat toxicity. We identified 15 conserved genes that function as suppressors or enhancers of CUG repeat-induced toxicity and modulate formation of nuclear RNA foci by CUG repeats. These genes regulated CUG repeat-induced toxicity through distinct mechanisms including RNA export and RNA clearance, suggesting that CUG repeat toxicity is mediated by multiple pathways. A subset is shared with other degenerative disorders. The nonsense-mediated mRNA decay (NMD) pathway plays a conserved role regulating CUG repeat RNA transcript levels and toxicity, and NMD recognition of toxic RNAs depends on 3′UTR GC nucleotide content. Our studies suggest a broader surveillance role for NMD where variations in this pathway influence multiple degenerative diseases.

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97 Dysregulation of microRNA bioprocessing in neurodegeneration

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Regulatory RNAs are fundamental in the normal brain and were suggested as a mechanism of neurodegeneration. We explored the causes and consequences of microRNA dysregulation in several forms of amyotrophic lateral sclerosis (ALS), by employing patient specimen, molecular biology approaches and mouse genetics. Our study reveals that microRNA expression is globally downregulated in human ALS motoneurons. Decreased activity of the Dicer-complex is a primary reason for loss of microRNA expression in ALS. Accordingly, experimental loss of Dicer1 and microRNAs activity in mice resulted in motoneuron degeneration and in denervation- dependent muscle atrophy, which are hallmarks of ALS. Furthermore, potentiating Dicer complex activity by a small molecule agonist recovers pre-miRNA processing and ameliorates neuromuscular deterioration in a classic mouse ALS model. Finally, we characterize the molecular pathways for control of microRNAs in the brain and show that Dicer activity is regulated post-translationally by stress signaling cascades. This study suggests a microRNA-based molecular mechanism that is involved in neurodegeneration and a framework for understanding how canonical microRNA maturation may be regulated by stress in chronic diseases.

98 A Novel Role for the Arginine Methyltransferase CARM1 in Nonsense Mediated Decay: Implications for Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is amongst the leading genetic causes of infant deaths and is characterized by specific degeneration and loss of spinal cord motoneurons. It is caused by the disruption of the "survival of motor neurons" gene (SMN1). Recently we uncovered an increase of CARM1 expression in SMA. We decided to investigate the impact of increased CARM1 and the massive alteration of splicing in SMA and the fact that CARM1 can regulate this mechanism. Thus, our objective was to identify splicing targets that are misregulated by CARM1 in the pathology.

While pursuing splicing targets regulated by CARM1, we uncovered a completely novel function for CARM1 as a regulator of nonsense-mediated mRNA decay (NMD). We have identified numerous genes whose decay is determined through CARM1's regulation of NMD. Among these targets we identified USPL1, a gene which has been found to be misspliced in SMA. The missplicing of USPL1 results in a variant harbouring a PTC and knockdown of the main effector of NMD, UPF1, confirm the variant as a NMD target. We have also been able to demonstrate the important role of CARM1 in the regulation of NMD, through RNAi and rescue experiments, for a number of well-established NMD targets. By employing the widely used β-globin NMD reporter, as well as tethering assays, we have further accumulated evidence strongly suggesting a direct role for CARM1 in the NMD mechanism. Coimmunoprecipitation experiments show an interaction between CARM1 and both UPF1 and UPF2, but not with UPF3 or components of the EJC, suggesting CARM1 may actually play a role in the early activating steps of NMD. Preliminary experiments using RIPs support this model and show that less UPF1 is recruited to a PTC-containing NMD reporter in the face of reduced CARM1 levels.

Finally, using data from our preliminary screens we continue to identify more NMD targets regulated by CARM1, that are also misregulated in SMA. Altogether our work not only reveals an additional, novel level of regulation in the complex NMD and splicing processes, but also demonstrates the importance of CARM1 in the disease pathology of SMA.

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99 The Fragile X Mental Retardation Protein regulates mRNA metabolism during corticogenesis affecting circuitry in the developing cortex

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Deficiencies in Fragile X Mental Retardation Protein (FMRP) lead to the most common cause of inherited intellectual disability, Fragile X Syndrome (FXS), with symptoms manifesting during infancy and early childhood. Here we show that FMRP regulates the positioning of neurons in the cortical plate during embryonic development, affecting their multipolar-to-bipolar transition (MBT). We identified mRNAs, crucial for MBT, as FMRP-regulated targets in embryonic brain. Furthermore, spontaneous network activity and high-resolution brain imaging (MRI) revealed defects in the establishment of neuronal networks at very early developmental stages, further confirmed by an unbalanced excitatory/inhibitory network. Our findings highlight the critical role of mRNA metabolism in the developing cerebral cortex and might explain some of the clinical features observed in patients with FXS such as alterations in synaptic communication and neuronal network connectivity.

100 The oncogenic kinase NPM-ALK-dependant repression of miR150 level promotes lymphoma cells growth

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Anaplastic Large cell Lymphoma (ALCL) is a rare type of aggressive lymphoma arising from an abnormal proliferation of T-lymphocytes. ALCL are divided into two sub-groups (ALCL-ALK- and ALCL-ALK+) depending on the expression of the ALK protein (Anaplastic Lymphoma Kinase). ALK is a tyrosine kinase receptor not present in normal lymphocytes. The abnormal expression of the ALK observed in ALCL can be caused by gene amplification or chromosome translocation such as t(2;5)(p23;q35) leading to the fusion of the nucleophosmine gene (NPM) to the ALK gene. The resulting fusion protein, NPM-ALK is constitutively active and induces T-cell lymphocytes transformation. We and others showed that some of the effectors of the NPM-ALK signaling are microRNAs. NPM-ALK modulates the expression of these microARNs and this contributes to the proliferative, cell survival and anti-apoptotic properties of the oncogenic kinase. We generated in the lab, a transgenic mouse model expressing the NPM-ALK protein in an inducible manner. These mice develop lymphomas upon NPM-ALK expression. Using micro-arrays we showed that miR150 is one of the most down-regulated genes in the mice with NPM-ALK+ lymphoma compare to the wild-type control mice. This microRNA is known for its role in hematopoietic cell lineage differentiation. We showed, using cell line models of human ALCL-NPM-ALK+ , that NPM-ALK negatively regulates the expression of miR150 at the transcription level by promoting the methylation of the miR150 gene. We demonstrated that this action of NPM-ALK involves the transcription factor Stat3 (Signal Transducer and Activator of Transcription-3) and Dnmt1 (DNA methyltransferase protein 1). Furthermore, we reported that, as a consequence of miR150 down-regulation by NPM-ALK, ALCL-ALK+ cell lines overexpress the c-myb protein, a miR150 target. C-myb is a transcription factor promoting cell proliferation. Interestingly, we observed that the transfection of miR150 mimic molecules inhibits the growth of xenografted ALCL-NPM-ALK+ tumors in mice. Finally, we showed that miR150 is down-regulated in human ALCL biopsies. Our work has clearly demonstrated that, within to the range of molecules targeted by NPM-ALK, miR150 is a key factor and that its downregulation is crucial for the ALCL-NPM-ALK+ lymphomas growth.

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101 Oncogenic properties of the RNA-binding protein UNR: Targets in Melanoma

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Upstream of N-Ras (UNR/CSDE1) is a conserved RNA binding protein involved in mRNA regulation at the levels of translation and stability. Here we provide evidence that UNR shows strong oncogenic properties using melanoma as a model system, and investigate the molecular mechanisms involved. UNR is over-expressed in melanoma cell lines compared to non tumoral melanocytes, as well as in malignant lesions from patients. UNR depletion reduces the oncogenic capacities of melanoma cells, such as anchorage-independent growth and clonogenicity. Strikingly, UNR expression alone in human primary fibroblasts induces transformation. We are currently undertaking xenograft experiments in mice to confirm these findings in vivo.

To gain insight into the molecular mechanisms underlying the transforming properties of UNR, we have identified its direct mRNA targets by iCLIP-Seq. UNR binds to around 2500 transcripts in melanoma, including both coding and non-coding genes. About 20% of the targets are also bound by UNR in *Drosophila,* suggesting conserved UNR functions. According to a general role in post-transcriptional regulation, UNR binds with strong preference to 3′ UTRs and coding sequences, while it is relatively depleted from introns and 5′ UTRs. Thus, although mammalian UNR has been described as an ITAF (IRES trans-acting factor), our data suggest that this role must be limited to a reduced number of targets. A proportion of targets encode factors implicated in cancer development and metastasis. Only a reduced number of UNR mRNA targets showed changes at the steady state level in UNR-depleted melanoma cells. Furthermore, direct validation of some cancer-related targets indicated reduced protein levels without changes in mRNA amounts, suggesting that UNR regulates oncogenicity at the level of translation. Ribosome profiling experiments are underway to confirm this hypothesis at a genome-wide scale.

102 An RNA binding protein hnRNPM promotes breast cancer metastasis via regulating alternative splicing

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Tumor metastasis remains the major cause of cancer-related death, but its molecular basis is still not well understood. Here we uncovered a splicing-mediated pathway that is essential for breast cancer metastasis. We show that the RNA-binding protein hnRNPM promotes epithelial-mesenchymal transition (EMT) and metastasis via alternative splicing regulation. We identify CD44 as a key target of hnRNPM. hnRNPM ablation inhibits EMT and breast cancer metastasis in mice, whereas enforced expression of the specific CD44s splice isoform in hnRNPM-depleted cells permits EMT and metastasis. We further demonstrate that the ubiquitously expressed hnRNPM directs CD44 exon skipping in a mesenchymal-specific manner, committing cells for a migratory mesenchymal phenotype, and its epithelial activity is prevented by the epithelial-specific ESRP1. Importantly, hnRNPM expression is associated with aggressive breast cancers and correlates with increased CD44s in patient specimens. These findings demonstrate a novel molecular mechanism through which tumor metastasis is endowed by the hnRNPM-mediated splicing program.

102A Splicing factor hnRNP A2/B1 modulates breast cancer metastasis controlling alternative splicing of invasion genes

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Previous studies suggest that alternative splicing is deregulated in cancer and that the activity of many oncogenes and tumor suppressor genes is modulated by alternative splicing. However, the contribution of splicing regulators to cancer development is largely unknown. Based on the evidence that splicing misregulation is a hallmark of cancer, we hypothesize that some splicing factors have the potential to be oncogenic by regulating splicing events which are critical for transformation and metastasis. We report that the splicing factor hnRNP A2/B1 is overexpressed in the metastatic effusion state of breast cancer, compared to the primary solid tumor state. we demonstrate that hnRNP A2/B1 is involved in the invasion properties of MDA-MB-435s cells. Morever, knockdown of hnRNP A2/B1 inhibits lung metastasis of the metastatic breast cancer cell line MDA-MB-435s. However, hnRNP A2/B1 knockdown did not affect lung colonization. These results demonstrate that hnRNP A2/B1 contributes to both invasion and early stages of metastasis. To identify the splicing targets of hnRNP A2/B1 in a genome-wide manner, we performed RNA-seq analysis on metastatic breast cancer cells before and after hnRNP A2/B1 knockdown. RNA-seq analysis shows that hnRNP A2/B1 directly or indirectly affects the splicing of hundreds of genes, some of which affects metastastasis. Using RNA antisense oligonucleotides that induced skipping of TCF7L2 exon 15, MKI67 exon 7, and MAGI1 exon 19, we show that these splicing changes which are regulated by hnRNP A2/B1 affects invasion and survival of metastatic breast cancer cells. Our results suggest that hnRNP A2/B1 is a prognostic marker for breast cancer progression, modulates the splicing of invasion genes and is an important target for the development of anti metastasis therapy.

103 Non-redundant functions of splicing factors in breast-cancer initiation and metastasis

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Alternative splicing (AS) is a key control point in gene expression, whose misregulation contributes to cancer malignancy, including breast cancer. Although certain splicing factors (SFs) and their targets are altered in cancer, the functional significance of these alterations remains unclear. The splicing factor SRSF1 is upregulated in human breast tumors and promotes transformation in vivo and in vitro. SRSF1 is a prototypical member of the SR protein family, composed of 12 structurally related proteins. However, little is known about differences and redundancies in their splicing targets and biological functions. We are investigating whether additional SFs also promote breast cancer, using transformation models that mimic the relevant biological context. In parallel, we are using next-generation RNA sequencing (RNA-seq) to systematically identify their oncogenic splicing targets.

We used SF-overexpressing human mammary epithelial MCF-10A cells grown in organotypic 3-D culture; these cells form polarized growth-arrested acinar structures, similar to the terminal units of mammary ducts. Various breast-cancer oncogenes are known to disrupt acinar growth and/or architecture. Interestingly, only a subset of SFs were oncogenic in this context, differentially affecting cell proliferation, apoptosis, or acinar organization, suggesting non-redundant functions. Furthermore, specific SFs increased cell migration or invasion, and were required for the maintenance of metastatic properties of breast-cancer cells. We are now characterizing AS targets relevant for SF-mediated transformation. Using the SpliceTrap/SpliceChange pipeline to quantify splicing variation in RNA-seq data, we first identified and validated hundreds of SRSF1-regulated AS events. To gain a better understanding of SRSF1 regulatory mechanisms, we performed de-novo discovery of the SRSF1 binding site, and constructed a Bayesian model predicting the positional effects of SRSF1 binding on cassette exons. In parallel, using RNA-seq, we defined the global repertoire of SF-regulated AS events in 3-D culture, and compared the target specificities of various SR proteins. Strikingly, SFs that promoted similar phenotypic changes shared a significant number of AS targets, suggesting that they regulate common pathways to promote breast-tumor initiation and metastasis.

In summary, we gained new insights into the regulatory mechanisms of SR proteins and identified novel oncogenic SF-regulated AS events that represent potential targets for therapeutics development.

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104 Attenuation of NMD activity during chemotherapeutic treatment

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Frontline chemotherapeutic treatments such as doxorubicin (Adriamycin) are known DNA damaging agents and are currently in use to treat a number of diverse cancer types. Nonsense-mediated mRNA decay (NMD) is an mRNA quality control mechanism that eliminates transcripts harboring a premature termination codon. In addition to its quality control function, NMD also controls the levels of a large swath of the transcriptome. These transcripts encode proteins of diverse structure and function. We have observed that during treatment with DNA damaging agents, NMD activity is attenuated and known endogenous NMD targets are post-transcriptionally upregulated. Concomitant with this upregulation of NMD sensitive transcripts are a number of post-translational changes to the key NMD trans-effector, UPF1. These include altered phosphorylation status as well as hydrolytic trimming of the UPF1, which functions to blunt NMD activity. We have confirmed that a number of NMD sensitive transcripts that are upregulated during the response to Doxorubicin encode proteins known to mediated cell cycle arrest and facilitate apoptotic progression. In order to begin to inventory the complete list of transcripts controlled by UPF1 hydrolysis, we are employing a chemical biological approach termed SNIPER (Single Nick in Proteome, developed by Jim Wells, UCSF) to recapitulate the hydrolytic processing of UPF1 in the absence of DNA damage. We hypothesize that further genes encoding proteins responsible for cell cycle arrest and apoptotic progression will be identified, explaining why NMD activity is attenuated during toxic DNA damage mediated by chemotherapeutics. These results indicate that NMD activity can be acutely tuned by the cell and that changes in NMD activity may be therapeutically beneficial.

105 PGRN Network-wide Project: Transcriptome Analysis of Pharmacogenes in Human Tissues

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Gene expression variation is crucial to the etiologies of common disorders and the molecular underpinnings of pharmacologic traits; however, the nature and extent of this variation remains poorly understood. The NIH Pharmacogenomics Research Network (PGRN) Network-wide RNA-seq project aims to create a community resource containing quantitative information on known and novel isoforms of genes involved in therapeutic and adverse drug response (pharmacogenes). Using 90 samples from 5 major tissues (liver, kidney, adipose, heart, and lymphoblastoid cell lines [LCLs]) of pharmacologic importance, some with extensive pharmacogenomic phenotyping, we performed RNA sequencing. The data were analyzed for expression quantification, splice junction analysis, and transcript reconstruction. We utilized the JuncBASE pipeline developed by members of our consortium to identify and classify splicing events. In samples from heart, kidney, liver and adipose tissues, similar numbers of transcripts and genes were detected; however, notable differences in expression levels of important pharmacogenes (see http://www.pharmgkb.org/search/annotatedGene/) across the various tissues were observed (FDR < 0.05). For example, CYP enzymes (e.g., CYP2C19 and CYP2D6) were highly expressed in the liver with low expression in other tissues. Other important drug metabolizing enzymes such as DPYD and TPMT showed more balanced gene expression patterns across the tissues. We observed that 72-93% of pharmacogenes are alternatively spliced within each tissue. There was substantial variation in both annotated and novel splicing events both between tissues and between individual samples of the same tissue. For example, we found evidence of a novel alternative last exon for SLC22A7, a gene involved in transport of various drugs, which is variably spliced in liver between individuals. In addition, given the importance of LCLs as a pre-clinical model for human genetic studies, we systematically investigated differential expression and splicing between LCLs and the other tissues. LCLs do not express many genes and splice variants present in the physiological tissues, and do express splice variants of genes of pharmacological interest that are not observed in these primary tissues. These studies provide mechanistic insights into pharmacogenomic findings and facilitate an understanding of the factors that lead to inter-individual differences in drug response.

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106 DGCR8 acts as a novel adaptor for the exosome complex to degrade double-stranded structured RNAs

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DGCR8 (DiGeorge critical region 8) protein is part of the Microprocessor complex together with Drosha and it is involved in the first step of microRNA (miRNA) biogenesis. MiRNAs originate from long primary transcripts, which fold in hairpin structures that are first cleaved in the nucleus by the Microprocessor, followed by a Dicer-processing step in the cytoplasm. DGCR8 recognizes the RNA substrates through two double-stranded RNA (dsRNA) binding motifs and acts as a molecular anchor to direct Drosha cleavage at the base of the pri-miRNA hairpin.

Recent characterization of the RNA targets of the Microprocessor by HITS-CLIP of DGCR8 protein revealed that this complex also binds and regulates the stability of several types of transcripts, including mRNAs, lncRNAs and retrotransposons [1,2]. Of particular interest is the binding of DGCR8 to mature small nucleolar RNA (snoRNA) transcripts, since the stability of these transcripts was shown to be dependent on DGCR8, but independent of Drosha. This raises the interesting possibility that there could be alternative DGCR8 complex/es using different nucleases to process a variety of cellular RNAs.

Mass spectrometry experiments revealed that DGCR8 co-purifies with subunits of the nuclear exosome, forming a mutually exclusive complex with Drosha. Biochemical characterization of this complex indicated preferential association of DGCR8 with the nucleolar form of the exosome, which contains the exonuclease hRRP6. Importantly, we confirmed that DGCR8 is essential for the recruitment of the exosome to a particular subset of nucleolar transcripts, such as snoRNAs. Interestingly, we also show that DGCR8/exosome complex, but not the canonical Microprocessor, control the stability of another nucleolar transcript, the human telomerase RNA component (hTR/TERC). Accordingly, cells lacking DGCR8 show a telomere phenotype, displaying an abnormal elongation of telomeres, which could be partially explained by upregulation of TERC. Altogether, this data suggest the existence of an alternative DGCR8 complex, in which DGCR8 acts as an adaptor to efficiently recruit the exosome to structured RNAs and induce their degradation.

1. Macias,S Nat. Struct. Mol. Biol., 19, 760-766.

2. Heras,S.R Nat. Struct. Mol. Biol., 20, 1173-1181.

107 piRNA- and lncRNA-mediated chromosomal fusions in the ciliate *Oxytricha*

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RNA appears to regulate all aspects of programmed genome rearrangement in the ciliate *Oxytricha* (Bracht et al. 2013). Maternally-inherited lncRNAs provide templates for both genome remodeling and DNA repair (Nowacki *et al.,* 2008), while also regulating gene dosage and chromosome copy number (Nowacki *et al.,* 2010). Furthermore, *Oxytricha* piRNAs provide the critical information to mark and protect over 225,000 DNA pieces of the germline (Fang *et al.,* 2012) so that they can rearrange according to the templates to form the somatic genome.

Chromosomal fusions, present in both normal and cancer cells, can produce aberrant gene products and RNAs. Though the mechanisms driving these fusions are poorly understood, recurrent fusions are widespread. Here, we demonstrate that both piRNAs and lncRNAs can specifically program somatic chromosomal fusions in the ciliate *Oxytricha trifallax*. Brief exposure of the germline to either lnc "template" RNAs or to 27nt piRNAs that simply bridge two different chromosomal loci can generate novel chromosome fusions in the offspring, or even the formation of circular chromosomes. Furthermore, these RNA-mediated inter-and-intra-chromosomal fusions are heritable over multiple sexual generations, illustrating the power of noncoding RNAs to program genome architecture. While *Oxytricha* is noted for its genome of 16,000 tiny chromosomes (Swart et al. 2013), these experiments allow us to evolve linkage in this organism, modulating chromosome length in an unprecedented fashion.

Swart et al. 2013 *PLoS Biology* 11:e1001473
Nowacki et al. (2008) *Nature* 451:153-158
Nowacki et al. (2010) *PNAS* 107:22140-4
Fang et al. (2012) *Cell* 151:1243-55
Bracht et al. (2013) *Cell* 152:406-16

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108 Human colorectal cancer-specific *CCAT1-L* lncRNA regulates long-range chromatin interactions in the *MYC* locus

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The human 8q24 gene desert contains multiple enhancers that form tissue-specific long-range chromatin loops with the *MYC* oncogene, but how chromatin looping in the *MYC* locus is regulated remains poorly understood. Here we demonstrate that a long noncoding RNA,*CCAT1-L*, is transcribed specifically in human colorectal cancers from a locus 515 kb upstream of *MYC*. *CCAT1-L* exclusively accumulates to its sites of transcription. While knockdown of *CCAT1-L* led to reduced transcription of *MYC*, *in-cis* over-expression of *CCAT1-L* with a modified TALEN method enhanced *MYC* expression and promoted tumorigenesis. Importantly, the *CCAT1-L* locus is located within a strong super-enhancer and is spatially closely located to *MYC*. Knockdown of *CCAT1-L* reduced long-range interactions between the *MYC*promoter and its enhancers. In addition, *CCAT1-L* interacts with CTCF and modulates chromatin conformation at these loop regions. Together, these results reveal an important role of a previously unannotated long noncoding RNA in gene regulation in the *MYC* locus.

109 Genome-wide screen identifies pathways that govern tRNA splicing and intron turnover

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tRNAs are major components of the cell's protein synthesis machinery. In yeast, tRNAs are transcribed in the nucleus. After the removal of the 5′ and 3′ ends and the addition of CCA and some modifications, end-matured tRNAs are exported from the nucleus to the cytoplasm and are delivered to the mitochondrial surface where intron splicing occurs. The subcellular movement of tRNAs involves the initial export of tRNAs from the nucleus to the cytoplasm, retrograde nuclear import of cytoplasmic tRNAs, and re-export of the imported tRNAs back to the cytoplasm. However, many aspects of tRNA metabolism and subcellular movement remain unknown. For example, the mechanisms that regulate tRNA splicing and intron turnover are poorly understood. We conducted a systematic and unbiased genome-wide screen in budding yeast to identify all the missing players involved in tRNA biology. We developed a rapid and sensitive Northern method that allows genome-wide analysis of mature tRNAs and their processing intermediates. The complete set of 4848 deletion strains and 757 strains with temperature-sensitive mutations of essential genes were analyzed for defects in tRNA biology. Biochemical analyses of some of the identified mutants have provided surprising insights. For instance, deletion of *TOM70* and *SAM37* cause accumulation of end-matured intron-containing tRNAs. We learned that the tRNA splicing defect occurs because Tom70 and Sam37 are required for proper localization of the tRNA splicing endonuclease complex onto the mitochondrial surface. We also learned that deletion of *XRN1*, encoding the cytoplasmic 5′ to 3′ exonuclease, causes accumulation of free tRNA introns. Further investigation uncovered, for the first time, the mechanism of tRNA intron turnover; surprisingly, it is a multi-step process requiring cooperation between tRNA splicing ligase, Rlg1, and Xrn1. Numerous additional mutations that affect other aspects of tRNA biology were identified. Thus, our genome-wide screen has led to discoveries of novel gene products that function in eukaryotic tRNA transcription, processing, and subcellular dynamics.

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110 The modular transcriptome: resolving the extensive library of functional, structured non-coding RNA domains in the human genome

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The majority (>80%) of our genome is dynamically transcribed into RNA in a developmentally coordinated and tissue-specific manner, producing an astounding diversity of processed transcripts. The most diverse class of transcriptional products are long non-coding RNAs (lncRNAs), yet their detailed functional characterisation lies in stark contrast to their abundance in the literature and public databases. Identifying the precise molecular mechanisms implicating lncRNAs is crucial to the advancement of genomics and personalised medicine, as exemplified by the fact that most reported genetic variants associated to complex diseases occur in non-coding regions of the genome with no evidence of evolutionary conservation. However, assigning function to lncRNAs has been limited by insufficient measures of purifying selection as well as unreliable structural predictions.

We expose how over 30% of mammalian genomes present the hallmarks of purifying natural selection at the level of RNA secondary structure using cutting edge bioinformatics algorithms. Under the hypothesis that the structural diversity of lncRNAs serves as a modular scaffold for the recruitment and targeting of epigenetic effector complexes, amongst others, we developed a new approach for the identification of common RNA structures within a subset of sequences that considers an ensemble of sub-optimal base-pairings. Applying it to RNA immuno-precipitation followed by deep sequencing (RIPseq) data reveals novel and statistically significant clusters of common RNA structure motifs, despite the lack of substantial sequence conservation. We are now identifying the occurrence of homologous protein-binding RNA structure motifs in numerous transcriptomic datasets, with the aim of cataloging their genomic distribution and experimentally validating their tertiary conformations. Given the abundance of evolutionarily conserved RNA structures and the pervasive nature of mammalian transcription, we propose that these RNA structure motifs form a network of functional domains for the recruitment of specific RNA-binding proteins.

111 Hypoxia regulated long non-coding RNAs in breast cancer: *Novel insights of hypoxic non-coding transcriptome*

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Transcriptional responses to hypoxia are central to the pathogenesis of many types of cancer. To date, pan-genomic analyses of these transcriptional responses have focussed on protein-coding genes and microRNAs. However, the role of other classes of non-coding RNAs, in particular lncRNAs, in the hypoxia response is largely uncharacterised. We undertook an integrated pan-genomic analysis of normoxic and hypoxic MCF7 breast cancer cells, employing RNA-seq of polyA selected and ribosomally depleted transcripts together with ChIP-seq for the major hypoxia-inducible transcription factor (HIF) and for chromosomal markers of active transcription (RNApol2 and histone H3K4 methylation). We establish a computational pipeline for total RNA-seq analysis to detect non-coding transcripts including piwiRNA, miRNA, tRNA, sn/snoRNA, and lncRNA. Analyses have revealed that hypoxia profoundly regulated all RNA classes. snRNAs and tRNAs are globally downregulated in hypoxia, whilst miRNAs, mRNAs and lncRNAs are commonly upregulated We describe a number of hypoxia regulated non-annotated RNA species, including several that are antisense to protein-coding RNAs. Significant numbers of lncRNAs were up-regulated in hypoxia and these were associated both with epigenetic marks of increased transcription and with HIF binding. The most hypoxia upregulated lncRNA was NEAT1, which is a direct transcriptional target of HIF-2 but not HIF-1. However, the role of hypoxic NEAT1 in cancer has not been previously studied. We demonstrate that hypoxic NEAT1 induction is common in breast cancer cell lines and xenografts. NEAT1 directly induces the formation of nuclear paraspeckles in hypoxia, contributes to tumourigenicity in cell proliferation and colony forming assays and reduces rates of apoptosis. Finally, in a large cohort of 2000 breast cancers, high levels of NEAT1 correlated with poor clinical outcome. Our findings extend the role of the hypoxic transcriptional response in cancer into the spectrum of non-coding transcripts. These results will provide new insights on functional and clinical potential of hypoxia regulated lncRNAs which may act as novel therapeutic targets in future.

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112 DNA damage response RNAs (DDRNAs) are novel necessary components of DNA damage response foci

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The DNA damage response (DDR) is a signaling cascade that follows the generation of genomic DNA double-strand breaks (DSBs) and promptly arrests cell proliferation to allow DNA repair. DDR activation at DNA lesions involves the generation of DDR foci, cytologically-detectable subnuclear structures constituted of several copies of different DDR factors. We have recently reported (Francia et al., *Nature* 2012) that a novel class of small non-coding RNAs, termed DDRNA, carrying sequences proximal to the damaged site and generated in a DICER- and DROSHA-dependent manner, are necessary for DDR focus formation and DDR activation at DSBs. Moreover, in a mammalian cell system in which a DSB can be generated at a defined locus, the ensuing DDR focus is sensitive to RNase A treatment and can reform upon reintroduction of sequence-specific DDRNAs.

Using ensemble and single molecule fluorescence microscopy, we now show that DDRNAs are novel integral components of DDR foci and stably accumulate at sites of DNA damage in a sequence-dependent manner. Chemically-synthesized fluorophore-labeled DDRNAs are observed in close proximity to DDR factors within DDR foci. Sequence-specific DDRNA accumulation at foci is dependent on transcription by RNA polymerase II, as demonstrated by the use of specific transcriptional inhibitors. Indeed, inhibition of RNA polymerase II prevents DDR focus formation and DDRNA localization at sites of damage, indicating that DDR foci formation is RNA-dependent. Consistent conclusions were independently obtained by single molecule detection and quantitative studies of microinjected fluorescent DDRNA in fixed and live cells. The essential role of DDRNA in DDR foci maintenance was also demonstrated by the use of sequence-specific antisense inhibitory oligonucleotides which, by pairing to DDRNAs, impede site-specific DDR focus formation and DDR activation in living cells. In agreement with the newly observed RNA-rich nature of DDR foci, and similar to other RNA-based subcellular granules, live imaging revealed that DDR foci show liquid-like dynamic properties, while other direct DNA binding factors at the damage site do not.

Collectively our results for the first time reveal that DDRNAs are small non-coding RNAs integral to DDR focus formation and maintenance, and that DDR foci share many features with known RNA granules.

113 Primate-specific lncRNA genes as causes of human disease: evidence from GWAS and reverse genetics

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Key accomplishments of the ENCODE (Encyclopedia of DNA Elements) Consortium include its Gencode reference catalog of 57,000 human genes, of which only 20,000 encode proteins, while the rest are non-protein-coding. Diverse functional roles and mechanisms have been identified for over 100 long non-coding RNA (lncRNA) genes, but this is still a small number, two orders of magnitude less than the total lncRNA repertoire of the human genome. An intriguing evolutionary property of lncRNA genes is their lack of conservation, relative to protein-coding genes: one-third of human lncRNAs are not conserved beyond primates. To investigate whether lncRNAs lacking previous evidence of function, and regardless of conservation, are heretofore underappreciated causative contributors to human disease, we intersected ENCODE's Gencode lncRNA resource with the complete NHGRI GWAS catalog of genome-wide significant disease-associated SNPs from over 1,000 GWAS studies. We found genome-wide significant associations with a small specific subset of diseases, including Hodgkin's Lymphoma, for which half or more of all associated SNPs reside in exons of lncRNAs. We call these "lncRNA-associated diseases". Another such disease was breast cancer, which we pursued in the MCF7 estrogen receptor alpha (ERα) positive cell line, a well-established breast cancer model. Through differential expression analysis, we identified 127 significantly estrogen-regulated lncRNAs. Overexpression and knockdown of 26 estrogen-responsive lncRNAs shifted breast cancer cells along the apoptosis-proliferation axis. Decreased cell growth and increased cell death were consistently observed upon knockdown of estrogen-induced, and overexpression of estrogen-repressed, lncRNAs. Overexpression of one primate-specific estrogen-repressed lncRNA reduced ERK1 and ERK2 phosphorylation in ERα positive breast cancer cell lines but not in an ERα negative control, consistent with the accompanying decline in cell viability, and indicating regulatory input into the MAP kinase pathway, which is central in cancer. Notably, 8 of these 26 lncRNAs had primate-specific exonic sequences or splice junctions. Multispecies sequence alignments indicate that those lncRNAs originated mainly after the prosimian split. LncRNAs represent novel candidate targets for cancer therapeutics, because, unlike protein-coding genes in canonical pathways, they are highly tissue-specific and unlikely to be required for the viability of healthy cells. {This work has been supported by 1U01-HG007031, NHGRI, NIH.}

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114 tRNA intronic circular (tric)RNAs: biogenesis, biology and biotechnology

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Among the three information-carrying biomolecules, RNA is the most diverse and versatile. Large numbers of circular RNAs were discovered in the past few years, yet very little is known about their functions in vivo. Intron-containing tRNAs exist in all three domains of life and proper splicing is required for their maturation. Here we report the discovery in higher eukaryotes of circular RNAs that are generated from tRNA splicing, which we called tRNA intronic circular RNAs (tricRNAs). tricRNAs range in size from 20 to over 100 nucleotides, and they accumulate to high levels in the cells. Some of these tricRNAs are highly conserved, suggesting that they have functions.

We show that a *Drosophila* tricRNA folds into a highly stable rod-shaped secondary structure, providing a good substrate for the small RNA biogenesis pathway. Analysis of RNA-seq datasets showed that this tricRNA is processed into three small RNAs that are 18-22nt long. Two of these RNAs show mutually exclusive stabilization, consistent with the guide/passenger arrangement in mi/siRNA duplexes. These small RNAs show tissue-specific expression patterns, suggesting functional relevance.

Two tRNA splicing ligation pathways exist in animals, the healing-sealing type and the RtcB direct ligation type. In order to identify the pathway required for tRNA intron circularization, we analyzed fly mutants for components in the two pathways. Our preliminary analysis suggests that the RtcB protein is the primary RNA ligase for tricRNAs.

tRNA splicing and tRNA intron circularization activities are highly efficient and present in most animal and archaeal species. Taking advantage of the tricRNA biogenesis pathway, we have developed a novel method for ectopic expression of 'designer' circular RNAs *in vivo*. We show that we can express circular RNAs in different cell types at high levels. This technology will be useful for studying the functions of circular RNAs in general, and potentially for making RNA drugs with superior stability. In summary, we have identified a class of circular RNAs with potentially important functions, and developed a circular RNA expression system with broad applications.

115 Strategies for Targeting the RNA in Expanded Repeat Disorders

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Expanded RNA repeats are involved in a range of disorders that include muscular dystrophy, fragile x syndrome, and amyotrophic lateral sclerosis. Thus, there is an urgent need to develop methods to design small molecules that can bind to these repeats and affect cellular function. Herein, we describe the development of potentially generalizable strategies to target this important class of RNA. These strategies include modular assembly, structure-based drug design, and traditional medicinal chemistry. When these approaches are applied to target the RNA that causes myotonic dystrophy type 1, bioactive compounds are obtained that improve disease associated defects in both cellular and animal models of disease.

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116 In vitro and in vivo correction of nonsense mutations by amlexanox

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Nonsense mutations are responsible of about 10% of genetic disease cases. The main consequence of a nonsense mutation is the fast decay of the mRNA carrying that mutation by a mechanism called nonsense-mediated mRNA decay (NMD). Several strategies have been studied in order to correct the presence or the consequences of a nonsense mutation. Among them the inhibition of NMD and the activation of the readthrough of the nonsense mutation can be associated to improve the correction efficiency of the nonsense mutation. Recently, we identified by screening a molecule called amlexanox with the double capacity of inhibiting of NMD and activating readthrough. We demonstrated this double property on several immortalized cell lines harboring a nonsense mutation in different genes.

We then studied the in vivo effect of amlexanox on nonsense mutation in a mouse model harboring a nonsense mutation in the dystrophin gene. We show that amlexanox is able to rescue the expression of dystrophin gene and also the function of this protein when the exposure to amlexanox is prolonged.

Finally, we investigated the mode of action of amlexanox on NMD and translation termination mechanisms in order to understand how this molecule can interfere with both. In particular, we seek for the direct target of amlexanox.

Interestingly, amlexanox is a drug already on the market for the treatment of mouth ulcers and some forms of asthma. This drug status could favor the development of amlexanox to be tested in clinical trials for the treatment of nonsense mutation related diseases.

117 A unique RNA structure formed by a long-distance interaction uncovers the therapeutic potential of a deep intronic sequence

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Humans carry two copies of *Survival Motor Neuron* gene: *SMN1* and *SMN2*. Loss of *SMN1* coupled with skipping of *SMN2* exon 7 causes spinal muscular atrophy (SMA), a leading genetic disease associated with infant mortality. We previously reported an intronic splicing silencer (ISS-N1) as a negative regulator of *SMN2* exon 7 splicing. ISS-N1 is the most studied and highly promising target for an antisense oligonucleotide (ASO)-mediated splicing correction in SMA. The 15-nucleotide long ISS-N1 harbours two hnRNP A1/A2 motifs, although the first residue of ISS-N1 located at the 10th intronic position (^{10}C) falls outside of putative hnRNP A1 motifs. Upon performing an antisense microwalk in the region encompassing ISS-N1, we serendipitously encountered a rare phenomenon. While a 14-mer ASO (F14) sequestering the first 14-residues of ISS-N1 promoted *SMN2* exon 7 inclusion, another 14-mer ASO (L14) that sequestered the last 14-residues of ISS-N1 promoted *SMN2* exon 7 skipping. We showed that the inhibitory effect of L14 is solely due to the ^{10}C, which is not sequestered by L14. We also demonstrated that the negative effect of L14 is contingent upon a rare long-distance interaction (LDI) in which the ^{10}C makes contact with downstream intronic sequences. Consequently, deletion of downstream intronic transformed L14 into a stimulatory ASO. We next established that the ^{10}C interacts with the downstream intronic sequences through an unique RNA structure that we term Internal Stem Through LDI-1 (ISTL1). Using site-specific mutations and SHAPE (Selective 2′-Hydroxyl Acylation analysed by Primer Extension) we confirmed the formation and functional significance of ISTL1. We also demonstrated that the 3′ strand of ISTL1 falls within an inhibitory sequence termed ISS-N2. We showed that an ASO-mediated sequestration of ISS-N2 fully corrects *SMN2* exon 7 splicing and restores high levels of SMN and Gemin2, a SMN-interacting protein, in SMA patient cells. These results expand the repertoire of ASO-based targets for SMA therapy and underscore the therapeutic potential of regulatory information trapped in secondary and high-order RNA structures located within an intron. Our findings also demonstrate that an ASO-based approach could be employed to favourably remodel the structure of deep intronic sequence for therapeutic splicing correction.

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118 RECTAS, a candidate of the therapeutic drug for Familial dysautonomia, rectifies aberrant splicing of *IKBKAP* gene

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Familial dysautonomia (FD), a devastating hereditary sensory and autonomic neuropathy, results from the intronic mutation in the *IKBKAP* gene. The mutation disrupts the 5′ splice site of intron 20 in the *IKBKAP* gene, leading to skipping of exon 20 and reduction of the IKBKAP protein (IKAP). Homozygous mutant cells from the FD patients, however, express both wild-type (containing exon 20) and mutant (skipping exon 20) IKBKAP mRNAs, because the intronic mutation does not completely abolish inclusion of exon 20. The ratio of wild-type mRNA to the mutant mRNA is different among tissues in the FD patients, suggesting that tissue specific factor(s) regulates the inclusion of exon 20 in the context of FD. To find out the regulators, we have set up multi-color fluorescence splicing reporter that reflects the inclusion and skipping of exon 20. This reporter with wild-type IKBKAP gene produced mRNA containing exon 20 and expressed GFP, whereas the reporter with the intronic mutation generated mRNA skipping exon 20 and expressed RFP. We conducted an expression screening by co-transfection of expression vectors of RNA binding proteins with the reporter vectors. We identified Rbm24 and Rbm38 as the inclusion factors and SRp46 as the skipping factor. These findings encouraged us to screen small molecules that enhance exon 20 inclusion and increase the amount of normal IKAP. We succeeded in identifying a small molecule that enhanced the inclusion of exon 20 and increased IKAP expression in cells from the FD patients. We named this small molecule RECTAS (RECTifier of Alternative Splicing). Furthermore, RECTAS recovered the defect in proliferation of cells derived from the FD patient. RECTAS was rapidly absorbed after oral administration (~30 min) and is stably retained (~8 hours) with effective dose for the *IKBKAP* splicing in the blood plasma in mice. These results suggest that RECTAS is a promising candidate of the therapeutic drug for FD.

119 Identification of Multiple Small Molecule Modulators of HIV-1 RNA Processing and Gene Expression

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Replication of HIV-1 is dependent upon the balanced splicing of the viral genomic RNA. Consequently, small molecule modulators of viral RNA processing could prove useful as novel therapeutics to augment current treatments or serve as salvage therapy. To identify such compounds, we screened a library of splicing modulators for their effect on HIV-1 Gag protein expression. Of the sixty compounds examined, we identified four (892, 791, 833 and 191) that strongly suppress HIV-1 Gag, Env, Tat and Rev expression. Subsequent examination revealed that all active compounds significantly reduced accumulation of unspliced and singly spliced viral mRNAs but with limited effect on HIV-1 multiply spliced (MS) mRNAs that encode Tat and Rev. To explore the discrepancy between MS RNA levels and expression of the encoded proteins, we have examined the effect of these compounds on viral protein stability and mRNA localization. Current results indicate that the compounds have limited or no effect on Tat/Rev protein stability with both 191 and 791 treatment inducing limited or no alteration of global protein synthesis or cell growth. Compound treatment also had limited effect on alternative spicing of >70 host RNA splicing events evaluated, suggesting that these compounds act in a highly selective manner. Subsequent tests confirmed anti-HIV activity in the context of a T cell line and preliminary experiments have shown efficacy in suppressing HIV-1 replication in PBMCs. Current experiments are focused on defining the mechanism by which these compounds block viral MS RNA expression These observations verify that HIV-1 RNA processing can be effectively targeted to block virus replication with minimum effects on host cell viability.

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120 RNA: The new revolution in nucleic acid vaccines

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Over the past two decades, vaccination with gene-based vaccines (e.g., viral vectors and plasmid DNA) has become a promising alternative to conventional vaccines based on whole organisms and recombinant proteins. However, so far, these types of gene-based vaccines have not lived up to their potential. Feasibility of manufacturing, interfering anti-vector immune responses and safety remain issues for viral vectors; and plasmid DNA vaccines have lacked potency in humans. Recent advancements have demonstrated that vaccines based on mRNA have the potential to combine the positive attributes of other types of vaccines. Although the RNA vaccine field is in its infancy, the prospects are promising. Naturally transient and cytosolically-restricted mRNA can now be produced at sufficient quantity and quality from a cell-free enzymatic transcription reaction for human clinical trials. In addition, product stability, large-scale production, and purification are no longer perceived as barriers to the wide spread implementation of the technology.

We have developed a self-amplifying mRNA vaccine platform and have utilized recent innovations in the systemic delivery of short interfering RNA (siRNA) using lipid nanoparticles (LNPs) to create a completely synthetic non-viral delivery system, which substantially increases vaccine potency. The broad utility of this novel vaccine technology has been demonstrated with genes encoding vaccine candidate antigens from several pathogens and was found to elicit broad and potent protective immune responses in multiple animal models, including non-human primates. To demonstrate the speed at which synthetic self-amplifying mRNA vaccine can be produced, we responded to the recent H7N9 influenza outbreak in China as soon as the viral sequence was posted on a web-based data sharing system. Using rapid and accurate cell-free gene synthesis, the viral antigen was produced and incorporated into the self-amplifying mRNA vaccine, allowing the generation of a vaccine candidate within 8 days. If self-amplifying mRNA vaccines prove safe, potent, well-tolerated, and effective in humans, this novel nucleic acid vaccine technology will enable a new generation of vaccines able to address the health challenges of the 21st century.

121 Inhibition of Individual 14q32 MicroRNAs Drastically Increases Neovascularization and Blood Flow Recovery after Ischemia

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Introduction and Hypothesis. Neovascularization, i.e. angiogenesis and arteriogenesis, is a multifactorial process. As microRNAs can regulate expression of up to several hundred target genes, we hypothesized that specific microRNAs may target not just single aspects of neovascularization, but neovascularization as a whole. We set out to identify microRNAs that target genes in all pathways of neovascularization. Using www.targetscan.org, we performed a reverse target prediction on a set of 197 genes involved in neovascularization. We found enrichment of binding sites for 27 microRNAs in a single microRNA gene cluster on the long arm of human chromosome 14. MicroArray analyses showed that 14q32 microRNAs were down-regulated during effective neovascularization in mice subjected to single femoral artery ligation.

Methods and Results. Gene Silencing Oligonucleotides (GSOs), were injected (1mg/mouse) to inhibit four 14q32 microRNAs, miR-487b, miR-494, miR-329 and miR-495, one day prior to double ligation of the femoral artery. Blood flow recovery was followed by Laser Doppler Perfusion Imaging. All 4 GSOs clearly improved blood flow recovery after ischemia. Mice treated with GSO-495 or GSO-329 showed increased perfusion already after 3 days (30% perfusion vs. 15% in control animals) and those treated with GSO-329 showed a remarkable full recovery of perfusion after 7 days (vs. 60% perfusion in control animals). In vivo arteriogenesis was enhanced as 3-fold increased collateral artery diameters were observed in adductor muscles of GSO-treated mice. Simultaneously, in vivo angiogenesis was also enhanced as we observed up to 10-fold increased capillary densities in the ischemic soleus muscles of GSO-treated mice. Furthermore, in vitro treatment with GSO-329, GSO-495 and GSO-487b led to increased proliferation of primary human arterial endothelial cells whereas treatment with GSO-494 led to increased proliferation of primary human arterial fibroblasts.

Conclusions. Inhibition of 14q32 microRNAs leads to drastic increases in post-ischemic blood flow recovery in vivo via stimulation of both arteriogenesis and angiogenesis. In conclusion, 14q32 microRNA inhibition may offer an alternative to growth factors in therapeutic neovascularization.

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122 Development of therapeutic RNA switches selectively activated in diseased cells

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Over the past decade, the total number of RNA interference (RNAi)-based preclinical and clinical trials has increased rapidly with 22 different therapeutic small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) reaching clinical trials for the treatment of at least 16 diseases. The first clinical trial (in 2004) for siRNA-based therapy ran only three years after the discovery of siRNA function in mammalian cells; much faster than the usual six to seven years for small-molecule drug candidate preparations. Altogether, this illustrates a promising future in novel RNAi therapeutics. We have computationally designed novel therapeutic RNA switches with embedded siRNA functionalities that could represent an important step towards a selective cure of cancers. In the absence of a specific mRNA that triggers the switch (e.g. CTGF, VEGF or TWIST, which are over-expressed only in cancer cells) these switches containing the Dicer substrate (DS) siRNA are inactive. However, the switches are innovatively designed to possess sequence regions that can bind to the trigger mRNA and induce the conformational changes leading to the release of siRNAs selected against human apoptosis inhibitor genes (e.g. BCL-2, FLIP, STAT3 and XIAP). We comprehensively analyzed several switches in vitro and in human breast cancer cells as a model system. Native PAGE experiments confirmed the successful formation of the switch construct and the release of DS siRNA during the incubation with a complementary mRNA fragment (CTGF) at 37oC. Furthermore, the successful processing of DS siRNA by a human recombinant Dicer was shown. The successful release of therapeutic moieties (siRNAs) was confirmed by the suppression of the target gene expression. Overall, this novel approach opens a new route for the development of conditionally activated nucleic-acids-based "smart" nanoparticles for a broad range of biomedical and nanotechnological applications.

123 Targeting Structured Oncogenic RNAs with Antibodies

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Non-coding RNAs (ncRNAs) have been recognized as important disease markers and therapeutic targets. The ncRNAs often form extensive structures and exert their oncogenic functions by interacting with protein partners, making the RNA-protein interfaces important targets for cancer therapy. Current RNA targeting methods include small molecules and RNA interference (RNAi), both with significant challenges. The small molecule method relies on an often sporadic small molecule library screening approach and is particularly challenging in selectively targeting the functional portion of the RNA structures. While RNAi approach is more systematic, it recognizes only the primary sequences of the RNA targets, making it difficult to target ncRNAs often containing extensive secondary and tertiary structures. This sequence-based nature, in combination with off-target effect, cellular nuclease degradation, potential immunological response, and challenges in cancer-specific delivery has set major hurdles in developing viable RNAi-based drugs.

Here we report a unique and effective approach to target structured RNAs with antibodies. With phage display selection and synthetic antibody libraries especially those tailored for RNA recognition, we have obtained specific antibody fragments (Fabs) for over a dozen RNA targets. To demonstrate the effectiveness of this approach to target functional RNAs for important biological process and potential disease pathway intervention, we have selected specific Fabs targeting human initiator tRNA as a proof-of-concept system. tRNAs were shown to be overexpressed consistently in human ovarian cancers and breast tumors. In particular, initiator methionyl tRNA (tRNAiMet) binding to the ribosome is a rate limiting step of protein synthesis and tRNAiMet has been demonstrated to be oncogenic. We will present the generation of a Fab (Fab7) that binds human tRNAiMet with 59 nM affinity. Fab7 does not show noticeable binding to an unrelated RNA P4-P6, E coli tRNA mixture, or even elongator methionyl tRNA (tRNAeMet), demonstrating high specificity. Using a human in vitro protein expression assay, we showed that GFP reporter expression can be significantly reduced by the addition of Fab7 in a concentration dependent manner while the addition of a control Fab has no effect. Further demonstration of the potential mediation of Fab7 in biological processes is being pursued.

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124 The structure of a yeast Pan2-Pan3 core complex reveals insights into its architecture and regulation

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The turnover of mRNAs is performed by a set of macromolecular complexes that act in a sequential and coordinated manner, progressively eroding the ends of the transcript until its degradation is complete. The first and rate-limiting step in mRNA turnover is the shortening of the poly(A) tail. Deadenylation is carried out by two evolutionary conserved protein complexes: Pan2-Pan3 and Ccr4-Not. While there has recently been much progress in understanding the architecture of Ccr4-Not, limited information is available at present on Pan2-Pan3.

Pan2-Pan3 is a complex of two multidomain proteins that are predicted to contain folded units as well as unstructured regions. The Pan2 subunit consists of an N-terminal WD40 domain, a central UCH (ubiquitin C-terminal hydrolase) domain and a C-terminal nuclease domain of the DEDD family. Experiments carried out ten to twenty years ago with complexes obtained from endogenous sources argued that the activity of Pan2 is simulated by the poly(A) binding protein (PABP, known as Pab1 in yeast). PABP binds to a low-complexity sequence of the Pan3 subunit. Pan3 also contains a pseudokinase domain, which is catalytically inactive but has retained an ATP-binding site that is important for RNA decay *in vivo*. How the activity of Pan2 is assembled and regulated within the complex is unknown.

We reconstituted recombinant *S. cerevisiae* Pan2-Pan3 and used biochemical *in vitro* assays to identify a minimal portion of the complex that would recapitulate catalytic activity. We determined the crystal structure of this 198 kDa assembly and found that it is formed by one Pan2 subunit and two Pan3 subunits. The structural analysis reveals the basis for the unusual 1:2 stoichiometry and the role of the UCH and the pseudokinase domains in positioning and regulating the nuclease domain.

125 Assembly of the PAN2-PAN3 complex involves an asymmetric bipartite interface

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The shortening of poly(A)-tails of mature mRNAs influences their translation status and is usually the first and rate-limiting step in eukaryotic mRNA turnover. Deadenylation is thought to occur in two phases, of which the initial one is catalyzed by the PAN2-PAN3 complex. Despite its important function and high conservation, structural information of this complex was so far limited to isolated PAN3. PAN2 is a multidomain protein comprising an N-terminal WD40 domain, a central ubiquitin specific protease domain, and a C-terminal 3′-5′ exoribonuclease domain of the DEDD family. The globular domains of PAN2 are connected by two low complexity regions. Although it is clear that the nuclease domain of the protein is responsible for PAN2 deadenylase activity, little is known about the role of the WD40 and USP domains in PAN2 function. Furthermore the molecular basis for the assembly of the PAN2-PAN3 complex remained unclear.

To address these open questions, we solved crystal structures of all three folded domains of PAN2, i.e. the WD40, protease and exonuclease domains, as well as a structure of the PAN2-PAN3 complex. The structures reveal that the PAN2 exonuclease domain forms a tight assembly with the protease domain. In this arrangement, canonical exonuclease elements are replaced by parts of the protease domain, suggesting that the two domains coevolved to function as one unit. In contrast to previous studies, we show that complex formation with the PAN3 homodimer is mediated by the N-terminal WD40 domain of PAN2 together with the subsequent linker region. Surprisingly, our biochemical and structural data suggest that the asymmetry of the PAN3 homodimer together with the placement of the PAN2 linker prevent the recruitment of a second copy of PAN2. Thereby a molecular ratio of 1:2 is established in the fully assembled PAN2-PAN3 complex, that could have mechanistic implications.

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126 New insights in free m7G cap metabolism

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In eukaryotes, mRNAs possesses a poly-A tail at their 3′ end and a methyl-7-guanosine cap (m7G) at their 5′ end. These two features play an essential role in the control of gene expression. Removal of the poly-A tail triggers mRNA degradation. After deadenylation, mRNAs can follow one of two different decay pathways: either 3′-5′ exosome-mediated degradation or m7G cap hydrolysis (mediated by the Dcp2-Dcp1 decapping complex) followed by 5′-3′ Xrn1-mediated degradation. The end-products of these two pathways are free m7GpppN and m7GDP (di-)nucleotides respectively. High-levels of such compounds interfere with many cellular processes like translation and splicing.

Free cap derivatives are further converted in m7GMP and other uncharacterized by-products. While m7GpppN was shown to be directly hydrolyzed into m7GMP by the DcpS scavenger enzyme, m7GDP is transformed in m7GMP by a mechanism that is still matter of debate.

In this study we aimed to clarify how m7GDP is eliminated from cells. We demonstrate that in yeast extracts, m7GDP is first converted in m7GTP, in an ATP dependent reaction. Then m7GTP is hydrolysed by DcpS in m7GMP. We also observe that *in vivo*, the absence of DcpS results in m7GDP accumulation. This confirms the essential physiological role played by the scavenger enzyme in m7GDP elimination. Furthermore, we show that this biochemical pathway is evolutionarily conserved in Eukaryotes.

Delineating this pathway led us to identify two new proteins involved in cap metabolism. The characterization of these proteins will be presented. In particular, our results indicate that DcpS is not the only scavenger decapping enzyme in Eukaryotes.

In yeast, m7GMP is further converted in uncharacterized by-products. We have observed that these uncharacterized compounds don’t contain the cap methyl group. The resulting product is therefore likely to be recycled in the nucleotide salvage pathway.

127 Codon optimization is a major determinant of mRNA half-life in *Saccharomyces cerevisiae*

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The control of mRNA half-life is a key aspect in determining the overall level of protein abundance in the cell. Importantly, general features that dictate transcript half-life are still largely unknown, though it is clear that the rate of degradation varies widely. A major pathway of transcript degradation involves initial shortening of the 3′ polyadenosine tail (deadenylation), followed by removal of the 5′ cap structure (decapping), and finally exonucleolytic digestion of the transcript body in the 5′-3′ direction. The rates of deadenylation and decapping differ between transcripts, and while deadenylation has been shown to be influenced by 3′UTR elements and associated binding partners, features controlling decapping rate are unclear. We have recently observed that under normal conditions mRNA decapping occurs co-translationally. This observation in conjunction with others suggests an integral relationship between protein synthesis rates and mRNA turnover rates might exist.

Using a genomic approach, we have been able to calculate rates of deadenylation as well as total mRNA decay. Using these data, we have sought mRNA features that correlate with turnover rate. Surprisingly, we see a strong contribution of codon usage towards mRNA half-life. Indeed, there is a striking correlation between the estimated optimality of codons and the effects of those codons on the decay of messages that bear them. Our analysis shows that occurrences of individual optimal or suboptimal codons can correlate with increased or decreased half-life respectively. Consistent with these data, we find a good correlation between effects on mRNA half-life and the adaptiveness value for each codon. The effects of these codons can be seen in the translation rates of mRNAs as well. Importantly, codon optimization appears to only effect mRNA decapping and has no bearing on deadenylation rate. Together, these data argue that the integral relationship between mRNA translation and decay occurs at the level of decoding; thus translational elongation rates are a major component in determining mRNA half-life.

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128 Magnetic tweezers reveal Upf1 as a highly processive translocase

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RNA helicases are involved in every aspects of RNP metabolism and many of them are essential for viability. Despite their common structural features, these enzymes possess many different functions. Fuelled by ATP, RNA helicases unwind RNA secondary structures, remodel RNA-protein interactions, or clamp protein complexes to RNA. The precise action of helicases for specific substrates in the presence of their protein partners often remains elusive. Upf1 is a multitasking DNA and RNA helicase, best known for its essential role in Nonsense-mediated mRNA Decay (NMD), a quality control mechanism that degrades mRNAs carrying a premature translation termination codon (PTC). In humans, Upf1 is recruited to stalling ribosomes prior to interact with the downstream Exon Junction Complex (EJC) via the NMD factors Upf2 and Upf3. The Upf1 helicase activity is tightly regulated. In the absence of binding partners, the N- and C-ter domains of Upf1 repress the helicase domain while Upf2 is able to partially restore Upf1 activity. However, even if Upf1 helicase activity is absolutely essential for NMD, its exact role is still mysterious.

We employ magnetic tweezers to manipulate single tethered DNA or RNA hairpin to analyze the biophysical characteristics of single molecules of Upf1. The substrate extension is used as a real-time reporter of the enzyme activity. We first observed that Upf1 helicase domain is able to (i) fully unwind long DNA (1200 bp) and RNA (156 bp) hairpins and (ii) translocate onto long single-stranded DNA or RNA regions of equivalent size. Upf1 translocates slowly with a remarkable processivity. In the presence of the N-ter CH domain, Upf1 becomes a tight clamp preventing hairpin rezipping while addition of Upf2 restores Upf1 translocase activity. Interestingly, single molecules and biochemical strategies showed that proteins binding to nucleic acids did not slow down Upf1 translocation, revealing that Upf1 is also an efficient and processive RNP remodeler.

These data bring to light the first eukaryotic RNA helicase able to translocate onto RNA processively. We propose that Upf1 employs its translocase activity to remodel aberrant mRNPs before their degradation during NMD.

129 Identification of cis-regulatory element within UPF1 target transcripts

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RNA degradation is an essential step in post-transcriptional regulation, involved in diverse biological processes such as differentiation and immune response. RNA-binding proteins (RBPs) and the other proteins associated with RNAs play important roles in the specific recognition of their RNA targets to regulate gene expression via RNA degradation.. UPF1, containing the RNA helicase domain, plays the central role in nonsense-mediated mRNA decay (NMD), which eliminates aberrant mRNAs harboring premature termination codon. Recent studies revealed that UPF1 is also involved in STAU1-mediated mRNA decay (SMD) to regulate physiologically normal RNA expression level. Thus, UPF1 is an important factor not only for the RNA surveillance but also for the regulation of gene expression through RNA degradation. We recently developed 5′-bromo-uridine (BrU) immunoprecipitation chase-deep sequencing analysis (BRIC-seq) to determine genome-wide RNA stability by chasing chronological decreases of BrU-labeled RNAs. In this work, we employed the combined analysis of RNA stability (BRIC-seq) and RNA-UPF1 protein interaction (RIP-seq) to identify bona-fide UPF1 target genes. BRIC-seq identified that 619 transcripts were stabilized in UPF1-depleted HeLa cells. RNA immunoprecipitation (RIP) showed that UPF1 protein interacted with 246 transcripts which stabilized in UPF1-depleted cells, suggesting that those 246 transcripts are directly regulated by UPF1-dependent RNA degradation. Surprisingly, only 16 of these transcripts were stabilized in STAU1-depleted HeLa cells and the others (230 transcripts) were not stabilized. This result indicates that these 230 transcripts could be regulated by unknown UPF1-dependent RNA decay besides SMD pathway. Moreover, we found that GC-rich motif is statistically enriched among 3′UTR of these UPF1 targets. The tet-off system was used to determine whether UPF1 could regulate the stability of these transcripts that contained GC-rich motif. HeLa Tet-Off cells were transfected with either a tetracycline-repressible plasmid (pTetBBB) expressing a stable b-globin reporter transcript or a plasmid (pTetBBB/GC-rich) containing an additional GC-rich motif in its 3′UTR. The pTetBBB/GC-rich transcript was relatively unstable in control cells. On the other hand, its transcript was stable in UPF1-depleted cells, suggesting that GC-rich motif is a degradative element which triggers RNA decay in UPF1-dependent manner.

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130 CLAMPing the transcriptome

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Genome-wide screens for synthetic lethal (sl) interactions with loss of exosome cofactors identified both Bre5 and Ubp3, which form a complex that has protein deubiquitinase activity on many substrates including RNA polymerase II (RNAPII). Bre5 has a potential RRM and we confirmed RNA binding *in vitro* and *in vivo*. The CRAC UV crosslinking approach identified many Bre5 targets, and showed enrichment over exon 2 of intron-containing pre-mRNAs. To determine the location of ubiquitinated RNAPII, we developed the crosslinking analysis of modified polymerase (CLAMP) technique; this provides data similar to ChIP, but strand specific and with nucleotide resolution. This confirmed that RNAPII ubiquitination is elevated over exon 2 regions of Bre5 target genes. Kinetic analysis revealed that the absence of Bre5 causes a delay in the release of RNAPII paused over exon II. The Bre5-Ubp3 sensitive ubiquitination site lies in an acidic loop close to the RNAPII active site. We propose that splicing-induced, exon 2 pausing of RNAPII, results in its ubiquitination of the active site to prevent further elongation. Following cotranscriptional splicing, deubiquitination by Bre5-Ubp3 bound to the nascent transcript, allows the polymerase to resume transcription.

In addition to mRNAs, yeast synthesizes two major classes of long non-protein coding RNAs (lncRNAs), termed CUTs (cryptic unstable transcripts) and SUTs (stable unannotated transcripts). To determine why lncRNAs and mRNAs have different fates in the cell, we followed the pathway of mRNA transcription, packaging, processing, nuclear export, translation and cytoplasmic turnover, in order to determine the point(s) at which lncRNAs diverge from mRNAs (Tuck and Tollervey, Cell, 2013). Analyses of 13 processing and packaging factors distinguished different lncRNA classes and identified groups of mRNA-like lncRNAs and lncRNA-like mRNAs. 3′ end formation emerged as a key decision point, in discriminating mRNAs from CUT lncRNAs, but this was not clearly the case for SUTs. CLAMP was applied to the determine occupancy of RNA Pol II with different CTD modifications (Ser5, Ser2, Ser7, Tyr1, Thr4). RNAPII on nascent SUTs show different modification profiles from mRNA or CUTs, suggesting that SUT fate is determined cotranscriptionally.

131 Post-transcriptional regulation of meiotic genes by a nuclear RNA silencing complex

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RNA is a central component of gene silencing pathways that regulate diverse cellular processes. In the fission yeast Schizosaccharomyces pombe, an RNA-based mechanism represses meiotic gene expression during vegetative growth. This pathway depends on the zinc finger protein Red1, which is required to degrade meiotic mRNAs as well as to target histone H3 lysine 9 (H3K9) methylation, a repressive chromatin mark, to a subset of meiotic genes. However, the mechanism of Red1 function is unknown. Here we use affinity purification and mass spectrometry to identify a Red1-containing Nuclear RNA Silencing (NURS) complex. In addition to Red1, this complex includes the Mtl1, Red5, Ars2, Rmn1, and Iss10 proteins and associates with several other complexes that are involved in either signaling or mediating RNA silencing. By analyzing the effects of gene knockouts and inducible knockdown alleles, we show that NURS subunits regulate RNA degradation and H3K9 methylation at meiotic genes. We also identify roles for individual NURS subunits in interactions with Mmi1, an RNA-binding protein that marks meiotic RNAs for destruction, and the nuclear exosome RNA degradation complex. Finally, we show that the levels of H3K9 methylation at meiotic genes are not sufficient to restrict RNA polymerase II access or repress gene expression during vegetative growth. Our results demonstrate that Red1 partners with other proteins to silence meiotic gene expression at the post-transcriptional level. Conservation of a NURS-like complex in human cells suggests that this pathway plays an ancient and fundamental role in RNA silencing.

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132 A third α-helix in Cyp33-RRM acts as an allosteric switch in MLL mediated transcription regulation

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The Mixed Lineage Leukemia factor (MLL) is a transcription activator of a series of HOX family genes. In its transcriptional active state MLL binds to a specific epigenetic mark on Histone H3 (H3K4me3) through its third Plant Homeobox Domain (PHD3). The absence of the PHD3 in fusion variants of MLL is associated with some very aggressive form of infant leukemia [1].

Cyclophilin 33 (Cyp33) is a member of the *cis-trans* peptidyl-prolyl isomerase family of Cyclophilins. N-terminal to the PPIase active Cyclophilin domain, Cyp33 contains an RNA Recognition Motif (RRM) domain. It was shown that Cyp33-RRM binds to both RNA and the PHD3 of MLL in a mutually exclusive manner [2] and that the PHD3 domain interacts in addition with H3K4me3. It is proposed that Cyp33 *trans*-isomerases a proline in MLL causing a conformational change enabling MLL-PHD3 to bind Cyp33-RRM and to promote the transcriptional repressive state of MLL. Furthermore it was shown that RNA binding to Cyp33-RRM not only stimulates its PPIase activity but can also rescues the expression of MLL target genes in cells. Hence, it is speculated that RNA binding to Cyp33 acts as a trigger to switch MLL to its transcriptional repressive state but also provides an escape route for MLL back to its active state.

Using solution NMR we determined four structures: Cyp33-RRM in free form, bound to RNA, to MLL-PHD3 as well as the ternary complex Cyp33-RRM, MLL-PHD3 and the epigenetically marked Histone H3 tail (H3K4me3). This ensemble of structures provides evidence that a C-terminal α-helix (α_3) of Cyp33-RRM adopts different conformations in each structure that can explain all the steps leading to a transcriptional repressive state. Indeed, RNA binding dislocates α_3 from the RRM β-sheet which in turn favors the formation of a ternary complex between Cyp33-RRM, MLL-PHD3 and H3K4me3. However, relocation of α_3 squeezes the spatially distant binding cleft for the H3K4me3 and therefore allosterically weakens the interaction of MLL to the Histone H3. This causes the epigenetic marks to be accessible for deacetylases in order to repress the transcription of MLL target genes.

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133 The spliceosomal U1 snRNP component Mud1 is autoregulated by promoting premature cleavage and polyadenylation of its own transcript

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Here we demonstrate in the budding yeast, *S. cerevisiae*, that the transcript encoding the spliceosomal U1 snRNP component Mud1 is normally subject to regulation by premature cleavage and polyadenylation (PCPA) at a location within an intron near the 5′ end of its transcript. In a strain carrying a mutation in *yth1*, a core component of the cleavage and polyadenylation (CP) machinery, full-length Mud1 mRNA levels increase by ~15-fold as a result of the decrease in PCPA and increase in read-through of the transcript. To better understand the complement of factors involved in this regulation we performed a high-throughput reverse genetic screen and identified mutations in many CP components, including CFT2, YSH1 and REF2, which allow for increased read-through of the Mud1 transcript. Similarly, mutations that function on or near the CTD of RNA polymerase, such as the cis-trans prolyl isomerase *ess1*, also relieve the natural PCPA of the Mud1 transcript. Because Mud1p is a stable component of U1 snRNP and recent work suggests a functional relationship between U1 snRNP and PCPA, we asked whether Mud1p levels were important for PCPA. Indeed, we demonstrate that ectopic over-expression of Mud1 increases PCPA of chromosomally-encoded Mud1. To our knowledge, this is the first example of a spliceosomal component functioning to promote CP, and contrasts with recently proposed models of spliceosomal antagonism of PCPA. Moreover, mutations that disrupt the U1 snRNA binding site in Mud1p also relieve PCPA of the Mud1 transcript, supporting the notion that Mud1p functions in promoting PCPA through its capacity as a component of the U1 snRNP. On the basis of these data, we present a model whereby the splicing and CP machineries compete with one another on the CTD of RNA polymerase for access to nascent transcripts, and wherein their activities are mutually exclusive with one another. Furthermore, we propose that Mud1p, which inspite of its stable association with the U1 snRNP has never been demonstrated to have a functional role in splicing, instead functions to regulate the competition between these two pathways through an as yet unknown mechanism.

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134 Detained introns are a novel, widespread class of introns posttranscriptionally spliced to regulate gene expression

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Deep sequencing of poly(A)-selected RNA from mouse embryonic stem cells (mESCs) revealed the presence of specific internal introns within transcripts in which downstream introns were absent. Quantitation of several such introns by qRT-PCR verified their elevated abundance as well as the presence of intact exon-intron borders. Because these introns remain unspliced in polyadenylated transcripts, we refer to them as "detained introns" (DIs). Using a novel computational approach, we identified thousands of DIs in human and mouse cells. DIs flank both constitutive and alternatively spliced exons, but are present in only 10-15% of any given class of alternative splicing. This suggests that rather than resulting from the intrinsically slower splicing of introns flanking alternative exons, DIs constitute a specifically regulated subset of introns spanning all types of splicing event. DIs ultimately decayed with slower kinetics than their more rapidly spliced neighbors, indicating that they are turned over by splicing or degradation of their precursor. Despite most DIs containing premature termination codons that would subject them to nonsense-mediated decay (NMD) upon translation, DI-containing transcripts are not NMD targets. This suggests that DIs remain localized in the nucleus until they are either spliced or the precursor degraded, a property that distinguishes DIs from retained introns. DIs in the transcripts encoding Clk SR-protein kinases were previously shown to undergo rapid splicing in response to small-molecule inhibition of Clk kinase activity[1]. We determined the genome-wide effect of Clk kinase inhibition on DI splicing using RNA-seq. Several hundred DIs exhibited differential splicing upon inhibitor treatment. Interestingly, about half showed increased levels of intron detention while the rest underwent increased splicing in response to the drug. Many of the DIs spliced more rapidly upon Clk inhibition are located in genes encoding splicing factors, particularly SR proteins, likely resulting in a global shift in splicing regulators that in turn affects the expression of many secondary target genes. These data suggest that among the pool of nuclear-detained, DI-containing transcripts, specific subsets can be rapidly mobilized in response to cellular signals or homeostatic autoregulation.

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135 Profiling the RNA maturation landscape in yeast

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In the last decade, it has become clear that RNAs play an even more significant role than previously thought; they are not only the conveyer of genetic information in the form of mRNA, but can themselves be regulators of gene expression and other important cellular pathways. But regardless of which type, all RNAs are transcribed and assembled into ribonucleoprotein (RNP) complexes, in order to be modified, processed and transported to their final destination within the cell. The process occurs along different pathways, defined by specific processing factors, which form discrete subsets of proteins that associate with each specie of RNA in a dynamic fashion to define the order of maturation events.

Yet while many factors and steps are known, given the vast amount of RNAs transcribed by RNA Pol II alone, and the, in comparison, relatively small number of known RNA maturation factors, several open questions remain; how much overlap between subsets of maturation factors exists for different Pol II transcripts? Are all mRNAs processed along the same pathway, or are there distinctions for different classes of transcripts and if so, what are the criteria?

Several studies have already provided some insight into the maturation of different RNAs, and recent work showed that several mRNA processing factors also bind to other Pol II transcripts. However, most previous studies either looked at the RNP RNome or Proteome separately, or at only a small subset of baits, leaving many gaps. To obtain a comprehensive view on mRNP maturation of *S.cerevisiae*, we have combined Proteomics with RNA sequencing to profile the protein as well as RNA interactome of 33 key mRNA maturation factors across the pathway, from capping, splicing, 3′end processing and quality control to mRNA export. Our results indicate that while there is a certain degree of overlap, not all processing factors are associated with the same RNAs. Together with the discovery of adaptive changes in specific bait interactomes and novel feedback loops, our data provides a more detailed overview of the vast RNA maturation landscape.

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136 Folding RNA by a Hierarchical Graph Sampling Approach

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A current challenge in RNA structure prediction is the description of global helical arrangements compatible with a given RNA secondary structure. We will present a hierarchical Monte Carlo sampling approach to describe RNA helical geometries by a coarse-grained sampling of 3D graphs guided by knowledge-based potentials derived from bend, twist, and radii of gyration measures based on known structures. The coarse-grained model using newly developed 3D graphs accelerates the global sampling of candidate RNA topologies. A comparison of our candidate graphs to reference graphs from both solved structures and predicted structures by currently available programs indicates promise for characterizing 3D global helical arrangements in large RNAs from a given secondary structure. The efficiency in graph sampling, however, implies an additional step of translating candidate graphs to atomic models. Such models can be built with the same idea of graph partitioning and build-up procedures already utilized for RNA design.

137 Nearest neighbor parameters for RNA from accurate atomistic simulations

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Nearest neighbor parameters are routinely used to predict the stability of nucleic acid double strands, with applications ranging from secondary structure prediction to determination of slippery sequences and miRNA binding sites. Several sets of such parameters have been fitted on optical melting experiments. As far as we know, no attempt has been made to obtain these parameters ab initio from accurate atomistic simulations. The reason for the lack of such works is that free energy calculations are computationally too demanding as they require the entire free energy surface of the system to be properly sampled. With state of the art techniques, such as metadynamics, it is possible to efficiently sample the part of the phase space which is relevant for our purposes. This work is aimed at reconstructing free energy landscapes describing the melting of a set of double stranded RNA molecules in aqueous salt solution by atomistic molecular dynamics.

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138 Computational Strategies for Analyzing RNA-Protein Complexes and Predicting RNA-Protein Interaction Networks

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The importance of RNA-protein interactions in epigenetic and post-transcriptional regulation of gene expression as well as in viral replication makes them potential targets for intervening in both genetic and infectious diseases in humans. We are using computational and experimental approaches to interrogate RNA-protein interactions, with two primary long-term goals: i) to identify determinants of recognition specificity in RNA-protein complexes; and ii) to understand how networks of RNA-protein interactions are regulated and integrated into cellular regulatory and signalling networks.

Recently, we have focused on developing databases and reliable computational tools for analyzing RNA-protein complexes and interaction networks. We have established two databases, ***PRIDB***[1], a database of interfaces from all structurally characterized protein-RNA complexes, and ***RPIntDB***[2], a database of experimentally-validated RNA-protein interactions, and two webservers, ***RNABindRPlus***[3], for predicting *interfaces* in RNA-protein complexes and ***RPISeq***[4], for predicting *partners* in RNA-protein interaction networks.

In investigating the "interface prediction" problem, we have shown that machine learning classifiers that use PSSM-based encodings of protein sequences consistently out-perform classifiers that use other sequence-derived encodings. Surprisingly, *RNABindRPlus*, a sequence-based ensemble method that combines an optimized Support Vector Machine classifier and a sequence homology-based classifier, out-performs available structure-based methods. On an independent dataset of 44 proteins, *RNABindRPlus* predicts interfacial residues with Specificity of 0.72, Sensitivity of 0.63, and MCC of 0.55. To address the "partner prediction" problem, we have developed a sequence-based method, *RPISeq* that uses a Random Forest (RF) classifier to predict whether a given pair of RNA and protein sequences interacts. On two non-redundant benchmark datasets extracted from PRIDB, *RPISeq-RF* classifiers achieved accuracies of 89.6% and 76.2%, with ROC AUCs of 0.96 and 0.92. These results indicate that computational tools can be reliable enough to identify key residues for targeted mutagenesis and to identify likely partners for RNAs or proteins of biomedical importance.

We will present results obtained using these approaches to analyse and predict interfacial residues in clinically important RNPs (e.g., Rev-RRE complexes) and to identify interaction partners in cellular signalling networks (e.g., long ncRNA-protein interaction networks implicated in cancer).

References

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139 A RNA Binding Score for predicting RNA binding probabilities of protein residues

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The understanding of the recognition principles of RNA binding to proteins is necessary to predict the binding interfaces. In the past decade, tens of computational prediction algorithms have been developed to predict RNA binding sites based on either protein sequences or protein structures. Most of the state-of-art methods depend on machine learning approach based on PSSM and other residue propensities, ranging from SVM, neural network to random forest and Naive Bayes. However, in order to discriminate RNA binding sites from non-binding sites, the existing programs are all-or-none classifications. Here, we propose a simple score to predict RNA binding probabilities based on a combination of protein sequences and structures.

The prediction score is based on physico-chemical and evolutionary principles. As amply demonstrated, RNA binding residues are accessible on protein surface, tend to be positively charged and are highly conserved in sequence. The derived score is a combination of residue accessibility surface, electrostatics potential and conservation entropy.

Importantly, the prediction score avoids comparison of all RNA binding residues and non-binding residues of different proteins together. Instead, it maximizes the prediction accuracy for each protein separately. It achieves similar or even better accuracy than the other best prediction programs.

Further, we have found that the RNA binding residues, when defined by commonly used distance cut-off, even for the same protein can vary in different PDB complexes. We define the residues that are always considered as RNA binding residues in different PDB complexes as conserved RNA binding residues. And the prediction score is also useful in predicting such residues.

With the prediction score, the residues around the RNA binding position can be plotted as an energy funnel: residues farther away from the binding position are scored lower. Therefore, it helps in the localization of the most central RNA binding region in a protein.

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140 Harnessing the Mutational Landscape of Structured RNA

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The development of efficient algorithms to compute the sequence-structure relationships in RNA molecules is a milestone in evolutionary studies, synthetic biology applications, or NGS error-correction methodologies. In the past decades, fast algorithms have been developed to characterize the conformational landscape of a fixed sequence. Yet the opposite remains extremely challenging.

We developed a new family of algorithms which, given a RNA secondary structure, explores the full sequence landscape in order to identify the most compatible ones. To identify those, we developed a novel scoring scheme combining classical stacking base pair to the recent isostericity scale, a measure of the geometrical base pair compatibility. In particular, the latter enables us to use the evolutionary information contained in a multiple sequence alignment.

A first declination aims to complement existing NGS error-correction pipelines. Implemented in the tool RNApyro, our algorithm computes which mutations would increase the most the probability that a sequence belongs to a certain structured RNA family. RNApyro was applied to point-wise errors correction of the ribosomal 5s and 16s rRNAs, and in both cases was able to successfully retrieve simulated errors, as evidenced by areas under the curves for the ROC over 0.8 in general, and of 0.95 for structured regions.

We also applied our techniques to the design of sequences folding into target secondary structures. Our tool, IncaRNAtion, is the first global method which considers the entire sequence space without bias and allows to control for the nucleotides distribution, and in particular the GC content. Our method is seedless, thus removing the bias of a starting point in local search heuristics. We show that IncaRNAtion samples sequences with their MFE close to the target and thermodynamically stable, and that its running time is comparable or better than local search methods. When IncaRNAtion is hybridized with a local search method, such as RNAinverse, it remarkably give better results while minimally affecting the nucleotide distribution and the time.

This novel family of algorithms constitute promising tools to study the sequence/structure relationship, paving the road for studies of RNA evolution and synthetic biology applications.

141 New tools and resources for RNA structure analysis, comparison, and prediction

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As increasing numbers of 3D structures of RNA molecules are solved at atomic resolution and increasing numbers of RNA sequences are identified in genomes and transcriptome studies, more powerful, yet easy-to-use tools are needed to connect RNA 3D structures and RNA sequences in biologically meaningful ways. We have developed an automated pipeline to analyze, annotate, compare, and evaluate RNA 3D structures. Once each week, all RNA structures deposited in the Protein Data Bank (PDB) are annotated with pairwise interactions and grouped by sequence and geometry into equivalence classes. These data are now available through the Nucleic Acid Database (NDB) Structure Summary pages. From each equivalence class, one high-quality structure is selected to represent the class in the NDB Non-Redundant sets. This structure is used for statistical summaries and by FR3D for structure searches. Every four weeks, all RNA internal and hairpin loops are extracted from the representative structures and clustered by geometry and conserved interactions into 3D motif groups. For each release of the RNA 3D Motif Atlas, a new set of probabilistic models of the sequence variation of each of the motif groups is constructed and incorporated into the JAR3D webserver. The success of recent efforts to improve the performance of JAR3D to correctly match known and novel sequences of RNA internal and hairpin loops to the correct motif group while minimizing false positive rates will be discussed. The automated pipeline makes it possible to incorporate new crystallographic data on an ongoing basis to improve our ability to predict 3D structure from sequence.

Links:

1. NDB website: http://ndbserver.rutgers.edu/; 2. NR sets: http://rna.bgsu.edu/nrlist; 3. FR3D: http://rna.bgsu.edu/webfr3d; 4. JAR3D website: http://rna.bgsu.edu/jar3d; 5. RNA 3D Motif Atlas: http://rna.bgsu.edu/motifs

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141A RNA Bricks - a database of RNA 3D motifs and their interactions

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The RNA Bricks database (http://iimcb.genesilico.pl/rnabricks), stores information about recurrent RNA 3D motifs and their interactions, found in experimentally determined RNA structures and in RNA-protein complexes. In contrast to other similar tools (RNA 3D Motif Atlas, RNA Frabase, Rloom) RNA motifs, i.e. 'RNA bricks' are presented in the molecular environment, in which they were determined, including RNA, protein, metal ions, water molecules and ligands. All nucleotide residues in RNA bricks are annotated with structural quality scores that describe real-space correlation coefficients with the electron density data (if available), backbone geometry and possible steric conflicts, which can be used to identify poorly modeled residues. The database is also equipped with an algorithm for 3D motif search and comparison. The algorithm compares spatial positions of backbone atoms of the user-provided query structure and of stored RNA motifs, without relying on sequence or secondary structure information. This enables the identification of local structural similarities among evolutionarily related and unrelated RNA molecules. Besides, the search utility enables searching 'RNA bricks' according to sequence similarity, and makes it possible to identify motifs with modified ribonucleotide residues at specific positions.

141B Staufen and Stau1 binding preferences defined using a new computational method applied to in vivo binding data

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Staufen is a Drosophila double-stranded RNA-binding protein with roles in early embryonic and neural development. It has five double-stranded RNA binding domains (dsRBD), three of which bind dsRNA in vitro. Using an unbiased computational analysis of double-stranded RNA structures, we discovered three types of structures enriched among RIP-chip targets of Staufen in Drosophila embryos: a short 12-bp stem with two or fewer unpaired bases or mismatches; a longer 19-bp stem with four or fewer unpaired bases or mismatches; and a 19-bp stem with no unpaired bases and up to four mismatches[1]. This last structure was extremely specific, having a 20-fold enrichment among Staufen-bound 3′UTRs, and was depleted for G-G mismatches, suggesting a role for non-canonical base pairs in defining Staufen targets. Collectively, our observations point to two different modes of binding by Staufen, possibly differing by the number of dsRBDs participating in binding[1].

The two mammalian Staufen homologs, Stau1 and Stau2, have fewer dsRBDs, raising the question of whether their binding specificity is conserved. We have previously reported enrichment for the 12bp stem in Stau2 targets in rat neurons[2]. Preliminary analysis of two independent datasets of human Stau1 RIP targets[3,4] suggests enrichment for all of our Staufen recognized structures.

I will report the full results on our analysis of human Stau1 targets as well as describing our computational pipeline which represents a new paradigm for defining the specificity of double-stranded RNA-binding proteins.

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141C *RNA-Puzzles* Round II: Assessment of RNA structure prediction of two large riboswitches

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RNA-Puzzles is a CASP-like collective blind experiment for the evaluation of RNA 3-dimensional structure prediction. The primary aims of RNA-Puzzles are to determine the capabilities and limitations of current methods of 3D RNA structure prediction based on sequence, to find whether and how progress has been made, and to illustrate whether there are specific bottlenecks that hold back the field. Ten puzzles have been set up and three assessments are published. Nine groups of modelers around the world participate in this collective effort. We now report a second round focusing on the prediction of two large riboswitches, the adenosylcobalamin and the T-box bound to a tRNA. No homologous structures existed in the databases at the time of the experiment. Although only two targets were selected, these targets provide a wealth of sub-domains (around 10), including both well-known modules like K-turns as well as new ones.

The 168nt adenosylcobalamin riboswitch consists of a ligand-bound structured core and a bent peripheral domain. Although the RMSDs of the prediction models range from 11.7 to 37.5 Å, the topology of the top ranked models are quite similar to the native structure. Top ranked models show much better scores in Deformation Index (DI) and non-Watson-Crick interaction network fidelity (nwc INF) than others, but surprisingly have worse clash scores.

The T-box and tRNA, 96 and 75nt in length respectively, form a large complex. The difficulty in prediction lies mainly in (i) the lack of homologous model for T-box and (ii) the interaction between T-box and tRNA. The RMSD range of the predictions is 6.8 to 17.4 Å and the top ranked models also have better DI score with worse clash scores.

The Das group performed best in both problems with their models ranked #1 at 14.5 and 7.6 Å, respectively. The Bujnicki group performed well in the second problem with the model ranked #1 at 10.2 Å and excellent clash scores with nwc INF around 0.5 like the models of the Das group. Further, the less well predicted models always had worse nwc INF score, demonstrating the importance of identifying non-Watson-Crick pairs and RNA modules.

141D RNA design rules from a massive open laboratory

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Self-assembling RNA molecules present compelling substrates for the rational interrogation and control of living systems. However, imperfect in silico models—even at the secondary structure level—hinder the design of new RNAs that function properly when synthesized. Here, we present a unique and potentially general approach to such empirical problems: the Massive Open Laboratory. The EteRNA project connects 37,000 enthusiasts to RNA design puzzles through an online interface. Uniquely, EteRNA participants not only manipulate simulated molecules but also control a remote experimental pipeline for high-throughput RNA synthesis and structure mapping. We show herein that the EteRNA community leveraged dozens of cycles of continuous wet laboratory feedback to learn strategies for solving in vitro RNA design problems on which automated methods fail. The top strategies—including several previously unrecognized negative design rules—were distilled by machine learning into an algorithm, EteRNABot. Over a rigorous 1-year testing phase, both the EteRNA community and EteRNABot significantly outperformed prior algorithms in a dozen RNA secondary structure design tests, including the creation of dendrimer-like structures and scaffolds for small molecule sensors. This project – enabled by the facile experimental manipulation of RNA – shows that an online community can carry out large-scale experiments, hypothesis generation, and algorithm design to create practical advances in empirical science.

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142 Adenosine to Inosine Editing Frequencies Controlled by the Speed of Splicing

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Systematic deep-sequencing projects have shown that the coding information of almost every gene product in multicellular organisms is modulated at the transcript level. The major factors leading to post-transcriptional changes of genetic information are alternative splicing and RNA-editing. In metazoa, the most prominent type of RNA-editing is adenosine deamination, mediated by the ADAR class of enzymes. Both, RNA-editing by ADARs and (alternative) splicing occur in the nucleus, and are most likely co-transcriptionally coupled. Moreover, mRNA splicing may control editing: On the one hand, several editing sites depend on the presence of intronic sequences that aid in guiding ADARs to their substrate sites. Thus, the speed of splicing may control the availability of binding sites for ADARs. On the other hand, the speed of splicing can control nuclear retention time. Extended nuclear retention, in turn, may increase editing by exposing mRNAs to the editing-competent nuclear environment.

To determine to what extent splicing kinetics affects editing we used a minigene approach. We chose several exonic editing sites that depend on intronic sequences and sites that do not depend on intronic elements (e.g. in exon 2 of *Kcna1* or in exon 9 of *Gabra3*). The edited exons and parts of their respective downstream introns were fused to different downstream 3′ splice-sites of varying strength. Subsequently, the constructs were transfected into editing-competent cells and the editing efficiency was determined using Sanger sequencing.

We demonstrate that the degree of editing depends on splicing kinetics when editing is guided by intronic elements: Constructs that splice less efficiently show a strongly increased editing frequency. However, for editing sites that do not depend on intronic elements (in this case the speed of splicing only influences nuclear retention time) the situation is less clear. While an increase in editing is observed when *Gabra3* exon 9 is coupled to introns of progressively slower splicing kinetics, the editing levels in *Kcna1* exon 2 are unaffected by the splice efficiencies of downstream introns. We therefore conclude that the speed of splicing strongly controls the editing frequencies of intron-depending editing sites while nuclear retention time only affects some, but not all editing events.

143 N6-methyl-adenosine RNA modification controls cell fate transition in mamallian embryonic stem cells

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Reversible chemical modifications on messenger RNAs have emerged as prevalent phenomenon. N6-methyl-adenosine (m6A) is the most abundant covalent modification of messenger RNAs and is linked to human diseases such as cancer and obesity. Little is known about when and how the pattern of m6A modification is set up during development. Here we assess the developmental origin of m6A by mapping the m6A methylome in mouse embryonic stem cells (mESCs). We found that thousands of messenger and long noncoding RNAs are m6A-modified, including transcripts encoding core pluripotency transcription factors such as Nanog, Sox2 and Myc. In mRNAs, m6A modification is enriched in the neighborhood of the STOP codon at defined sequence motifs. To elucidate potential mechanisms of m6A function, we asked whether m6A-marked transcripts differ from unmodified transcripts by leveraging published genome-wide datasets in ESCs. We found that m6A marks transcripts with short half-life, including transcripts that need to be turned over upon differentiation.

To understand the role of m6A in early development we targeted Mettl3, one of the known m6A methylases, with the CRISPR-genome editing tools to generated loss of function *Mettl3* mESCs mutants. Loss of Mettl3 led to incomplete m6A erasure and significant loss of methylation in genes involved in regulation of transcription. Loss of Mettl3 did not affect self-renewal, but resulted in impaired ESC exit from self-renewal towards differentiation to several lineages. Mettl3 KO cells did not differentiate into cardiomyocites or neurons in vitro, and subcutaneous injection of Mettl3 KO cells formed tumors consistent in morphology with teratomas that tended to be larger than tumors derived from wild type cells. Furthermore KO tumors were predominantly composed of poorly differentiated cells with very high mitotic indices and numerous apoptotic bodies, whereas wild type cells differentiated predominantly into neuroectoderm. Thus, we propose that m6A is a mark of transcriptome flexibility important for stem cell fate transitions.

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144 Genome-wide pseudouridine sequencing (Pseudo-Seq) reveals regulated pseudouridinylation of mRNAs in yeast and humans

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RNA molecules contain a wide variety of modified nucleotides that modulate RNA structure and function. Pseudouridine (Ψ), the most common of these modifications, comprises ~1% of the nucleotides in cellular RNA, and is predominantly found in structured non-coding RNAs including rRNA, tRNA and snRNA. In contrast, cellular mRNAs were not previously known to contain Ψ. Recent studies have shown that artificial pseudouridylation of mRNAs at stop codons (ΨAA, ΨAG, and ΨGA) leads to specific re-coding of stop codons as sense codons for serine, threonine, phenylalanine and tyrosine. Thus, mRNA pseudouridylation affects the fidelity of decoding on the ribosome, and has the potential to expand the genetic code. To determine whether endogenous mRNAs contain Ψ, we have developed Pseudo-Seq, a high-throughput method to map the locations of Ψ's genome-wide with single nucleotide resolution. Pseudo-Seq accurately identifies known sites of pseudouridylation within rRNA, snRNA and tRNA, and reveals hundreds of novel Ψ's in mRNAs; almost 100 new Ψ's in non-coding RNAs were also identified, including many sites within snoRNAs. Genetic analysis in yeast allowed us to assign a subset of these new modification sites to conserved pseudouridine synthases, Pus1 and Pus7. Notably, a subset of Ψ's in both mRNAs and ncRNAs are regulated in response to environmental signals (nutrient deprivation in yeast and serum starvation in human cells). Work is ongoing to determine the effects of mRNA pseudouridinylation on protein coding using targeted mass spectrometry. Our results reveal widespread mRNA pseudouridylation and suggest a possible mechanism for regulated rewiring of the genetic code through inducible mRNA modifications.

145 Pseudouridylation of yeast U2 directly affects the ATPase activity of Prp5p during pre-mRNA splicing

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Pseudouridine (Ψ) is the most abundant internal modification identified in RNA. Yeast U2 snRNA contains three conserved Ψs (Ψ35, Ψ42, and Ψ44) in the branch site recognition region (BSRR), which base-pairs with the pre-mRNA branch site during splicing. Here, we show that blockade of pseudouridylation at these positions, either individually or in combination, reduces the efficiency of pre-mRNA splicing, leading to defects in cell growth. Simultaneous blockage of Ψ42 and Ψ44 has the most significant effect on splicing and cell growth. Genetic analysis suggests that these Ψs interact with Prp5p, an RNA-dependent ATPase involved in monitoring the U2 BSRR-branch site base-pairing interaction. Subsequent biochemical analysis indicates that Prp5p has reduced affinity (presumably through SF3b) to U2 snRNA that lacks Ψ42 and Ψ44. In addition (or perhaps consequently), the ATPase activity of Prp5p is also reduced when U2 lacking Ψ42 and Ψ44 is used, resulting in inefficient spliceosome assembly. Further in vivo DMS probing analysis reveals that pseudouridylated U2, when compared with U2 lacking Ψ42 and Ψ44, adopts a slightly different structure in the branch site recognition region. Taken together, our results indicate that the Ψs in U2 snRNA contribute to pre-mRNA splicing through directly affecting the binding/ATPase activity of Prp5p.

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146 Proteins that contain a functional Z-DNA binding domain localize to cytoplasmic stress granules

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Long dsRNAs may be hyper-edited by adenosine deaminases that act on RNA (ADARs), where up to 50% of adenosine residues may be converted to inosine. However, while numerous RNAs may undergo hyper-editing, the role for inosine-containing hyper-edited dsRNA in cells is poorly understood. Nevertheless, editing plays a critical role in mammalian cells, as highlighted by the analysis of ADAR-null mutants. In particular, the long form of ADAR1 ($ADAR1^{p150}$) is essential for viability. Moreover, a number of studies have implicated $ADAR1^{p150}$ in various stress pathways. We have previously shown that $ADAR1^{p150}$ localized to cytoplasmic stress granules in HeLa cells following either oxidative or interferon-induced stress.

We have now carried out experiments that demonstrate that the Z-DNA/Z-RNA binding domain ($Z\alpha^{ADAR1}$) exclusively found in $ADAR1^{p150}$ is necessary and sufficient for localization of $ADAR1^{p150}$ to stress granules during either oxidative or interferon-induced stress[1]. Moreover, we show that fusion of $Z\alpha^{ADAR1}$ to either GFP or PTB4 (polypyrimidine binding protein 4) also results in their localization to stress granules. In contrast, $ADAR1^{p110}$, which lacks the $Z\alpha^{ADAR1}$ domain, does not localize to stress granules. In addition to $ADAR1^{p150}$, Z-DNA/Z-RNA binding domains have so far been identified in only four other proteins (E.g. ZBP1, E3L), which have all been implicated in immune pathways. We have thus shown that the Zα domain from ZBP1 and E3L is also sufficient for localization to stress granules. Finally, we have shown that Z-RNA or Z-DNA binding is important for stress granule localization. However, we anticipate that Z-RNA is the binding partner of ADAR1p150 as they co-localize in the cytoplasm. We have thus identified a novel role for Z-DNA binding domains in mammalian cells, which results in localization to stress granules. Moreover, we speculate that localization of $ADAR1^{p150}$ to stress granules will be important for its role in mammalian cells that make it essential for viability.

(1) Ng, S.K., Weissbach, R., Ronson, G. and Scadden, A.D.J. (2013). Proteins that contain a functional Z-DNA binding domain localize to cytoplasmic stress granules. *Nucleic Acids Research*, **41**(21):9786-99.

147 Two methylase paralogs, two diverging functions: from tRNA biogenesis to ribosome assembly

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Transfer and ribosomal RNAs undergo numerous post-transcriptional modifications which are often essential for structure and function. One such modification, 3-methylcytidine (m^3C) is highly conserved at position 32 of the anticodon loop of serine and threonine tRNAs within eukarya. Interestingly, *Trypanosoma brucei* has two homologs of the *Saccharomyces cerevisiae* m^3C methyltransferase; a situation thus far unique to the kinetoplastid lineage. Using molecular and genetic approaches, we have identified the *T. brucei* C_{32} tRNA methylase (Trm140) and established the importance of the second homolog (MTase37). We observed that C_{32} methylation occurs in the nucleus prior to tRNA export to the cytoplasm. Previous work from our lab demonstrated C_{32} is also edited to uridine by the tRNA editing deaminase, TbADAT2/3. Provided our previous observation of both a nuclear and cytoplasmic localization of the TbADAT2/3 deaminase and its involvement in both A to I and C to U editing, we explored the possibility of a possible connection between editing and methylation. Using in vitro methylation assays, we show recombinant TbTrm140 can efficiently methylate C_{32} of a synthetic tRNA substrate only in the presence of TbADAT2/3. Additionally, *in vivo* experiments have shown this methylation to be biologically important in translation elongation. Down-regulation of the second ortholog, MTase37, by RNAi leads to severe cytokinesis defects and affects steady-state ribosomal RNA levels. Sucrose gradient analysis has revealed absence of MTase37 protein leads to defects in large subunit (60S) biogenesis and/or assembly and a decrease in 80S and polysome associated mature ribosomes. These results exemplify the importance of RNA modifications in fine-tuning RNA function and highlight evolutionary connections between modification and editing.

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148 Diverse roles of the prion-like protein, Mod5, in tRNA-modification and RNA-silencing

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Mod5 is a highly conserved tRNA modifying enzyme that resides primarily in the cytoplasm in eukaryotes where it modifies a small subset of tRNAs, by transferring a isopentenyl group from dimethylallyl pyrophosphate to A37 adjacent to the anticodon. In yeast a small population of Mod5 is bound to nuclear tRNA gene transcription complexes and nascent pre-tRNAs, and is required for silencing RNA polymerase II transcription near tRNA genes (1). Yeast Mod5 can misfold into heritable prion-like aggregates which confer resistance to the fungicide, fluconazole (2) . We have demonstrated that the human homolog of Mod5, TRIT1, complements both the tRNA-modification and tRNA gene-mediated silencing functions in yeast (1). We are currently investigating the folding behavior of Mod5/TRIT1 in yeast and in human cells, addressing the following questions: (1) Does prionization affect the known nuclear or cytoplasmic functions of Mod5? (2) Is the prionization tendency conserved from yeast to humans? (3) Is prionization affected by cellular stress responses? Others have shown that tRNA modification in the cytoplasm is significantly reduced in prion-Mod5 cells, suggesting that prion-Mod5 lacks tRNA modification capabilities presumably due to the insolubility of the Mod5 amyloid aggregate (2). However, our studies demonstrate that Mod5 prionization does not affect tRNA gene-mediated silencing in the nucleus suggesting that either the nuclear pool of Mod5 is aggregation-resistant or that prion-Mod5 in the nucleus remains functional for silencing. It is not currently known whether selection of misfolded Mod5 by fungicide treatment is an evolutionary response to compounds that inhibit the ergosterol pathway or rather that Mod5 evolved prion-like capabilities as part of a more general stress response. Our preliminary data suggests that oxidative stress may also affect Mod5 prionization status, suggesting that prionization of Mod5 may be part of a larger protein misfolding response to cellular stressors.

1. Pratt-Hyatt, et al. (2013) Mod5 protein binds to tRNA gene complexes and affects local transcriptional silencing. *Proc Natl Acad Sci U S A*, **110**, E3081-3089.

2. Suzuki, et al. (2012) A yeast prion, Mod5, promotes acquired drug resistance and cell survival under environmental stress. *Science*, **336**, 355-359.

149 A specialized mechanism of miRNA mediated translation in cellular Quiescence

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MicroRNAs are well documented as translational repressors. However, under certain cellular condition such as G0 (quiescent state), microRNAs can mediate translation activation of specific mRNAs. These mRNAs are translationally up-regulated by an FXR1a-associated microRNP complex (microRNA-protein complex), in quiescent (G0) mammalian cells and immature Xenopus laevis oocytes. The mechanism of this translation activation by microRNAs during the G0 state remains largely unknown. Here we show that microRNA-mediated activation requires short or no poly(A) tails on target mRNAs in oocytes and mammalian THP1 G0 cells, which holds true for endogenous targets of microRNA-mediated activation. Polyadenylated mRNAs are repressed, possibly due to poly(A) binding protein (PABP)-mediated enhancement of microRNA-mediated downregulation. Overexpression of PAIP2, which removes PABP from poly(A) tails, rescues microRNA-mediated upregulation of polyadenylated mRNAs in oocytes. Similarly inhibition of the deadenylase, poly(A) ribonuclease, PARN, prevents upregulation of translation activation in oocytes. Importantly, we also observed that the interaction of FXR1-associated microRNP with p97, a paralog of the translation factor eIF4G without PABP-interacting domains, is required for translation activation. This mechanism is required for maintenance of the immature state in oocytes, with implications for related physiological function in G0 mammalian cells. Taken together, these data reveal a specialized mechanism of microRNA-mediated activation where the FXR1a-associated microRNP targets specific shortened poly(A) mRNAs for p97 mediated translation.

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150 Translational regulation by the mRNA-binding protein Cpeb4 controls terminal differentiation of erythroid cells

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In mammalian cell differentiation, transcriptional networks establishing transcriptomic dynamics are well characterized. Our knowledge on post-transcriptional regulatory events translating transcriptomic changes into cellular proteomes and ultimate phenotypes, however, are still very limited. Using differentiation of primary erythroid cells as a model, we show that the sequence-specific mRNA-binding protein Cpeb4 is essential for terminal erythroid cell differentiation and is induced by the erythroid important transcription factors Gata1 and Tal1. By interacting with the translation initiation factor eIF3 Cpeb4 represses the translation of a large set of mRNAs, including its own mRNA. Thus transcriptional induction and translational repression of Cpeb4 combine to form a negative feedback regulatory circuit to control Cpeb4 protein levels within a narrow range, presumably to ensure proper levels of translation of developmentally-important mRNAs. Our study reveals that translational control synchronizes with transcriptional regulation to precisely control gene expression during mammalian cell differentiation.

151 Folding and function of a preQ$_1$ riboswitch-regulated messenger RNA at the single molecule level

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Riboswitches, regulatory RNA motifs present in the 5′-UTRs of many bacterial mRNAs, change their folded structure in response to stimuli, which in turn causes a change in the expression of downstream genes. However, the molecular details of their folding and function are still largely unexplored.

The exceptionally small, translationally-acting preQ$_1$ riboswitch found in the 5′-UTR of TTE1564 from *Thermoanaerobacter tengcongensis* tightly binds 7-aminomethyl-7-deazaguanine (preQ$_1$), a precursor in the biosynthesis of the hyper-modified nucleobase Queuosine. This riboswitch's aptamer domain, which overlaps with a portion of the ribosome binding site, becomes more compact in the presence of preQ$_1$ and thus is expected to interfere with ribosome binding and result in down-regulation of TTE1564.

We have used a combination of single molecule fluorescence resonance energy transfer and computational approaches to characterize the dynamics of folding of the riboswitch aptamer domain, and found the preQ$_1$ ligand to bind early, inducing a conformational fit of the RNA. In addition, we have developed a novel single molecule fluorescence tool that allows us to observe the secondary structure fluctuations of individual mRNA molecules and have used it to directly probe changes in the accessibility of the Shine-Dalgarno (SD) sequence (used by the ribosome to bind mRNA) as a function of preQ$_1$. We observe that the SD sequence in each mRNA molecule undergoes distinct bursts of high and low accessibility with complex, 6-parameter kinetics, leading to fine-tuned riboswitching behavior that is subtly influenced by the preQ$_1$ concentration. We are now also investigating the impact of ribosomal protein S1 on riboswitching. S1 has long been known to play a role in binding to single stranded regions of RNA as an early step in translation initiation. However, more recent work has underscored the importance of S1 and its interactions with structured messenger RNAs. We observe that S1 has affinity for preQ$_1$-riboswitch containing mRNA and, importantly, that this affinity decreases in the presence of preQ$_1$. These findings suggest that understanding S1's interactions with RNA secondary structures during ribosome initiation is critical for understanding the mechanism of translationally-acting riboswitches

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152 Evolutionary conserved patterns of sequence complementarity between eukaryotic mRNA UTRs and rRNAs and their implication for gene translation regulation

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There are several key mechanisms regulating eukaryotic gene expression at the level of protein synthesis. Interestingly, the least explored mechanisms of translational control are those that involve the translating ribosome per se, mediated for example via predicted interactions between the ribosomal RNAs (rRNAs) and mRNAs. We took advantage of robustly growing large-scale data sets of mRNA sequences for numerous organisms, solved ribosomal structures and computational power to computationally explore the mRNA–rRNA complementarity that is statistically significant across the species and that has a potential to form interactions between mRNA and the translating ribosome. Our predictions reveal highly specific sequence complementarity of 18S rRNA sequences with both 5′ and 3′ UTRs of mRNA forming well-defined and specific 3D patterns on the rRNA sequence of the 40S subunit. We discuss physiological significance of these structurally conserved patterns and, in the context of previously published experimental results, propose that they modulate scanning of the 40S subunit through 5′ UTRs of mRNAs and, in the case of 3′ UTRs, a contact between mRNA and the post-termination 40S subunit during translation termination.

153 What makes an efficient ribosome binding site an efficient ribosome binding site? A study of the randomized libraries exceeding 1 000 000 5′-UTRs

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Regulation of gene expression at the level of translation accounts for up to three orders of magnitude in its efficiency. Previously, we systematically compared the impact of several known mRNA ribosome binding site (RBS) features, such as length and location of the Shine Dalgarno sequence (SD), secondary structure, start codon etc, on translation initiation; the experiments were done in a system with internal control based on dual cerulean and red (CER/RFP) fluorescent proteins. Based on these results, we proposed a model for estimating translation efficiency and predict translation efficiency for large set of natural 5′-UTRs. Among a set of 50 natural 5′-UTRs cloned into the CER/RFP reporter system we've found several outliers that possesses much worse or much better translation efficiencies than could be predicted. Consequently, we concluded that known elements composing bacterial RBS are non sufficient to adequately describe translation efficiency. We chose a set of outlier 5′-UTRs to investigate what makes them different from those whose ribosome binding efficency was successfully predicted. Selected 5′-UTRs were randomly mutated within the reporter plasmid and E. coli cells transformed with this library were sorted according to the ratio of the CER and RFP fluorescence, subjected to next generation sequencing (NGS) and analysis. Several unexpected features which strongly affect bacterial translation were found.

To investigate mRNA RBS elements contributing to translation efficiency further we created an unbiased set of reporter construct libraries exceeding a million randomized RBS variants. After sorting and NGS we not only investigated a detailed anatomy of classic bacterial RBS, but also found several completely unusual sequences, which provide strong translation efficiency.

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154 Accurate measurements of ribosome footprints and mRNA abundances reveal the limited extent of translational control

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Ribosome footprint profiling provides snapshots of translation at sub-codon resolution on a genome-wide scale. The positional information in these profiling data can identify differences in the rates at which codons are decoded during translation elongation, as well as global differences in elongation rates associated with specific mRNA features. Ribosome-profiling data can also be combined with mRNA abundance measurements to calculate the translational efficiency (TE) of each mRNA. Such calculations have previously suggested that the TEs of yeast mRNAs span a roughly 100-fold range, indicating pervasive translational regulation that makes mRNA levels a poor proxy for protein levels. However, attempts to identify mRNA features that explain this 100-fold range of TEs have been largely unsuccessful. Here, we use an optimized strategy to measure ribosome profiles, mRNA abundances, and TEs that more accurately reflect *in vivo* translation in *Saccharomyces cerevisiae*. Our positional data reveal that elongation rates are extensively modulated during translation in ways that were missed in previous datasets due to protocol-specific biases. Most notably, our results provide the first demonstration that tRNA abundances impact the rate of elongation along endogenous mRNAs *in vivo* (with codons corresponding to lower-abundance tRNAs being decoded more slowly), thereby confirming a decades-old hypothesis about the functional role of codon-usage bias. After accounting for codon usage, we find that translation elongation is further slowed during the early phase of translation, and also within inter-domain linkers, which may facilitate co-translational folding of the nascent polypeptide. By incorporating accurate mRNA abundance measurements, our analyses indicate that the TEs of most yeast mRNAs span only a 5-fold range and, correspondingly, that mRNA levels are a generally good predictor of protein levels. Thus, in exponentially growing yeast cells, translational control plays a more minor role in determining protein abundances than originally suggested. We use these TEs to generate a statistical model in which more than half of the variance in TEs is explained by a small number of mRNA features, including 5′-end secondary structure and mRNA length. Collectively, our results reveal key features of translational control in yeast and provide a general framework for executing and interpreting ribosome-profiling studies.

155 Core structure of the U6 snRNP and investigation of U4/U6 di-snRNP biogenesis

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The spliceosome is a dynamic assembly of five small nuclear ribonucleoproteins (U1, U2, U4, U5 and U6 snRNPs) that removes introns from eukaryotic precursor messenger RNA (pre-mRNA). U6 RNA participates directly in splicing catalysis within fully assembled spliceosomes. After each splicing reaction, U6 RNA dissociates from the spliceosome and forms the U6 snRNP, which contains the Prp24 and Lsm ring proteins. Prp24 chaperones the pairing of U6 with U4 RNA, forming the U4/U6 di-snRNP, which is recruited into another round of spliceosome assembly. We are using a combination of structural, biochemical, and genetic approaches to elucidate the mechanism of U6 RNA recycling and assembly into the spliceosome.

We have determined a 1.7 Å resolution crystal structure of the U6 snRNP core, containing 70 nucleotides of U6 snRNA and all four RNA recognition motif (RRM) domains of the Prp24 protein. The structure reveals several novel ribonucleoprotein structural motifs, provides the first example of how a tetra-RRM protein can engage a cognate RNA, confirms the existence of the U6 telestem, and reveals a novel topology of interlocked rings of protein and RNA that sequesters the 5′ splice site-binding region of U6.

We previously showed that a stabilizing mutation in the U6 internal stem-loop, A62G, impedes U4/U6 assembly and confers a cold-sensitive growth phenotype in yeast. This cold-sensitivity can be suppressed by *cis*-acting mutations in U6 and *trans*-acting mutations in Prp24. Mapping of suppressor mutations onto the crystal structure reveals that they localize primarily to the protein-RNA interface, suggesting they operate through destabilization of the U6 snRNP. This finding supports a model proposed by Guthrie and coworkers that Prp24 and U4 RNA compete for binding to U6 RNA.

We have found that an *in vitro* assay for Prp24-dependent annealing of U4/U6 can recapitulate the cold-sensitive phenotypes observed *in vivo*. This *in vitro* system will allow us to determine the molecular basis for the observed phenotypes, and the mechanism of action of the suppressor mutations. It will also allow us to test our model that a basic groove on the surface of Prp24 nucleates U4/U6 annealing.

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156 An early assembly intermediate containing tri-snRNP forms during both exon- and intron-defined splicing, and its conversion to a stable B complex involves structural rearrangements triggered by tri-snRNP/5′ splice site interaction

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Previous studies from our lab revealed that cross-exon spliceosomal complexes contain U4/U6.U5 tri-snRNP in addition to U1 and U2. A 5′ss-containing RNA oligonucleotide (5′ss oligo) added in trans, binds the tri-snRNP and stabilizes its interaction, converting the 37S cross exon complex into a stable 50S complex (denoted B-like), whose protein/snRNA composition are similar to cross-intron B complexes. Here we investigated the requirements for stable B/B-like complex formation. 2D PAGE of cross-exon complexes demonstrated that whereas U1 snRNP is lost, several so-called B-specific proteins are recruited upon tri-snRNP stabilization, resulting in only a small change in overall mass. Electron microscopy revealed major structural differences between 37S and 50S exon complexes, with the former exhibiting a more elongated shape, while the structure of B-like complexes was highly similar to that of cross-intron B complexes. Thus, within the exon complex, the tri-snRNP/5′ss interaction triggers a structural rearrangement leading to stable tri-snRNP integration. The latter can be achieved by adding the 5′ss oligo to purified 37S exon complexes in the absence of splicing extract and ATP, indicating that all components required for tri-snRNP stabilization are present in the 37S complex. Thus, a subset of the B-specific proteins do not appear to contribute to stable tri-snRNP integration. Mutation of the 5′ss oligo indicated that base pairing with the U6 ACAGAG box is not sufficient to trigger the observed structural rearrangement, but instead the latter likely requires Prp8/5′ss contacts. Using a dominant negative, ATPase-deficient hPrp28 mutant, we blocked cross-intron spliceosome assembly prior to stable B complex formation, but after initial docking of the tri-snRNP. Affinity purification of this novel 37S cross-intron assembly intermediate revealed that it is compositionally similar to the 37S cross exon complex, indicating that the cross-exon and cross-intron pathways both involve an intermediate where tri-snRNP has docked, but is not yet stably-associated. The 37S cross-intron complex is also converted into a 50S complex with stably-integrated tri-snRNP when a 5′ss oligo is added in trans, and their structures are currently under investigation. Our data provide new insights into the requirements for stable B complex formation, and the switch from a cross-exon- to cross-intron-defined spliceosome.

157 Brr2 and Prp8 retinitis pigmentosa alleles impact the efficiency and fidelity of multiple steps in the splicing cycle

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Defects in splicing are responsible for many human diseases. In retinitis pigmentosa (RP), a common form of heritable blindness, a subset of autosomal dominant RP alleles affect core splicing components of the U4/U6-U5 tri-snRNP including the Brr2 helicase and Prp8, which regulates Brr2 activity through its C-terminal Jab1/MPN domain. Brr2 RP alleles map to both to the first ATPase domain as well as to the Brr2 ratchet helix, responsible for unwinding U4/U6 during catalytic activation of the spliceosome. Prp8 RP alleles map to the Jab1/MPN domain. When introduced into yeast, our lab and others have shown that these Brr2 and Prp8 RP alleles decrease Brr2 helicase and/or ATPase activity, suggesting that RP pathogenesis arises from a defect in the efficiency of spliceosome activation. However it is not known whether this defect directly impacts splicing fidelity. We used the well-characterized ACT1-CUP1 splicing reporter system, in which the actin intron must be accurately and efficiently removed to allow growth on otherwise toxic levels of copper, to determine if Brr2 and Prp8 RP alleles exhibit changes in splicing fidelity. We show that a subset of Brr2 RP alleles that map to the Brr2 ratchet helix show decreased copper tolerance in response to alterations in the branch-site, but not the 3′ splice site, indicative of a defect in the first step of the splicing reaction. We further show that particular Prp8 RP alleles have decreased copper tolerance specifically in response to an altered 3′ splice-site, suggesting that that this growth defect reflects alterations in the regulation of Brr2 activity at the second step of splicing. We hypothesize that in humans these RP mutations create hyperaccurate spliceosomes in which suboptimal splice-sites used in alternative splicing in the retina are precluded. Taken together, our data link Brr2 and Prp8 as a regulators of splicing efficiency and fidelity at multiple points in the splicing cycle, and implicate the regulation of splicing fidelity as contributing to pathogenesis in retinitis pigmentosa.

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158 Crystal structure of a eukaryotic group II intron lariat

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The splicing of both group II and spliceosomal introns results in the formation of an intramolecular 2′-5′ phosphodiester bond resulting in a branched intron RNA called the lariat. Lariat formation affects 5′ splice site selection and defects in this process result in aberrant splicing and human disease. Formation of this linkage during RNA splicing is also highly conserved across all kingdoms. Here we present the crystal structure of 623-nucleotide eukaryotic group II intron in the lariat form. We can visualize the architecture required to place the bulged adenosine over the 5′ end of the intron to facilitate lariat formation. The structure also reveals new functional roles for the conserved 5′ end as well as domains 2 and 3. In the catalytic core, we can see multiple novel monovalent and divalent ions comprising the active site. This represents the first structure of a 2′-5′ branched RNA molecule.

159 The spliceosomal DExD/H-box ATPase Prp16 chaperones splice site selection

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Gene expression requires high fidelity at all stages. In contrast to fidelity mechanisms in transcription and translation, fidelity mechanisms in splicing remain poorly understood. To ensure fidelity, the spliceosome employs DExD/H-box ATPases to discriminate against suboptimal splice sites, but it has remained unclear how these factors promote the high specificity of intron excision. By assaying substrate conformation by single molecule fluorescence energy resonance transfer, we revealed that the DExD/H-box ATPases Prp16 and Prp22 proofread and promote splice site selection at the catalytic stage through a common mechanism of substrate undocking. Further, although Prp16 is canonically required only for exon ligation, we unexpectedly discovered that in the rejection of suboptimal branch sites, Prp16-dependent undocking allowed re-docking and selection of alternative branch sites, establishing that spliceosomal DEAH-box ATPases can function as RNA chaperones to facilitate alternative splicing. This re-docking activity additionally permitted us to readily investigate the mechanism of Prp16-dependent branch site undocking, independent of exon ligation. Our preliminary results indicate that Prp16, like the spliceosomal DExD/H-box ATPases Prp2 and Prp22, acts on single-stranded substrate RNA downstream of the catalytic core. These data strongly suggest a common mechanism for DExD/H-box ATPases at the catalytic stage involving 3′ to 5′ translocation along the splicing substrate to transiently disrupt catalytic core interactions and to facilitate splice site selection.

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160 Identification of the 3′SS substrate binding site in the spliceosome second-step active site

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The spliceosome removes introns via two trans-esterification reactions, and there have been long-standing questions about the relationship between the first- and second-reaction active sites. In the first reaction, the two substrates (the branch site nucleophile and the 5′SS) are bound and positioned by base pairing with U2 snRNA and U6 snRNA, respectively. For the second reaction, much less is known about substrate-binding sites. In particular, the 3′SS UAG/ is recognized early in assembly by U2AF35 in many organisms, but this interaction is thought to be disrupted upon stable U2 snRNP binding and complex A formation; essentially nothing else is known about 3′SS UAG/ binding subsequent to this or specifically for the second reaction.

We previously developed an orthogonal, or second-copy, spliceosome system to investigate first-step catalysis using complete replacement of the branch sequence–U2 snRNA duplex. To investigate the second step, we envisaged a model in which, after the first-step catalysis, the formed lariat structure is removed from its first-step binding site and translocates on the triplet repeat GUAGUA in U2 snRNA, revealing the binding site for the 3′SS UAG/, in which the UA would be base-paired to U2 snRNA and the ultimate G of the intron replaces the bulged branch-site adenosine in a geometry similar to the first-step nucleophile. We tested this model using 64 combinations of mutant 3′SS and cognate branch–U2 snRNA pairs. Indeed, traditional W-C compensatory changes provide evidence of pairing between the 3′SS UAG/ and U2-GUAGUA. This can only be observed in the context of additional cognate changes between branch site and U2 snRNA for the first step.

Thus, these data identify the binding site for the 3′SS UAG/ for the second step: the 3′SS binds to U2-GUAGUA, replacing the lariat structure in the second-step core. The dual role of U2-GUAGUA in both catalytic steps explains its conservation throughout Eukarya, and translocation of the lariat structure by three nucleotides explains its triplet-repeat nature. First- and second-step core geometries are similar/mostly the same. Collectively, these data are consistent with one active site for both steps, with only minor differences.

161 Transient RNA structure features are evolutionarily conserved and can be computationally predicted

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State-of-the-art methods in RNA secondary-structure prediction focus on predicting the final, functional structure. However, ample experimental and statistical evidence indicate that structure formation starts immediately during transcription and this **co-transcriptional folding** influences the resultant final RNA structure. Thus, identifying the transient structures that are formed co-transcriptionally may bring insight into understanding how co-transcriptional folding leads to the final conformation *in vivo*. As RNA secondary-structures are currently best predicted by comparative approaches, we therefore investigated whether homologous RNA genes not only assume the same final structure, but also share structural features during the co-transcriptional folding *in vivo*. For this, we compiled a non-redundant data set of 32 transcripts deriving from six different RNA families which constitutes the most comprehensive data set with experimentally confirmed transient and alternative RNA structures so far. We present solid statistical evidence that homologous RNA genes from related organisms fold co-transcriptionally in a similar way (J.Y. Zhu *et al*., Nucleic Acids Res, 2013). In particular, we show that some transient structures are highly conserved with levels similar to those of the final, functional structure. Moreover, we find that the predicted co-transcriptional folding pathways of homologous sequences encounter similar transient structure features, and that these features often coincide with known transient features. We thus also predict candidates for these evolutionarily conserved transient features of co-transcriptional folding pathways *in silico*.

We further expand 4 alignments from the aforementioned dataset using search via covariance model and manual curation in order to share them with the RNA community. These alignments either update the existing Rfam datasets with annotation of transient structures, or introduce new RNA family: (1) Trp operon leader, where alternative structures are coordinated to regulate the operon transcription in response to tryptophan abundance (2) HDV ribozyme, where the self-cleavage activity is modulated via transient structures involving the extended 5′ flanking sequence (3) 5′ UTR of Levivirus maturation protein, where a transient structure temporarily postpones the formation of the final structure that inhibits the translation of maturation protein (4) SAM riboswitch, where the downstream gene expression is regulated by alternative structures upon binding of SAM.

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162 Accurate and generic approaches for novel motif discovery in large RNAs using differential SHAPE

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Modeling RNA secondary structure, assessing the accuracy of RNA structural models, and discovering new functional motifs are challenging problems confounded by the length and complexity of large RNAs, including viral RNA genomes. Pseudoknots, or non-nested RNA base pairs, are overrepresented in functionally important regions of RNA but are particularly challenging to model. Improvements in structural modeling accuracy can be achieved by the inclusion of SHAPE (selective 2′-hydroxyl acylation analyzed by primer extension) data, however some of these models remain imperfect. We have created experimental and algorithmic approaches that improve the accuracy of large-scale RNA structure modeling, allow for the detection of pseudoknots within a large RNA, and identify regions of well-folded structure. First, comparing reactivity differences between the SHAPE reagents 1M6 and NMIA (differential SHAPE) allows for the detection of local non-canonical and tertiary interactions. Second, windowed folding approaches allow pseudoknot discovery to be scaled to arbitrarily large RNAs. Third, Shannon entropies of base pairing can be used to evaluate the well-determinedness of a given fold. Including differential SHAPE on a test set of challenging RNAs with well-established secondary structures results in prediction sensitivities exceeding 93% with essentially zero long-range errors. Using a windowed pseudoknot modeling algorithm, we discovered three new pseudoknots in the HIV-1 RNA genome, which were verified in viral competition experiments and SHAPE-detected structure disruption. Using differential SHAPE and Shannon entropies, we implemented a windowed searching algorithm to find regions with low SHAPE reactivity (well-structured) and low Shannon entropy (single-structure) in the HIV-1 genomic RNA. We recovered known structures in the U5, RRE, and U3 regions and also identified multiple previously unreported structured elements. Differential SHAPE, windowed folding algorithms, and concurrent SHAPE and Shannon entropy analysis are concise and powerful approaches for creating accurate, high quality models of complex RNAs, and for discovering new motifs, even in well studied RNAs.

163 Three classes of conformations are a necessary structural feature of the Retinoblastoma 5′ UTR

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Structural conservation of specific motifs is a hallmark of RNA evolution. Base pairs in eukaryotic 5′ and 3′ untranslated regions (UTRs), however, generally do not co-vary sufficiently to reveal important structural motifs. The 5′ UTR of *RB1*, an important tumor suppressor, is typical in that no RNA structural motifs are identified from evolutionary analysis alone. Several specific mutations in this 5′ UTR are causative of retinoblastoma in individuals suggesting translation control as a potential tumor driver in these individuals.

SHAPE-directed structure determination indicates that the human *RB1* 5′ UTR adopts three distinct structural conformations. Retinoblastoma-associated mutations collapse the structural ensemble into single clusters with varying degrees of structural diversity as determined by suboptimal structural sampling analysis with SHAPE data. These data confirm that the human *RB1* 5′ UTR is a novel riboSNitch and that SNP-induced structural change can drive cancer. The fact that the cancer-associated mutations collapse, rather than expand, the structural ensemble is also a novel feature of this particular riboSNitch. These changes in fact suggest that adopting more than one distinct conformation is functionally important to *RB1* mRNA post-transcriptional regulation.

The *Bos taurus* (cow) and *Trichechus manatus latirostris* (manatee) are significantly divergent in sequence to human when comparing *RB1* 5′ UTR homologs. SHAPE-directed structure determination of cow and manatee *RB1* 5′ UTRs reveal multiple alternative clusters of conformations analogous to those observed in the human wild-type, but not disease-associated, UTR. Our data suggest that adopting three equivalently probable conformations is necessary for healthy *RB1* regulation in humans and that multiple conformations are selected for during evolution in this tumor suppressor.

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164 Three-dimensional structure determination of large RNAs using small-angle X-ray scattering

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RNA plays important roles in biology. However, knowledge of three-dimensional structure of RNA is very limited comparing to protein counterpart. The disparity is due to technical limitation of the current methods. Here we present a novel method for the structure determination of RNA using small angle X-ray scattering (SAXS) data and secondary structural information as input. SAXS offers obvious advantages as it is recorded with a high accuracy in solution at a synchrotron facility with a very little sample without a need for crystallization and has basically no size limit. In our program, each residue in an RNA is treated as a "glob". The "glob" approximation allows fast evaluation of Debye equation in a simulated annealing. The program efficiently samples various conformations directed towards minimizing the Chi square difference between the experimental and calculated SAXS data. At the level of approximation considering each nucleotide as a "glob", our method is able to determine RNA structure with various structural complexity at 4-5 Å resolution. The output of the calculation is RNA structural coordinates, comparing to the bead models and molecular envelopes using current popular programs. The development of this program represents a significant step forward in RNA structural biology.

165 Characterizing transient base-pair rearrangements in RNAs by Nitrogen relaxation dispersion NMR

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Microsecond-to-millisecond time scale dynamics plays a particularly import role in functions of biomolecules. The relaxation dispersion NMR spectroscopy has been widely used to characterize transient states in proteins that are sparely populated (as low as ~0.1%) and short-lived (lifetimes ranging between microseconds and milliseconds). Although the amide ^{15}N relaxation dispersion measurement has gained extensive success in studying protein dynamic, its counterpart in nucleic acids, the imino ^{15}N type experiment, has never been reported in RNAs. In this work, we applied this approach to two RNA systems: a 29-nt ribosomal A-site internal loop and a 56-nt P5abc domain of Tetrahymena group I intron ribozyme. In both cases, we revealed the existence of excited states involving secondary structure rearrangements with population of 3%~4% and exchange rate of a couple of milliseconds. Due to the relatively smaller number and well resolved spectra of imino resonances, this approach paves the way for future studies of secondary structure reshuffling in larger RNA systems.

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166 Kinetics and Thermodynamics of Domain Docking in the Juctionless Hairpin Ribozyme

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The hairpin ribozyme is a well-studied self-cleaving catalytic RNA that consists of two internal loops, denoted A and B. The two loops undergo major structural rearrangements to form an intricate RNA-RNA tertiary interface. In nature the two loops are connected by a four-way junction. As a complement to spectroscopic studies, we are examining the docking properties of a junctionless (trans-docking) system in which the two loops reside on separate molecules. By utilizing temperature-dependent surface plasmon resonance (SPR) we have been able to characterize the thermodynamics and kinetics of docking for the junctionless hairpin ribozyme. We have also investigated the effect of the 2′-*O*-methyl modification commonly used to prevent cleavage in biophysical and structural studies. For this purpose, cleavage-inactivating mutations in the catalytically critical residue A38 were introduced. In agreement with recent single-molecule kinetic fingerprinting studies, we find that the active site 2′-*O*-methyl results in a significant destabilization of the bound species compared to the native 2′-OH species. For the native species, the docking interaction is completely enthalpically-driven, with the entropy term small and unfavorable. Interestingly, docking of the 2′-*O*-methyl species is substantially less favorable enthalpically, with partial compensation from a favorable entropic term. Activation energies were determined for docking and undocking in the 2′-OH species but were undeterminable in the 2′-*O*-methyl species due to nonlinear Arrhenius curves, suggesting a significant disruption of the energy landscape between the two species. Comparison of our results with other systems emphasizes the diversity of thermodynamic contributions for RNA-RNA tertiary structure formation, as well as the unexpectedly significant effect of an inactivating modification on the energy landscape for tertiary structure formation.

167 Quantitative analysis of RNA modifications during bacterial ribosome assembly

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RNA components of the *E. coli* ribosome contain a total of 36 nucleosides which are chemically modified using site-specific protein enzymes. Ribosomal RNA (rRNA) modifications are introduced during the complex multi-step biogenesis process, where modifications are coupled to RNA folding, RNA processing, and protein assembly events temporally and functionally. To better understand these dependencies, we have developed a stable isotope labeling and mass spectrometry (MS) method for quantitative analysis of individual rRNA modifications in 16S and 23S RNA. Our work enables convenient monitoring of both rRNA methylations and pseudouridines by MS using a novel metabolic labeling approach.

Uisng quantitative MS analysis of assembly intermediates isolated from the wild type *E. coli* cells we have revealed that 16S and 23S rRNA are modified at different stages of the small and large subunits assembly and in a schedule comparable with binding of the ribosomal proteins. Furthermore, by combining our MS approach with genetic perturbations to the assembly pathway, such as deletions of the ribosome assembly factors, we begin to understand how rRNA modifications are integrated in the existing framework of ribosome assembly. Overall, our results demonstrate the utility of quantitative MS for mechanistic studies of rRNA modifications during assembly and biogenesis. We are extending this approach to quantitatively monitor RNA modifications in yeast and human cells.

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168 Widespread N^6-methyladenosine-dependent RNA Structural Switches Regulate RNA-Protein Interactions

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RNA-binding proteins (RBPs) control all aspects of RNA biology through binding at the single-stranded RNA binding motif (RBM). However, RBMs are often buried within their local RNA structures, and the mechanism regulating RBP-RBM interaction remains unclear. N^6-methyladenosine (m^6A) is the most abundant mRNA modification with still poorly characterized function. Here we present the regulation of mRNA/lncRNA-RBP interactions by m^6A-dependent RNA structural switch, termed m^6A-switch. We show that m^6A can alter its local RNA structure and increase the RBM accessibility to facilitate its interaction with heterogeneous nuclear ribonucleoprotein C (hnRNP C). Through combining PAR-CLIP and MeRIP-Seq, we identify over 35,000 m^6A modified hnRNP C binding sites. In addition, reducing global m^6A-RNA methylation decreases hnRNP C binding activity transcriptome-wide. We identify over 2,500 m^6A-switches for hnRNP C binding in mRNA/lncRNA among a wide range of cellular processes. These findings uncover a novel mechanism for regulating RNA-RBP interactions through m^6A-induced RNA structural remodeling.

169 Structure and function of 23S rRNA m^{6}A methyltransferase RlmJ

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Ribosome assembly in *Escherichia coli* involves post-transcriptional modifications of ribosomal RNA (rRNA) by site-specific enzymes, but the detailed understanding of the mechanism of many of these enzymes and the function of the modifications still lack. We have structurally and functionally characterized the S-adenosyl methionine (SAM)-dependent methyltransferase (MTase) RlmJ that methylates the N^6 atom of A2030 in domain V of *E. coli* 23S rRNA.

The 1.9 Å crystal structure of RlmJ represents the first structure of a mono-methylating MTase acting on the N6 position of an RNA substrate. The structure consists of a Rossmann-fold MTase domain with an inserted helical subdomain unique to the RlmJ family. Upon binding of the cofactor SAM and a substrate analogue adenosine monophosphate (AMP), structural rearrangements occur in four loop regions causing constriction of the active site. The N-terminal motif X tail undergoes a large movement to cover the SAM-binding site and trigger active-site changes in motifs IV and VIII. AMP binds in a partly accommodated state with the target N6 atom 7Å away from the sulfur atom of the cofactor. The active site of RlmJ is similar to m^{6}A MTases acting on DNA substrates, suggesting that these enzymes have a common evolutionary origin. RlmJ binds its substrate base similarly to DNA MTases T4Dam and M.*Taq*I.

The SAM- and adenosine-binding sites involve an intricate network of hydrogen-bond interactions between the conserved residues Y4, H6 and K18 in motif X and D164 in the catalytic motif IV. The critical role of this interaction network for MTase activity was confirmed by site-directed mutagenesis.

We showed by primer-extension analysis that RlmJ can methylate A2030 of *in vitro* transcribed 23S rRNA, demonstrating that no prior nucleotide modifications or ribosomal proteins are needed in its substrate. Further, in a tritium-labelling assay we demonstrated adenosine-specific methylation activity on a minimal substrate consisting of a 27-nucleotide hairpin corresponding to helix 72. The current status of the project will be presented.

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170 A comprehensive catalogue of the *C. elegans* dsRNAome

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Recognition of double-stranded RNA (dsRNA) as a pathogen associated molecular pattern (PAMP) and subsequent activation of innate immunity pathways is an evolutionarily ancient host defense mechanism in metazoans. However, recent studies in organisms ranging from worms to humans reveal that perturbation of dsRNA-mediated signaling pathways can activate innate immunity in the absence of pathogens, suggesting an endogenous pool of dsRNA is also capable of activating the innate immune response. Multiple human diseases including obesity, diabetes and cancer, display a chronic low-grade inflammation that has been linked to activation of dsRNA sensors, which highlights the relevance of endogenous dsRNA signaling pathways to human health. However, progress toward understanding the mechanisms underlying endogenous dsRNA signaling in *C. elegans* and other organisms is currently hindered by an incomplete annotation of dsRNA-producing genes. In order to identify all dsRNAs expressed in *C. elegans*, we have developed a bioinformatics workflow to detect clusters of RNA editing sites in RNA-seq data, since RNA editing by Adenosine Deaminases that act on RNA (ADAR) is strictly limited to dsRNA. Using a combination of alignment algorithms to map both unique and repetitive reads, we detected as many as 1,775 editing-enriched regions (EERs). We found that the majority of EERs were associated with protein coding genes, approximately 6.5% of all genes in *C. elegans*, and that EERs are primarily embedded within long introns and 3′UTRs. A significant fraction of EERs are located less than 1000 base pairs downstream of protein coding genes and are likely to represent a previously undetected population of edited 3′UTRs. Many EERs are predicted to possess strong internal secondary structures as well as sequence complementarity with other EERs, indicative of both intramolecular and intermolecular dsRNAs. In addition to numerous EERs associated with coding genes, our analysis identified a population of EERs distant from protein coding genes with no overlapping genome annotation and little to no coding potential. By combining basic RNA sequencing with freely available bioinformatics tools, our workflow provides an easily accessible approach for the identification of dsRNAs, and more importantly, a comprehensive catalogue of the dsRNAome of *C. elegans*.

171 The RNA editing enzyme ADAR1 is a key regulator of innate immune responses to RNA

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Both ADAR1 and ADAR2 can deamination of adenosine (A) to inosine (I) within double stranded (ds) RNA. ADAR1 is the enzyme responsible for promiscuous editing of long dsRNA, where up to 50% of A residues can be converted to I. It is estimated that in humans 0.4% of RNA editing events result in recoding while the majority, which is over 100 million editing events, predominantly occurs within transcripts encoding Alus. The questions arises what is the biological role of this widespread promiscuous editing that is catalysed by ADAR1?

Mutations in ADAR1 have been shown to cause the autoimmune disorder Aicardi Goutières syndrome (AGS). Patients with AGS display heightened levels of type-I interferon (IFN) and IFN stimulated genes (ISGs). We have characterised the editing activity of these ADAR1 mutants identified in the AGS patients and find that they have reduced editing activity. Interestingly, the mutations have a greater effect on the IFN-inducible cytoplasmic isoform, ADAR1 p150 than on the constitutive ADAR1p110 isoform and this is consistent with clinical observations of the disease.

The Adar1 mutant mouse is embryonic lethal, dying by E12.5 with an immune phenotype similar to that observed in the AGS patients. We have rescued the embryonic lethality to birth by generating a double homozygous mutant with Adar1 and another mutant in the innate immune pathway. The heightened innate immune response is rescued in the double homozygous mutant at E12.5. This indicates that the aberrant immune response underlies the embryonic lethality in the Adar1 mutant mouse. To elucidate ADAR1's role in the immune response we generated Adar1-/-; p53 -/- MEFs. By reintroducing various ADAR isoforms into the Adar1-/-; p53 -/- MEFs we find that to rescue the aberrant immune response requires both catalytic activity and the presence of an ADAR protein within the cytoplasm.

Overall, A-to-I editing by ADAR1 is an essential RNA modification required by the cell and in its absence there is an aberrant immune induction that results in autoimmune disease. This immune regulatory function of ADAR1 is evolutionary conserved and is found in Drosophila.

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172 Editing of miRNAs fine-tunes synaptic gene regulation

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Highly polarized cells, such as differentiated neurons, requires a sophisticated network of regulatory events to fine-tune gene expression in response to developmental as well as environmental cues. One of the most powerful regulatory network is the post-transcriptional silencing of genes by microRNAs (miRNA). Primary miRNAs, consisting of small stem loop structures of non-coding RNAs, are perfect targets for the ADAR editing enzymes. We, and others, have shown that several miRNAs are subjected to adenosine-to-inosine (A-to-I) editing by the adenosine deaminases ADAR1 and ADAR2 in mammals. Inosine is structurally similar to guanosine and ADAR editing is thus a functional A to G change. Intriguingly, several of the miRNAs subjected to editing are encoded within a polycistronic cluster, called the miR-370-410, compromising over 50 different miRNAs. Two of these, miR-376b and miR-381, are edited within the crucial target recognition seed-sequence and are therefore no longer fully complementary to the same mRNA targets. We provide evidence that editing acts as a negative regulator of these miRNAs and is thereby fine-tuning translation of the RNA-binding protein, Pumilio 2, important for neuronal homeostasis as well as memory and learning. We will also discuss the impact of editing of miR-381 in its regulation of the brain-derived neurotrophic growth factor (BDNF), important for neuronal survival and synapse formation. In neurons, dendritic miRNAs and mRNAs are crucial for the regulation of local protein synthesis at synapses. Editing of synaptic miRNAs such as miR-376b and miR-381 could be a way to fine tune the growth of dendrites necessary for synaptic plasticity.

173 Understanding alternative polyadenylation in preeclampsia

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Preeclampsia is a disorder that affects 2-16% of pregnancies worldwide, with high mortality rates for both mother and fetus. One cause of preeclampsia is increased levels of a protein called soluble Fms-related tyrosine kinase 1 (sFlt1). sFlt1 is encoded by the gene Fms-related tyrosine kinase 1 (FLT1), which produces two major protein isoforms: (1) a transmembrane bound full–length Flt1, a tyrosine kinase receptor participating in vascular endothelial growth factor (VEGF) signaling; and (2) a truncated and blood circulating form, sFlt1, which lacks the transmembrane anchoring domain and acts as an antagonist to full-length Flt1 protein. Relative production of these isoforms is the result of alternative polyadenylation, either in the 3′-UTR to create the mRNA encoding full-length Flt1 or within an upstream intron to create the mRNA encoding sFlt1. In order to understand why sFlt1 levels increase during preeclamptic pregnancies, we want to determine whether (1) there is an overall increase in FLT1 gene expression and/or (2) there is a preferential switch in polyadenylation sites from those in the 3′-UTR to those in the upstream introns. We would also like to determine what other genes are differentially expressed in preeclampsia versus non-preeclampsia conditions. To this end, we made polyadenylation site sequencing (PAS-Seq) and RNA-Seq libraries using RNA from normal and preeclamptic placentae, allowing us to compare alternative polyadenylation sites and measure gene expression in a transcriptome wide manner.

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174 Pin1 regulates mRNA 3′ end processing during the DNA damage response

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The DNA damage response (DDR) involves rapid functional and structural changes in a number of nuclear proteins, resulting in a coordinated control of gene expression and DNA repair. mRNA 3′ end processing, an essential step in eukaryotic RNA metabolism, regulates the steady-state levels of different mRNAs and contributes to the cells rapid response to stress. Poly(A)-specific ribonuclease (PARN), the major nuclear deadenylase, regulates the length of mRNAs 3′ end poly(A) tail and, consequently, mRNA stability and gene expression. Previously, we showed that PARN is functionally connected to the tumor suppressor p53. While PARN keeps p53 levels low by destabilizing p53 mRNA in non-stress conditions; the increase in p53 levels after UV treatment results in PARN deadenylase activation in a transcription-independent manner. Together these results show a feedback loop between PARN deadenylase and its target p53 mRNA.

Interestingly, Pin1 has also been shown to regulate the steady state levels of mRNAs in the p53 signaling pathway. Pin1 is a prolyl isomerase that catalyzes the cis/trans isomerization of peptidyl-prolyl peptide bonds in phosphoproteins, such as phosphorylated p53. Previous studies have suggested a potential role for Pin1 in mRNA 3′ end processing. Together these studies indicate a functional overlapping between PARN deadenylase and Pin1. Consistent with this, our studies reveal that Pin1 forms a complex with PARN and p53 under DNA damaging conditions. Pin1 inactivation by juglone treatment or by siRNA-mediated knockdown decreases deadenylation before and after UV treatment in nuclear extracts from HCT116 cells, indicating that Pin1 is an activator of nuclear deadenylation. Finally, Pin1 inactivation reverses the previously described UV-induced inhibition of mRNA 3′ cleavage. These preliminary results suggest that Pin1-associated p53 might participate in the regulation mRNA 3′ processing, and therefore gene expression, during the DDR. As Pin1 is overexpressed in most human cancers and downregulated in the neurons of Alzheimer's disease patients, these studies will give us a better understanding of the role of mRNA 3′ processing and these regulatory factors in these diseases.

175 Genome-wide analysis of RNA polymerase II distribution in CDS-associated polyadenylation sites in *Arabidopsis*

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In plants, alternative polyadenylation (APA) plays roles in the regulation of flowering time, oxidative stress responses, and the expression of genes involved in RNA processing. The link between various biological processes and APA modulation indicates that poly(A) sites selection is under active control at which gene expression can be regulated. Coding region-APA is the event least understood, such should lead to transcripts without an in-frame stop codon, and affected mRNAs should be subject to rapid degradation via the nonstop decay mechanism. However, in some cases, an in-frame stop codon is created using the poly(A) tail sequence producing different protein isoforms.

In Arabidopsis, poly(A) sites that lie within coding regions are associated with a non-canonical putative polyadenylation signal. This, and the paradoxical outcomes of polyadenylation within coding regions, raises questions as to how these RNAs are formed. To explore this, we are conducting a study of the association of different forms of RNA polymerase II with sequences associated with coding region polyadenylation. The C-terminal domain (CTD) of RNA Pol II is composed of consecutive repeats of the sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser and the main stages of the transcription process are associated with distinct phosphorylation and dephosphorylation states of CTD. Specifically, the nonphosphorylated form of Pol II is involved in the preinitiation complex formation; Ser7P-CTD occurs early in transcription; Ser5P-CTD participates in transcription initiation and early elongation, and the Ser2P-CTD modification recruits factors for mRNA polyadenylation and transcription termination. By comparing the distributions of these forms of Pol II in genes with and without coding region poly(A) sites, we expect to determine whether polyadenylation at these sites involves canonical mechanisms.

Chromatin immunoprecipitation coupled to deep sequencing (ChIP-seq) using specific antibodies, each directed against one of the different phosphorylated forms of CTD, has been performed to analyze changes in RNA Pol II occupancy along the lengths of CAPs-associated transcription unit. Early results suggest that the distributions of the Ser7-P and Ser2-P forms of Pol II around coding region poly(A) sites resembles those seen around sites situated within 3′-UTRs. This supports the hypothesis that coding region polyadenylation involves mechanisms similar to those used for canonical polyadenylation.

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176 Post-transcriptional control of gene expression including alternative polyadenylation plays a major and unexpected role in the cellular stress response

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Post-transcriptional steps of the RNA processing pathway have emerged as important hubs for fine-tuning gene expression in response to different stimuli and to play a critical role in human pathophysiology. Here, we investigated the role of post-transcriptional regulation of gene expression in response to stress by specific pre-mRNA-seq and mRNA-seq analysis. We found more than half of all mRNAs which are up-regulated by stress to be unchanged at the pre-mRNA level and thus to be controlled by post-transcriptional mechanisms. These post-transcriptionally up-regulated genes were significantly enriched for transcription factors in general and for C2H2-Zn-finger proteins in particular. These findings suggest that the early phase of the stress response is induced by post-transcriptional mechanisms that rapidly stimulate a specific transcriptional program that is critical for the later phase of the stress response.

Based on previous results of our group, which implicated regulated 3′ end processing as an important component of the stress response (Danckwardt et al. Mol Cell 2011), we have extended the RNA-seq data set by a specific analysis of poly(A) site usage. We established a high-coverage transcriptome-wide poly(A) site map and detected a large number of previously not annotated, high-confidence poly(A) sites. Many of these novel sites become apparent as a consequence of stress-induced alternative polyadenylation (APA) thereby controlling 3′UTR length thus likely affecting mRNA stability or the length of the C-terminal coding region. Taken together, our data show that post-transcriptional regulation of gene expression generally and alternative mRNA 3′end processing specifically represent fundamental mechanisms for maintaining cellular homeostasis under stress conditions.

177 Regulation of COX-2 expression by post-transcriptional mechanisms

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Prostaglandins are a class of molecules that mediate cellular inflammatory responses and control cell growth. The oxidative conversion of arachidonic acid to prostaglandin H2 is carried out by two isozymes of cyclooxygenase, COX-1 and COX-2. COX-1 is constitutively expressed, while COX-2 is transiently induced by external stimuli, such as pro-inflammatory cytokines. COX-2 is also overexpressed in numerous cancers, including lung cancer. MicroRNAs (miRNAs) are known to be responsive to various stimuli, and their pattern of expression can vary in cancer, which, in turn, can regulate gene expression of their target mRNAs. MiR-146a has been implicated in immune responses, and was predicted to target COX-2 mRNA. We have demonstrated that miR-146a expression is highly downregulated in lung cancer cells as compared to normal lung cells. Conversely, lung cancer cells have high levels of COX-2 protein and mRNA expression. Synthetic miR146a can specifically ablate expression of COX-2 protein and COX-2 biological activity as measured by prostaglandin production. Therefore, we propose miR-146a downregulation contributes to the upregulation and overexpression of COX-2 in lung cancer cells. Since potential miRNA-mediated regulation is a functional consequence of alternative polyadenylation site choice, understanding the molecular mechanisms that regulate COX-2 mRNA alternative polyadenylation and miRNA targeting will gives us key insights into how COX-2 expression is involved in the development of a metastatic condition.

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178 Mis-regulation of Alternative Polyadenylation in a Microsatellite Expansion Disease

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Microsatellites, or simple tandem repeats of 2-10 bp, are common but unstable genomic elements characterized by very high mutation rates due to DNA replication, recombination and repair errors induced by the non-canonical structures formed by these repetitive DNA sequences. Although tandem repeat instability is associated with >40 hereditary, primarily neurological, diseases, the molecular basis for many of these disorders remains unclear. Previously, we demonstrated that expression of C(C)UG expansion (exp) RNAs in the unstable microsatellite disease myotonic dystrophy (dystrophia myotonica, DM) leads to inhibition of MBNL protein activity and dysregulation of developmental alternative splicing. Here, we have performed PolyA-seq analysis of wild-type versus Mbnl1; Mbnl2 double knockout (DKO) mouse embryo fibroblasts to show that Mbnl protein expression is required for the normal regulation of alternative polyadenylation (APA). HITS-CLIP mapping and minigene polyadenylation reporter analyses indicate that altered polyA site (pA) selection induced by Mbnl depletion results from direct binding of these proteins to sites overlapping, or upstream, of target RNA polyA sites to promote pA skipping or selection, respectively. Aberrant APA is also a characteristic feature of a transgenic poly(CUG) mouse model of DM and in human DM skeletal muscles where loss of MBNL1 and MBNL2 activity results from sequestration of these factors in intra-nuclear CUGexp RNA foci and persistence of embryonic APA patterns in adult muscle. These observations indicate that MBNL proteins regulate both alternative splicing and alternative polyadenylation of specific RNA targets during development and suggest that DM is a pre-mRNA processing disorder.

179 Molecular Characterization of the pre-mRNA 3′ end core cleavage factor: Interactions, assembly and activity of CPSF73, CPSF100, and Symplekin

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Regulation of gene expression is vital for proper cellular differentiation, cellular proliferation, and organismal development. At the post-transcriptional level this regulation involves proper processing of pre-mRNAs. These processing events can include 5′ end modification, splicing, and 3′ end processing. A core cleavage complex consisting of three proteins (CPSF73, CPSF100 and Symplekin) is required for 3′ end cleavage of all pre-mRNAs. CPSF73 is the endonuclease responsible for catalyzing the cleavage reaction, CPSF100 forms a heterodimer with CPSF73, and Symplekin acts as a scaffolding protein. Currently we are characterizing molecular interactions in the core cleavage complex required for both assembly and catalysis, *in vivo* and *in vitro*, in the model organism *Drosophila melanogaster*. The first goal is to understand which regions of the cleavage factor components are required for complex assembly. To this end, we created stably transfected *Drosophila* S2 cell lines expressing HA-tagged full-length, and N-terminally and C-terminally truncated core cleavage factor proteins. Using immunoprecipitation (IP) experiments we show that over-expressed, HA-tagged, full-length proteins are successfully incorporated into core cleavage complexes containing endogenous binding partners. Additionally, IP of N- and C-terminal Symplekin, CPSF73 and CPSF100 deletion mutants reveals co-immunoprecipitation of some full-length, endogenous core cleavage factor components. For example, amino acids 525-756 of CPSF100 (C-CPSF100) bind to full-length Symplekin more tightly than amino acids 1-524. Amino acids 272-1165 of Symplekin (C-Symp) interact with full-length CPSF73, but amino acids 1-271 of Symplekin do not. Amino acids 466-685 of CPSF73 (C-CPSF73) bind to Symplekin, while amino acids 1-465 do not. From these experiments we can construct an accurate *in vivo* core cleavage complex model. Analysis of mutant core cleavage complex activity is also being investigated. To assess the activity of only mutant complexes, endogenous proteins are first depleted by RNAi and mutant complexes are created with RNAi-resistant proteins. RNA is then isolated from these cells and the 3′ ends of endogenous histone mRNAs are mapped using S1 assays. Collectively, these experiments are beginning to elucidate a general model of regional binding interactions as well as specific direct interactions required for assembly and activity of the core cleavage complex.

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180 hnRNPs repress alternative polyA sites to promote formation of longer 3′UTRs

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Alternative polyadenylation is increasingly being recognized as an important mechanism to control gene expression, however the factors controlling it are still incompletely understood. For this study we used a high-throughput sequencing method (pA-seq) to identify the polyadenylation sites regulated by TDP-43 and TIA1/L1 RNA binding proteins. We further used the individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) to find that these proteins bind in the vicinity of the promoter-proximal polyadenylation (polyA) sites to promote the choice of promoter-distal pA site. Analysis of sequence motifs at the repressed pA sites revealed enriched clusters of short motifs, or multivalent motifs, similar to the ones bound by TDP-43 and TIA1/L1 when they regulate alternative splicing. A swap of the multivalent motifs in a reporter minigene confirmed that both proteins can repress the same polyA site if their binding site is positioned close to the polyA site. We conclude that multivalent RNA motifs located in the vicinity of promoter-proximal polyA sites recruit hnRNPs such as TIA/TIAL and TDP43, which repress the use of these polyA sites and thereby promote formation of longer 3′ UTRs.

181 The Poly(A)-Binding Protein Nuclear 1 (PABPN1) and the Nuclear Exosome Promote 3′ End Maturation of the Human Telomerase RNA

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Telomerase, the enzyme that maintains telomere integrity, has been extensively studied because it is activated in many cancers. In humans, the core telomerase complex consists of the human telomerase catalytic subunit (hTERT) and the human telomerase RNA (hTR), which are sufficient to reconstitute telomerase activity *in vitro*. In contrast, the biogenesis of the telomerase holoenzyme requires a variety of interacting partners *in vivo*. Accordingly, the 3′ end of hTR harbors a typical RNA structure found in H/ACA box snoRNAs that is recognized by a set of conserved factors, including the human dyskerin protein. As yet, however, the mechanism and factors involved in transcription termination and 3′ end maturation of hTR remain elusive. We previously identified a 3′ end maturation pathway for fission yeast snoRNAs that depends on the poly(A)-binding protein, Pab2, and the nuclear exosome, a complex of 3′-5′ exonucleases. Given the role of Pab2 and the nuclear exosome in 3′ end processing of snoRNAs in *S. pombe*, we examined whether the human Pab2 homolog, PABPN1, contributes to hTR synthesis. We show here that depletion of PABPN1 from human cell lines resulted in reduced levels of mature hTR and decreased telomerase activity, but in the accumulation of 3′-extended telomerase RNA. Immunopurification experiments indicated that PABPN1 interacts with 3′-extended telomerase RNA and associates with hTERT in a RNA-dependent manner. We also found that cells deficient for a component of the RNA exosome (hRRP40) or hMTR4, a cofactor for the nuclear exosome, led to the accumulation of 3′-extended telomerase RNA as well as mature hTR. Notably, we show that 3′-extended telomerase RNA co-purify with hTERT and dyskerin. Altogether, our results shed new light into the processing and maturation of the human telomerase RNA, and support a model in which PABPN1 and the nuclear exosome promote the 3′ end trimming of precursor transcripts into mature hTR.

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182 Hydrogen Deuterium Exchange Mass Spectrometry (HDXMS) and cross-linking in structural studies on the FLASH-Lsm11 complex and its interaction with polyadenylation factors

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Animal replication dependent histone pre-mRNAs are processed at the 3′ end by endonucleolytic cleavage that is not followed by polyadenylation. Cleavage depends on binding of U7snRNP to histone pre-mRNA and is catalyzed by CPSF73. U7snRNP is comprised of U7snRNA and a unique Sm ring where Lsm10 and Lsm11 proteins replace the canonical SmD1 and SmD2. We demonstrated that N-terminal domain of Lsm11 associates with FLASH and recruits the Histone Cleavage Complex (HCC)- a subset of polyadenylation factors: Symplekin, CstF64, and all six CPSF subunits, to the U7snRNP.

Bacterially expressed FLASH-Δ28NFLASH (amino acids 28-139) and N-terminal domain of Lsm11 (amino acids 1-169) form a complex, which interacts with the HCC from mammalian nuclear extracts. To characterize the interaction between FLASH and Lsm11 and to identify structural features of the FLASH/Lsm11 platform, we used Hydrogen Deuterium Exchange Mass Spectrometry (HDXMS). This method maps the interaction surfaces in two interacting proteins and provides information about conformational changes that occur during binding. Individual proteins or protein complexes are placed in deuterated reaction buffer for a defined time and immediately quenched by lowering pH (2.5) and temperature (0°C). Subsequent proteolysis generates deuterated peptide fragments that can be distinguish by mass spectrometry.

By comparing the same peptides in two different states, before and after binding, we mapped the FLASH binding site on Lsm11 and identified the beginning of the binding site for Lsm11 on FLASH. Although Δ28NFLASH is sufficient to stimulate in vitro processing, HDXMS results suggest that stable interaction with Lsm11 requires an additional downstream fragment, which we are currently defining. Based on the kinetics of the hydrogen deuterium exchange we conclude that N-terminus of Lsm11 is disordered, with only two peptides folding into secondary structures. FLASH undergoes slower exchange, suggesting that it is mostly structured.

We are also trying to identify components of the HCC that directly contact the FLASH/Lsm11 platform by conducting cross-linking experiments followed by Mass Spectrometry. These studies will characterize critical interactions among polyadenylation factors that result in forming two alternative 3′ end processing machineries, one crafted for cleavage and polyadenylation, the other dedicated to the U7-depended processing of histone pre-mRNAs.

183 U1 snRNP-dependent Regulation of Intronic Polyadenylation Linked to Tumor Dissemination

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Regulation of 3′ end processing (cleavage/polyadenylation) efficiency and alternative 3′ end processing are increasingly considered as important steps in gene regulation. Several examples highlight the essential contribution of 3′ end processing in physiological (e.g. immunity and inflammation) or pathological processes (e.g. cancer and viral infection). However, very little is known concerning the regulation of alternative polyadenylation (APA) associated with tumor dissemination and metastasis development.

We have performed a microarray-based analysis at the single exon level as well as a 3′RNA-Sequ procedure in several human/murine breast tumor cell lines with different migration/invasion properties. We found that 30% of regulated events generating alternative mRNA isoforms are related, besides alternative splicing and alternative promoter usage, to APA. Computational analysis revealed that transcripts differentially 3′-end processed between non invasive and highly metastatic tumors were preferentially involved in specific processes like motility and cell-to-cell interactions. The contribution of APA to *in vitro* cell migration and invasion was validated for some of these events by trans-well migration/invasion assays. Our data showed that the shift in polyadenylation site usage was mediated by U1 snRNP which was recently shown to be implicated in RNA surveillance in addition to its role in splicing. Moreover, our data demonstrated that functional knockdown of one of the U1 snRNP components decreased dramatically the *in vitro* cell migration and invasion capacities. Altogether, our study identified the pattern of APA regulation associated with tumor progression and showed the physiopathological significance of APA-encoded isoforms in breast cancer. The study of APA events may therefore provide novel prognostic markers for metastatic relapse.

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184 Rational experiment design for sequencing-based RNA structure characterization

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Structure mapping is a classic experimental approach for determining nucleic acid structure, which has gained renewed interest in recent years following advances in chemistry, genomics, and informatics. The approach encompasses numerous techniques that use different means to introduce nucleotide-level modifications in a structure-dependent manner. Modifications are then assayed via cDNA fragment analysis, using capillary electrophoresis or next-generation sequencing (NGS). The recent advent of NGS has dramatically increased the throughput, multiplexing capacity, and scope of RNA structure mapping assays, thereby opening new possibilities for genome-scale, *de novo*, and *in vivo* structural studies of RNA.

From an informatics standpoint, NGS is more informative than previous technologies by virtue of delivering direct molecular measurements in the form of nucleic acid sequence counts. Motivated by these new capabilities, we introduce a novel model-based *in silico* framework for quantitative design of large-scale multiplexed NGS RNA structure mapping assays, which takes advantage of the direct and digital nature of NGS readouts. We use it to characterize the relationship between controllable experimental parameters and the precision of structure mapping measurements, with particular emphasis on assays that generate cDNA fragments of fixed and *a priori* known locations. Our results highlight the complexity of these dependencies and shed light on relevant tradeoffs and pitfalls, which can be difficult to discern by intuition alone. We then use our approach to highlight the advantages and challenges associated with emerging NGS-based *in vivo* techniques, which use random priming to generate richer and more comprehensive cDNA fragment sets.

We demonstrate our approach by investigating the robustness of SHAPE-Seq measurements, obtained by multiplexing SHAPE (selective 2′-hydroxyl acylation analyzed by primer extension) chemistry, in conjunction with NGS.

185 Long-range Nucleotide Covarations in Drosophila Genomes Detected By A Novel Computational Approach

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We developed a two-stage computational approach for detecting long-range compensatory base changes (covariation) in nucleotide sequence alignments. The first stage consists of an index-based approach (called CovaRNA) for the fast detection of conserved Watson-Crick complementarity in sequence alignments on a genome-wide scale. A second tool, called CovStat, is able to compute a measure of statistical significance for the observed covariation pattern to have occurred by chance. We applied both tools to alignments of *Drosophila* genomes. We found a plethora of pairs of regions with covariation, many of them being located far apart (including different chromosomes). We find an enrichment for covariation in noncoding RNAs as well as in regions with *cis* natural antisense transcripts, suggesting that the generation of endo-siRNAs is an important driver of conserved base pairing. The results suggest that covariation is a hallmark for a multitude of processes involving nucleotide base pairing, and argue that the found long-range covariation clusters correspond to an under-appreciated layer of genomic complexity.

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186 Abstract Withdrawn

187 RNA Bricks - a database of RNA 3D motifs and their interactions

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The RNA Bricks database (http://iimcb.genesilico.pl/rnabricks), stores information about recurrent RNA 3D motifs and their interactions, found in experimentally determined RNA structures and in RNA-protein complexes. In contrast to other similar tools (RNA 3D Motif Atlas, RNA Frabase, Rloom) RNA motifs, i.e. 'RNA bricks' are presented in the molecular environment, in which they were determined, including RNA, protein, metal ions, water molecules and ligands. All nucleotide residues in RNA bricks are annotated with structural quality scores that describe real-space correlation coefficients with the electron density data (if available), backbone geometry and possible steric conflicts, which can be used to identify poorly modeled residues. The database is also equipped with an algorithm for 3D motif search and comparison. The algorithm compares spatial positions of backbone atoms of the user-provided query structure and of stored RNA motifs, without relying on sequence or secondary structure information. This enables the identification of local structural similarities among evolutionarily related and unrelated RNA molecules. Besides, the search utility enables searching 'RNA bricks' according to sequence similarity, and makes it possible to identify motifs with modified ribonucleotide residues at specific positions.

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188 Nucleic Acid Database (NDB): Redesign and focus on RNA

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ABSTRACT

The Nucleic Acid Database (NDB) (http://ndbserver.rutgers.edu) is a web portal providing access to information about the structures of nucleic acids and their complexes. In addition to the primary structural information obtained from the Protein Data Bank (PDB), NDB contains annotations and derived structural data for nucleic acids. NDB annotations include classifications based on nucleic acid type, conformation and secondary structure. Annotations related to proteins and ligands bound to nucleic acids are also available. The NDB includes derived geometric data and derived data on RNA structural features including pair-wise nucleotide interactions for each RNA structure, equivalence classes and non-redundant sets of RNA structure files, and RNA 3D motifs extracted from the structures. These features have been integrated into the search capabilities. The results from these searches are presented as structure selection reports, structure summary reports, and other featured reports. NDB also provides standards for describing nucleic acid features, and a list of tools for the analysis of nucleic acids.

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189 Identification and visualization of alternative events from RNA-Seq data using EASANA: Impact of SF3B1 mutations in uveal melanoma

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Uveal melanoma is the most common primary cancer of the eye and is divided into prognostically distinct subgroups on the basis of their transcriptome signatures. *SF3B1* is one of the very few genes that is commonly mutated in uveal melanoma. *SF3B1* gene encodes subunit 1 of the splicing factor 3b protein complex, which is a component of the U2 small nuclear ribonucleoprotein complex that participates in the splicing of pre-mRNAs. *SF3B1* was found to be recurrently mutated in myelodysplastic syndromes (Papaemmanuil *et al.*, N Engl J Med. 2011), in chronic lymphocytic leukemia (Wang *et al.*, N Engl J Med. 2011), in ER+ breast cancers (Ellis *et al.*, Nature 2012) and more recently in uveal melanoma (Harbour *et al.*, Nature Genetics 2013). Despite genome-wide analyses by RNA-Seq or splicing-sensitive microarrays, very few or no splicing defects were found to be associated with *SF3B1* mutation in these pathologies (or lack of further validations, mandatory for these kind of results).

Here, we reexamine publicly available RNA-Seq data from Harbour *et al.* (8 RNA-Seq samples: 3 uveal melanoma patients with R626C *SF3B1* mutation and 5 patients with wild-type *SF3B1)*. RNA-Seq data were analyzed for gene expression levels and regulated exons using GenoSplice EASANA analysis and visualization tool that allows identifying and visualizing different types of alternative events linked to RNA processing mechanisms. We show that *SF3B1* mutations in uveal melanoma are associated with alternative events of more than 150 genes. Eight events were selected for further extended RT-qPCR validations using total RNA extracted from Institut Curie patients (58 WT *SF3B1* patients and 16 patients with *SF3B1* mutation: R625H, K666T and K700E; Furney *et al.*, Cancer Discovery 2013). These eight events correspond to alternative terminal exons (*UQCC*, *ADAM12* and *GAS8*), alternative 3′ acceptor splice sites (*CRNDE* and *ANKHD1*), alternative cassette exons (*GUSBP1*), alternative first exon (*F8*) and intron retention (*ABCC5*).

Overall, this analysis identified formerly undetected alternative events occurring in the uveal melanoma tumors mutated for *SF3B1*, consistent with a role of *SF3B1* in alternative splicing regulation.

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190 Consistent alternative splicing isoform switches in tumor samples provide novel signatures in 10 cancer types

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Cancer genome projects have been instrumental to uncover the heterogeneity of genetic alterations in tumors [1], motivating the development of individualized treatments [2]. However, alternative pre-mRNA splicing alterations, which bear major importance in terms of the understanding of cancer [3], have not been exhaustively studied yet in the context of recent cancer genomics efforts.

We have used RNA sequencing data from The Cancer Genome Atlas (TCGA) project for more than 1000 tumor and normal samples to characterize the alternative splicing of genes in 10 different cancer types. After careful quality assessment of the samples and data normalization, we have applied a new algorithm based on the consistent reversal of relative expression of splicing isoforms to define alternative splicing isoform switches. Cross-validation allows us to find predictive models based on isoform switch rules, which include known tumor suppressors (*TPM1*, *TSC2*, *NUMB*, *FBNL2* and *QKI)* and which correctly classifies 100% of samples in each cancer type as well as for the subtypes in breast cancer (luminal A, luminal B, Her2+ and basal), colon cancer (hypermutated and non-hypermutated), and lung squamous cell carcinoma (primitive, classical, secretory and basal). We further investigate how these isoform switches may remodel the network of protein interactions and predict a number of interactions that are disrupted in specific cancer types. We provide an effective way to process and interpret large RNA sequencing datasets from cancer genomics projects, and a set of new signatures based on alternative splicing isoform switches that may suggest novel strategies for diagnosis, prognosis and therapy in cancer medicine.

1. Stephens et al. (2012). The landscape of cancer genes and mutational processes in breast cancer. Nature, 486(7403), 400-4.

2. Hudson (2013). Genome variation and personalized cancer medicine. J Intern Med. 274(5): 440-50.

3. Bechara et al. (2013) RBM5, 6, and 10 differentially regulate NUMB alternative splicing to control cancer cell proliferation. Mol Cell. 52(5):720-33.

191 Common Secondary Structure Prediction for RNA Homologs with Domain Insertions: Dynalign II

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The ENCODE (the Encyclopedia of DNA Elements) project shows that possibly more than half of the human genome is transcribed as non-protein-coding transcripts. This motivates the development of new methods to identify and study non-coding RNA in the human genome. Automated comparative prediction of RNA secondary structure is an important tool for non-coding RNA identification and functional studies that can also be executed fast enough for genome scale research.

Domain insertions, however, where a structural motif is inserted in a sequence (with respect to homologs) are prevalent. This phenomenon can affect the accuracy of RNA secondary structure prediction and has not been effectively accounted for. In this project, we introduce a novel methodology for handling domain insertions during the process of common RNA secondary structure prediction. We develop and demonstrate the methodology by developing Dynalign II, an update to the dynamic programming algorithm-based Dynalign algorithm for common secondary structure prediction for two RNA homologs. Our update introduces recursions that explicitly allow and account for inserted domains. This update is also accomplished at negligible increase in computational cost, using precomputed information from single sequence structure prediction.

Benchmarks obtained on ncRNA families with domain insertions validate the proposed method. Dynalign II has improved accuracy over Dynalign, attaining an 80.8% sensitivity (compared with 14.4% for Dynalign) and a 88.3% positive predictive value over base pairs occurring in inserted domains for tRNA, and a 69.3% sensitivity (compared with 44.8% for Dynalign) and a 60.0% positive predictive value (PPV) over base pairs in inserted domains for RNase P. Overall, Dynalign II also exhibits statistically significant improvement in sensitivity and PPV compared with Dynalign. This newly proposed method can be a starting point for improvement of RNA related automated comparative genomic analysis tools.

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192 Detecting cis-antisense elements using RNA sequencing and comparative genomics in the Archaea

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As high-throughput sequencing of archaeal genomes has increased, methods for finding regulatory elements has lagged substantially. We have performed small-RNA sequencing for a range of archaea that has illustrated the diverse distribution of *cis*-antisense RNAs in more than 15 species. Using these unique data, we have made the novel observation that more than 70% small non-coding RNA transcripts possess archaeal poly-T transcription terminators for the model archaeon *Pyrococcus furiosus*. We have built a hidden Markov model to predict these poly-T transcription termination sites for all archaeal protein coding genes and cis-antisense transcripts. Using comparative genomics to find conservation of poly-T terminators, we show that this method can be used to effectively predict novel *cis*-antisense genes in many archaeal genomes, even those without transcription data.

193 3D Modeling of Group I Intron structures by comparative modeling with ModeRNA and de novo RNA folding with SimRNA

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Group I introns is a family of widespread non-coding RNA molecules well known for self-splicing from the host precursor RNA. Thus far only Azoarcus, Tetrahymena and Twort are known and well studied structures of this family. Commonly, group I introns are classified into 14 subfamilies [1] based on conserved core sequences and peripheral structures. However, introns from particular groups have high length diversity and weak sequence similarity, which makes structure prediction for these RNAs very difficult.

To provide 3D structural models of representatives of group I introns from all families, we used a combination of comparative and de novo RNA structure modeling. We manually prepared alignment of 11 representatives (from subfamilies with unknown structures) with sequences and structures of representatives with known structures. ModeRNA [2] software was used to generate initial models of group I intron core structures by a comparative modeling approach. We defined the structural core based on the available secondary and tertiary structures, P4-P6 domain containing P4, P5 and P6 and P3-P9 domain containing P3, P7, P8 and P9. Azoarcus, Tetrahymena and Twort sharing significant similarity and common secondary structure with the representatives were chosen as templates for modeling. Fragments of models without counterparts in templates were then added and folded with a de novo modeling approach, as implemented in the SimRNA method (Boniecki, Bujnicki, and coworkers, manuscript in preparation).

The generated models of group I intron structures accurately depict the global topology, secondary and tertiary interactions. Expectedly, the accuracy is highest in the core, with RMSD between 3-4 Å, whereas deviations are larger for peripheral regions that differ substantially between different introns. The results of this analysis provide a 3D perspective for studying group I introns and for interpretation of their sequence evolution in a structural context.

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2. Rother M, Rother K, Puton T, Bujnicki JM, 2011: RNA tertiary structure prediction with ModeRNA. Briefing in Bioinformatics (2011) 12 (6): 601-613.

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194 RNA transcript complexity is associated with Chronic Obstructive Pulmonary Disease

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Chronic obstructive pulmonary disease (COPD) is a complex disorder with both genetic and environmental factors. The contribution of RNA post-transcriptional processes to COPD predisposition is not well characterized. Hundreds of genes are associated with COPD predisposition, suggesting that multiple molecular mechanisms can cause the phenotype. We established a comprehensive list of gene loci associated with COPD and compared this list with other human disease gene loci, including those associated with Type 2 Diabetes and Parkinson's disease. Our analysis reveals that COPD-associated loci have a high level of transcript diversity. These genes also have low GC content in the region of the first exon, potentially pointing to non-transcriptional regulation. Since COPD is a chronic condition aggravated by environmental factors, it is not surprising that subtle changes in multiple post-transcriptionally regulated pathways could impact risk. RNA plays an important regulatory and functional role in normal human biology; our data reveal a role for RNA regulation in COPD disease etiology.

195 eRNA: a collection of web servers for comparative RNA structure prediction and visualisation

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In computational RNA secondary structure prediction, a large variety of unique and novel tools have been published. For users however, running such tools can occasionally be a challenge due to both technical and physical system requirements. Web servers in turn can provide an easy and effective solution for users wanting to run specific programs.

e-RNA (www.e-rna.org) is such a web site, and offers a free and open-access collection of five published RNA sequence analysis tools, each solving specific problems not readily addressed by other available tools. Given multiple sequence alignments, Transat detects all conserved helices, including those expected in a final structure, but also transient, alternative and pseudo-knotted helices. RNA-Decoder uses unique evolutionary models to detect conserved RNA secondary structure in alignments which may be partly protein-coding, such as those commonly found in viral sequences. SimulFold simultaneously co-estimates the potentially pseudo-knotted conserved structure, alignment and phylogenetic tree for a set of homologous input sequences. CoFold predicts the minimum free energy structure for an input sequence while taking the effects of co-transcriptional folding into account, thereby greatly improving the prediction accuracy for long sequences. R-chie is a program to visualise RNA secondary structures as arc diagrams, allowing for easy comparison and analysis of conserved base-pairs and quantitative features.

Standardized input interface across all tools ensures easy usage and familiarity, along with example input for each tool. The e-RNA server dispatches user jobs to a cluster, where up to 100 jobs can be processed in parallel. Upon job completion, users can retrieve their results via a bookmarked or emailed link, where users may retrieve intermediate files, raw output data and visualized output graphics. When available, downloadable versions of each tool are available for users to run locally if they so desire. e-RNA is located at http://www.e-rna.org

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196 Improved quality assessment of RNA 3D models

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The understanding of the importance of RNA molecules has dramatically changed over the recent years. As in the case of proteins, the function of an RNA molecule is encoded in its three-dimensional structure, which in turn is determined by the molecule's sequence.

Therefore, there is a need to develop computational methods that are able to provide reliable models of RNA molecules based only on nucleic acid sequences. A standard workflow of structure prediction looks as follows: based on a nucleic acid sequence, a set of RNA 3D models is produced, the quality scores of models are computed, and based on the scores the final prediction is made.

In this report, we present a new computer program for assessing the quality of RNA 3D models. First, we generated alternative models (decoys) using an unfolding procedure starting with a native structure with SimRNA for five RNA structures taken from the PDB database. Next, for each alternative model energies were calculated using the following programs: RASP, RNAkb, SimRNA, FARNA/ROSETTA, NAST and measurements were recorded as: radius of gyration, clash and secondary structure agreement between secondary structure of a model and secondary structure predicted for a model's sequence.

With the use of above-mentioned values and the RMSD between a given model and the native structure, a linear regression model was calculated. Later, this model was benchmarked using testing decoys of structure 1XJR (47 nt) yielding predicted RMSDs that can be then used as a measurement of the quality of the model (large predicted RMSD equals low quality). The highest correlation with RMSDs was observed for predictions generated by our model that makes the method a useful tool for assessing the quality of models. Our method was able to correctly identify, in the set of alternative models, models similar to the native structure.

To the best of our knowledge, this is the first time where such analysis has been performed and based on the results a computer program has been developed. At this stage of testing, it appears to be a promising tool for RNA 3D structure prediction.

197 Computational analysis of the key components of the splicing and NMD machinery in *Ustilago maydis*

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Molecular mechanisms regulating alternative splicing and NMD are still not fully understood, recent data suggest that these processes are highly conserved in different organisms. *Ustilago maydis* is a basidiomycete and the best-known smut, which has become a model to study molecular and cellular eukaryotic mechanisms. As a result of the extensive studies with *U. maydis*, a vast number of molecular tools are available including the genome sequence. We have identified in *Ustilago maydis* the putative proteins that regulate splicing and NMD in this model. When comparing *Ustilago* and human, identity for most putative splicing factors ranges between 40% and 60%. Remarkably, the NMD factor UPF1 shows 65% identity between *Ustilago* and human. Other factors are highly conserved as well. In order to provide supporting evidence that the homologs identified could perform equivalent biological activities, we conducted bioinformatic analysis to compare chemical and biochemical characteristics from the fungal and human homologs. We performed molecular dynamics assays, including contact maps and dynamic simulations for snRNP U1-70K and UPF1 proteins from *H. sapiens* and *U. maydis*. Our results indicate that the putative factors identified in *U. maydis* show a very similar structure, mechanic stability, physicochemical properties and spatial organization in comparison to the human homolog. This high homology could suggest an important degree of conservation between human and fungal control of alternative splicing and NMD.

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198 *RNA-Puzzles* Round II: Assessment of RNA structure prediction of two large riboswitches

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RNA-Puzzles is a CASP-like collective blind experiment for the evaluation of RNA 3-dimensional structure prediction. The primary aims of RNA-Puzzles are to determine the capabilities and limitations of current methods of 3D RNA structure prediction based on sequence, to find whether and how progress has been made, and to illustrate whether there are specific bottlenecks that hold back the field. Ten puzzles have been set up and three assessments are published. Nine groups of modelers around the world participate in this collective effort. We now report a second round focusing on the prediction of two large riboswitches, the adenosylcobalamin and the T-box bound to a tRNA. No homologous structures existed in the databases at the time of the experiment. Although only two targets were selected, these targets provide a wealth of sub-domains (around 10), including both well-known modules like K-turns as well as new ones.

The 168nt adenosylcobalamin riboswitch consists of a ligand-bound structured core and a bent peripheral domain. Although the RMSDs of the prediction models range from 11.7 to 37.5 Å, the topology of the top ranked models are quite similar to the native structure. Top ranked models show much better scores in Deformation Index (DI) and non-Watson-Crick interaction network fidelity (nwc INF) than others, but surprisingly have worse clash scores.

The T-box and tRNA, 96 and 75nt in length respectively, form a large complex. The difficulty in prediction lies mainly in (i) the lack of homologous model for T-box and (ii) the interaction between T-box and tRNA. The RMSD range of the predictions is 6.8 to 17.4 Å and the top ranked models also have better DI score with worse clash scores.

The Das group performed best in both problems with their models ranked #1 at 14.5 and 7.6 Å, respectively. The Bujnicki group performed well in the second problem with the model ranked #1 at 10.2 Å and excellent clash scores with nwc INF around 0.5 like the models of the Das group. Further, the less well predicted models always had worse nwc INF score, demonstrating the importance of identifying non-Watson-Crick pairs and RNA modules.

199 Abstract Withdrawn

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200 Sailfish: Software for Rapid, Alignment-free Quantification of Isoform Abundance

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Sailfish is a computational method for quantifying the abundance of transcripts (previously-annotated RNA isoforms) from RNA-seq data that works by assigning k-mers from reads to specific transcripts. Because Sailfish does not map reads, which is a time-consuming step in all current methods, it provides quantification estimates often more than 20 times faster than those methods. We show using both simulated and real data that this can be accomplished without loss of accuracy.

A Sailfish index is built from a particular set of reference transcripts and needs to be rebuilt only when that reference or the value of k changes. The quantification phase of Sailfish applies an expectation-maximization (EM) procedure to determine maximum likelihood estimates for the relative abundance of each transcript in the reference, measured in Reads Per Kilobase per Million mapped reads (RPKM), Transcripts Per Million (TPM) and K-mers per Kilobase per Million mapped k-mers (KPKM).

We are exploring extending the application of the Sailfish approach to use gene segment annotation files that substitute individual exons, junctions of length 2k-2 and alternatively spliced intervals for full transcripts, in order to detect and quantify novel RNA processing events. We also present further work exploring alternative choices of k in specialized contexts to trade-off between the ambiguity of the k-mer origin and sensitivity to polymorphisms and to sequencing errors.

The speed of Sailfish makes routine reanalysis of data from archived experiments possible in the face of new genome annotations such as newly discovered isoforms, RNA editing sites, polymorphisms, etc. and Sailfish makes it practical to revisit data in order to address specific questions. Applications of this sort on a number of data sets will be presented.

Sailfish is free and open-source software and is available at www.cs.cmu.edu/~ckingsf/software/sailfish (short url: ongen.us/SFish).

201 Estimating Intron/Exon retention levels using IntEREst

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RNA sequencing is widely used for studying gene expression analysis as well as identifying and quantifying various mRNA processing intermediates in the cells. To estimate the levels of the partially spliced or unspliced transcripts in a cell from RNA-Seq data we developed IntEREst (Intron-Exon Retention Estimation) pipeline in R. IntEREst can be run in parallel on multiple computing resources to obtain an optimal performance.

We employed IntEREst to investigate the global analysis of the nuclear processing of the unspliced U12-type introns by the exosome, a ribonuclease complex involved in RNA turnover. The U12-type introns are a distinct subset of nuclear introns and constitute >0.5 % of all introns (~800 introns in humans). They are removed via a separate spliceosome (so called U12-dependent spliceosome) while the normal U2-type introns are removed by the U2-dependent spliceosome. Earlier studies have shown that the splicing rate of the U12-type introns in the nucleus is slower than that of the U2-type, suggesting that the U12-type introns can regulate the levels of their "host" mRNAs in the cell. Consistently, an elevated level of unspliced U12-type introns have been detected in the steady-state mRNA populations in various organisms. Here we investigated the hypothesis that due to slower splicing, the unspliced mRNAs containing U12-type introns in the nucleus are preferentially degraded by the exosome complex.

We investigated the retention levels of the U12- and U2-type introns in nuclear and cytoplasmic fractions in human Hep-2 cells. To investigate the effect of exosome on intron retention we knocked out the Rrp41 or Dis3 subunits of the exosome. GFP knockdown served as the control. Approximately 400,000,000 SOLiD4 paired reads (50 bps + 35 bps, ~100-200 bps library) were mapped genome-wide and the retention of U12- and U2-type introns were estimated using IntEREst. We found that exosome inactivation preferentially stabilizes unspliced U12-type introns as opposed to the U2-type introns in the same transcripts. Moreover, the effects of Rrp41 and Dis3 knockdowns were not identical, suggesting different functional roles for the two subunits. Our data also suggested that unspliced intron retention is significantly overrepresented with U12-type introns compared to the U2-type introns.

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202 Principal component decomposition to visualize the RNA suboptimal ensemble

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Certain ribonucleic acid (RNA) molecules are evolved to adopt a single conformation and carry out a specific function in the cell. However, a majority of RNA, and in particular messenger RNAs (mRNA), are not thought to adopt a just a single conformation. A robust method for visualizing the results of sampling Boltzmann-weighted suboptimal structures of RNAs remains a contemporary challenge in the field. This is especially true when attempting to visualize how mutations, chemical or enzymatic probing data, and evolutionary constraints affect the structural ensemble. Dimensionality reduction on base pairing information for each suboptimal structure facilitates the visualization of conformational space. We are investigating the effect of RNA length and variability in the structural ensemble on our ability to robustly visualize the suboptimal ensemble. This method enables us to visualize mutations in an RNA that may lead to shifts in the structural ensemble in a novel way. In addition we are able to visualize the effect of empirical data on structures produced using suboptimal sampling algorithms facilitating the interpretation of this complex conformational space.

203 Developing computational approach to predict coincidence of structural and primary signatures in mRNAs

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More than a simple carrier of the genetic information, messenger RNA also harbor functional *cis* elements that evolved to control different post-transcriptional processes. Such *cis* regulatory elements, which are targeted by trans-regulatory molecules such as microRNAs and RNA binding proteins (RBP), can be resided throughout the mRNA molecule. They are typically defined by their primary sequence and secondary structure characteristics. Indeed, the structural confirmation of an mRNA can greatly influence its interactions with trans regulators. Therefore, to decipher the regulatory logic operating at the RNA level, it is essential to develop analysis tools that can consider both the sequence and structural features of an mRNA of interest.

To address this question, we recently developed a web server called SPARCS to identify the secondary structural profile of mRNA coding regions by generating sequences preserving both the amino acid sequence and the dinucleotide frequencies (Zhang et. al, 2013). This tool thus allows us to observe the structural landscape of the coding region since the available sequence shuffling tools are only applicable in UTR regions.

We are now implementing an analysis pipeline combining SPARCS-generated structural predictions with RBP binding site searches from a database. For this, we are integrating information form a recent systematic survey of RBP target motifs described by Ray et al. 2013. To discover the potential RBP binding sites in the transcript, we enumerate all possible candidates with the consideration of evolutionary conservation information (PhastCons Scores). Based on all Position Weighted Matrix (PWM) in the Cisbp-rna database, we assign hit score and conservation score at each position of the transcript and then select the candidates with an effective threshold. To augment the secondary structure information with local 3D motif information, we will incorporate RNA-MoIP, an integer-programming framework that extracts from a database (Reinhard et al, 2012). Examples will be shown on RNAs that show certain RNA localization patterns.

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204 Synthesis and Evaluation of 8-substituted Adenine Derivatives as RNA Binding Molecules

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MicroRNAs (miRNAs) are attractive drug targets because they are involved in many biological processes. To control functions of miRNAs, new miRNA-binding molecules are desired. On the course of development of lead molecule, there are two challenging steps: design and evaluation. Designing of RNA-binding molecules often be very difficult because of the low reliability of a calculated target RNA structure. One of the solutions of this problem is searching the lead compound using a high throughput screening (HTS), and we have developed a fluorescent indicator displacement (FID) assay to assess the binding abilities of library compounds. In the assay, 2,7-disubstituted 9*H*-thioxanthen-9-one derivatives (X2SS) are used as fluorescent indicators, and fluorescence of that are quenched upoun binding to RNA.

In this study, we designed and synthesized 8-substituted adenine derivatives and evaluated their binding abilities to miRNAs. These derivatives can be non-planar molecules depending on the substituents and were easily prepared by Pd-catalyzed coupling reactions. With the obtained small compound library of 8-adenine derivatives, binding ability to miRNA was evaluated by FID assay. Although we finally got a few compounds with moderate binding ability to miRNA, the conditions of FID assay seemed to be needed further optimizations to clarify substitution effects. Here, we report synthesis of 8-substituted adenine derivatives and evaluation of their binding abilities to Pre-miRNA29a using FID assay, SPR, and titration experiment.

205 Massive parallel sequencing based hydroxyl radical probing of RNA accessibility (HRF-Seq) using Fenton chemistry and synchrotron irradiation

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The hydroxyl radical footprinting (HRF) is a well established method for assaying nucleic acid backbone accessibility, which found its applications in studying the tertiary and quaternary structure of RNA and DNA. Traditionally, the signal is detected with either slab-gel or capillary electrophoresis, which considerably limits the throughput. Here we present a method of HRF signal detection utilizing the massive parallel sequencing, called HRF-Seq, which allows for a simultaneous analysis of multiple, long RNA molecules. The HRF-Seq workflow starts with the hydroxyl radical treatment of the RNA ensemble of interest, followed by a randomly primed reverse transcription, adapter ligation, PCR amplification and detection of cDNA 3′ ends and priming sites with Illumina sequencing. We describe a novel computational method of alleviating the PCR bias which uses the random barcodes introduced during the ligation. Moreover, we show the normalization procedure which unifies the signal over the regions of varying coverage and corrects for the background terminations. The HRF-Seq correlates well with the slab-gel electrophoresis and with the RNA backbone accessibility measured from the known crystal structures with the resolution that allows for the observation of differential reactivity of sides of helix. The results indicate that the method can generate useful constraints for the automated three dimensional RNA structure modeling in a high-throughput manner. As a next step we are applying the HRF-Seq method for in vivo RNA probing of mouse liver RNA. Samples were probed with synchrotron generated X-ray beams and the quality of RNA shows clear dose-response relationship with the irradiation time. Further analysis of the probed RNA is ongoing.

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206 Large scale variant discovery in microRNA genes to identify microRNAs regulating neuronal processes

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By performing large scale targeted sequencing of brain expressed microRNA genes in patients with neurological disorders, we aim to identify variants that interfere with miRNA biogenesis and/or function. For this purpose, we developed a multiplex PCR assay that enables simultaneous amplification of 290 miRNA genes. We are screening a cohort of 349 patients with two different neurological phenotypes (schizophrenia and idiopathic generalized epilepsy) and 1316 population matched healthy individuals to increase the likelihood of finding variants that might influence the functionality of miRNA genes involved in neuronal processes. After targeted amplification and incorporation of barcodes, the genes are sequenced on the MiSeq platform. Read mapping and variant calling is performed with our internal pipeline, using BWA, SAMtools and GATK. Variant filtering, performed using GenomeComb, is based on quality, coverage and frequency of the variants in patients compared to control populations. Variants are further prioritized based on the proximity of the variant to the mature and seed sequence of the miRNA and on the predicted impact of the variant on the secondary structure of the miRNA precursor using an in house developed tool miRVaS. The screening and preliminary analysis of the schizophrenia samples is completed, the screening of epilepsy patients and controls is in progress. In total 427 variants were found in the schizophrenia patients. After a preliminary filtering using 1000 Genomes European samples as a control population, two interesting patient specific variants and three known variants that have different frequency in the patients were found. These variants could either affect the targeting or the biogenesis of the miRNA. After full data analysis of the 3 phenotypes, a final set of miRNA variants will be selected to experimentally investigate the influence of the variants on the transcriptome and proteome level to assess the function of the wild type miRNA in neuronal processes.

207 Deoxyribozymes for Preparation of Fluorescent Pre-mRNAs without Splinted Ligation

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Fluorescent techniques can offer unique insights into the mechanisms and dynamic properties of biological systems. Fundamental to many fluorescent studies is the installation of site-specific fluorophores within the biomolecules of interest. Short fluorescent RNAs can be prepared by chemical synthesis, but long RNAs containing site-specific modifications are usually prepared by the ligation of smaller RNA fragments using ligases and DNA splints. This process can be inefficient, time consuming and costly. As a novel alternative, we are using the 10DM24 deoxyribozyme as a catalyst to prepare long, site-specifically modified RNAs. 10DM24 generates a branched RNA by addition of a GMP moiety to the 2′ hydroxyl of a targeted nucleotide within an RNA strand. We have shown that 10DM24 can also transfer fluorescent derivatives of GMP to specific sites within a large, capped pre-mRNA splicing substrate. Notably, this method allows for incorporation of fluorophores suitable for single molecule fluorescence imaging such as TAMRA or Cy3. In order to develop 10DM24 as a standard tool for preparing RNAs for biophysical studies we have additionally characterized the sequence specificity of the reaction, the yield of fluorophore incorporation, and the susceptibility of the fluorescent products to degradation by debranchase. We believe that this approach can be used to rapidly and efficiently prepare fluorescent RNAs for bulk and single molecule fluorescence studies of a variety of systems.

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208 Single Molecule, Real-Time Sequencing of Full-length cDNA Transcripts Uncovers Novel Alternatively Spliced Isoforms

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Alternative splicing of mRNA molecules is a tool used by eukaryotic organisms to expand the protein coding potential of their genomes. In humans, for example, nearly all multi-exon genes are alternatively spliced. Different mRNA isoforms from the same gene can generate changes in RNA stability as well as produce proteins that can have distinct properties such as structure, function or subcellular localization. Thus, understanding the biology of an organism requires knowing the full complement of isoforms. Microarrays and high-throughput cDNA sequencing have become incredibly useful tools for studying transcriptomes, yet these technologies provide small fragments of transcripts and building complete transcripts has been challenging (1).

We have developed a technique that is capable of sequencing full-length, single-molecule cDNA sequences. The method employs PacBio SMRT® Sequencing which has the capability to sequence individual molecules with read lengths that average 8 kb and can reach as long as 40 kb. Thus we are able to generate sequence for complete individual transcripts from the polyA-tail to the 5′ end of the RNA molecule. Full-length cDNA sequencing allows for unambiguous identification of alternative splicing events, alternative transcriptional start and polyA sites, and transcripts from gene fusion events. Knowledge of the complete set of isoforms from a sample of interest is key for accurate quantification of isoform abundance when using any technology for transcriptome studies (2).

Using a deep dataset of full length cDNA sequences from the MCF-7 human breast cancer cell line and a comparative study of human brain, heart, and liver, we demonstrate the ability to obtain full-length cDNA sequences from transcripts longer than 10 kb. Even in extensively profiled sample types, the method has been able to uncover large numbers of novel alternatively spliced isoforms and previously unannotated genes.

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209 Integrating mRNA-protein interactions, translation efficiency and protein mass spectrometry to measure cellular stress response

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A major focus of research in systems biology is concerned with changes in protein expression under different conditions. Regulation of translation and protein degradation are major contributors to protein concentrations. For example, during steady-state conditions in mammalian cells, computational and experimental work demonstrate that ~40% of gene expression variation can be attributed to translation and protein degradation control, while ~30-40% are due to transcriptional regulation (Schwanhausser et al., 2011; Vogel et al., 2010; Vogel and Marcotte, 2012). In perturbed systems, post-transcriptional processes appear to play a similar role: about half to two-thirds of yeast proteins monitored in their response to oxidative stress show strong discrepancies between mRNA and protein expression profiles (Vogel et al., 2011). In general, these discrepancies can be explained by down-regulation of translation and up-regulation of protein degradation - but the details and the global principles are still under active investigation and many regulators are still unknown.

To better understand these changes we have set out to profile and integrate multiple layers of cellular control in HeLa cells exposed to two different stress conditions; oxidative stress using H_2O_2 and Tunicamycin. To profile these changes we are using Ribosome Profiling to measure compare translational efficiency, occupancy profiling of RNA binding protein interactions and protein mass spectrometry.

The 3 main goals of these experiments are:

1) How do protein and mRNA concentrations compare in their response to different stressors? How does this relationship change during the immediate stress response and later time points?

2) How can a change in protein concentrations (relative to mRNA concentrations) be explained by changes in translation efficiency (ribosome occupancy)? Does a change in translation occur early or late during the stress response? Which mRNAs are affected early, which late?

3) How does ribosomal profiling (assessing mRNAs purely bound by ribosomes) compare to protein-RNA occupancy profiling (assessing mRNAs bound by ribosomes and other proteins)? What is the relationship between sites within the mRNAs that are occupied by ribosomes and sites occupied by other proteins? How does this relationship change under stress?

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210 *Cryptococcus neoformans* var. *grubii* transcriptome structure

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Cryptococcus neoformans is a pathogenic basidiomycetous yeast responsible for more than 600 000 deaths each year. It exists as two serotypes (A and D) representing two varieties (i.e. *grubii* and *neoformans*, respectively). The genome of the serotype D has been sequenced and annoted a few years ago using a collection of 21000 cDNA molecule sequences and the sequence of the serotype A genome is publically accessible for some years now. However, no analysis of the transcriptome had been performed for this last serotype and most of the annotation was based on bioinformatics analysis and comparison with the serotype D sequence. We here performed an RNA-Seq based analysis of the *C. neoformans* var. grubii transcriptome structure. We observed that more than 92% of the genome was transcribed at least on one strand. Automatic and manual curation was used to remodel the genome annotation. As expected, few genes were added and few were deleted but the most spectacular change was the identification of more than 8000 new introns. Although most of these new introns are located in the CDS altering more than one third of the proteome, we also identified more than 2000 introns in the UTRs. We also used the poly(A) containing reads to locate the polyadenylation sites of more than 80% of the genes. Examination of the sequences around these newly identified sites revealed the presence of a new poly(A) site associated motif (ATGHAH). Moreover, alternative polyadenylation sites appeared to be common. Finally, 1208 miscRNA were identified. These miscRNA are spliced and polyadenylated but they do not seem to have obvious coding capacities.

211 Identification of the RNA targets of RBM45, a new RNA-binding protein implicated in ALS and FTLD

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Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset motor neuron disease characterized by premature loss of the upper and lower motor neurons. Frontotemporal lobar degeneration (FTLD) is a progressive degenerative brain disease characterized by behavioral, personality and language disorders. A major advancement to our understanding of the ALS and FTLD pathogenesis was the identification of mutations in the RNA-binding proteins TDP-43 and FUS in familial cases of ALS and FTLD, and the observation of cytoplasmic aggregates of these proteins in familial and sporadic ALS and FTLD. However, the motor neuron degeneration mechanism remains unknown. Our lab has recently identified a new RNA-binding protein, RBM45, with pathologic alterations in ALS and FTLD. RBM45 containing cytoplasmic inclusions were observed in both ALS and FTLD patients [1]. RBM45 also co-localized with TDP-43 and ubiquitin inclusions in affected neurons. Domain analysis shows that RBM45 contains 3 RNA-recognition motifs, sharing similar structural elements with TDP-43 and FUS. However, very little is known about the physiological functions and RNA targets of RBM45.

We have used a modified PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation) method to study the RNA-mediated pathways of RBM45 in cultured cells. We have found over 4000 clusters from the CLIP dataset. The transcript regional preference and the binding motif will be discussed. The RNAs bound and regulated by RBM45 will also be compared with the RNA targets of TDP-43 and FUS. Future mechanistic studies of RBM45 are warranted to further define the roles of RBM45 in RNA-mediated neurodegeneration, which will broaden therapeutic options for ALS and FTLD.

References:

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212 Coupling demethylation to RNA sequencing reveals a missing class of modified tRNA fragments and dynamics of RNA modification

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RNA fragments derived from transfer RNAs are increasingly recognized as important indicators of tRNA processing. In addition, a number of studies have shown potent activities for tRNA fragments as signaling or regulatory molecules. The prevalence of tRNA fragments in small RNA sequencing libraries has helped spur these discoveries, but tRNA fragments with nucleoside modifications similar to mature tRNAs may commonly be underrepresented in sequencing libraries when these modifications interfere with reverse transcription. Here, we show that pre-treatment of small RNAs using the *Escherichia coli* dealkylating enzyme AlkB reveals a large class of modified tRNA fragments that were either missing or grossly underrepresented in sequencing results for untreated samples. The principal substrates for *E. coli* AlkB are 1-methyladenosine (m1A), which is among the most common tRNA modifications in many organisms, and 3-methylcytosine (m3C), which is less common, but equally problematic for reverse transcription. Treatment with AlkB more than doubled the total proportion of tRNA-derived reads and substantially increased the apparent diversity of tRNA fragments in samples from the model eukaryote *Saccharomyces cerevisiae*. For most cytoplasmic tRNA isotypes in *S. cerevisiae*, modified tRNA fragments appear to outnumber unmodified fragments by a factor of two to ~twenty-fold. Furthermore, the specific tRNA isotypes affected by AlkB treatment provided a high-throughput readout of m1A and m3C modification for tRNA fragments in *S. cerevisiae*. These new partial tRNA modification data are remarkably consistent with modification profiles of the corresponding *mature* tRNAs previously determined by independent biochemical characterization. We have also applied this method to multiple human cell types, and found unexpected complexity in partial tRNA abundance as well as evidence for dynamic modification patterns. Our general strategy, targeting specific modifications for removal prior to RNA sequencing, provides a rapid, high-throughput approach to profiling changes for nearly any class of modified RNA. As a first example, this study demonstrates the new potential for studying the processing and modification of tRNA, the most complex and densely modified non-coding RNA at the heart of cellular information flow.

213 Targets of RNA-binding proteins Identified By Editing (TRIBE); a novel technique

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Identifying the targets of RNA-binding proteins (RBP) is challenging. The current gold standard is HITS-CLIP (**HI**gh **T**hroughput **S**equencing – **C**ross**L**inking and **I**mmuno**P**recipitation). Despite its myriad advantages, it is not particularly suited for identifying RNA targets within small numbers of discrete cells *in vivo*. This goal is important because the targets of many RBPs are likely to vary between tissues and cells types.

We are developing an entirely different approach, which involves a fusion protein between an RBP and the catalytic domain of the RNA-editing enzyme ADAR (adenosine deaminase). ADAR catalyses the conversion of adenosines to inosines, which are interpreted by the cellular machinery as guanosines. As the RBP-ADAR-cd (catalytic domain) fusion protein lacks the RNA recognition features of ADAR, the specificity of the RNP should determine the editing specificity of the fusion protein. Moreover, the RNA targets should be permanently marked after the interaction, making it possible to visualize transient interactions. RBP-ADAR-cd substrates are identified like normal RNA editing events, namely by RNA sequencing and comparison to genomic DNA sequence. We call this technique TRIBE (**T**argets of **R**NA-binding-proteins **I**dentified **B**y **E**diting).

Initial experiments conducted in the *Drosophila* S2 cell line using the RBP Hrp48 (a hnRNPA/B homolog) fused to the ADAR-cd show that the fusion protein maintains the ability to edit RNA. The frequency of A-to-G editing events was increased upon induction of the RBP-ADAR-cd fusion protein, and the same transcripts were consistently edited across multiple biological repeats indicating the fusion protein has specificity for certain transcripts. There is a higher rate of editing among top Hrp48 targets as determined RNA immunoprecipitation, and a comparison of Hrp48 targets as determined by CLIP also indicates that bona fide Hrp48 targets are edited. However, there are CLIP targets that remain unedited, and there are novel edited RNAs. These are either false-positives or Hrp48 targets that escape CLIP identification.

Preliminary results indicate that novel editing events were detected upon expression of the fusion protein in specific subsets of fly brain neurons followed by sequencing of head RNA , suggesting that the technique may facilitate the identification of cell-specific RBP targets.

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214 Tumour RNA Disruption: An Effective Biomarker for Monitoring Clinical Response and Disease-free Survival After Neoadjuvant Chemotherapy in Locally Advanced Breast Cancer

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Neoadjuvant chemotherapy in locally advanced breast cancer patients can promote strong reductions in tumor RNA content and integrity, a phenomenon we term RNA disruption. We have developed the RNA Disruption Assay (RDA), which quantifies RNA disruption as an RNA Disruption Index (RDI) and stratifies RNA disruption into 3 zones of clinical importance. Zone 1 is a level of disruption inadequate to obtain a pathologic complete response (pCR), zone 2 is an intermediate level, while zone 3 contains the vast majority of pCR responders. We have observed that RDA is able to identify >4-fold more chemotherapy non-responders than clinical response assessments by calipers. Moreover, at 108 months of follow up, we have observed that disease-free survival (DFS) was approximately 2-fold greater (an additional 2 years) for patients with a zone 3 level of tumour RNA disruption than in patients with tumor RNA disruption in zone 1, even for patients with hormone receptor positive (HR+) tumours that seldom exhibit a pCR. Our findings suggest that RDA is superior to pCR as a chemotherapy response biomarker, raising the prospect of its use as a tool to identify patients with non-responding tumours early in chemotherapy, who can be spared the toxic side effects of the ineffective regimen and switched to potentially more effective treatments. Insights into the biology of RNA disruption will also be presented, including the ability of a wide variety of structurally distinct chemotherapy agents to induce time-, dose-, and caspase-dependent rRNA degradation products that are distinct from those observed during autolytic degradation of RNA.

215 Understanding T4 RNA ligase bias to improve small RNAseq library construction

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Next generation sequencing (NGS) is a powerful tool for the detection and sequence characterization of small RNAs (sRNA). However, the use of NGS data to determine the relative quantity of different microRNAs (miRNAs) in a sample has been shown to be inconsistent with quantitative PCR and Northern Blot results. Several recent studies have concluded that the major contributor to this inconsistency is bias in sRNA library construction for NGS. The bias is primarily derived from the adapter ligation steps; specifically where single stranded adapters are sequentially ligated to the 3′ and 5′-end of sRNAs using T4 RNA ligases. In our current study we investigated the nature of the ligation bias by using a defined mixture of 962 equimolar miRNAs in NGS library construction. Our results shed even more light on the nature of the ligation bias and allowed us to design adapters that significantly reduce it. We prepared small RNA libraries with spike-in control oligonucleotide mixtures from multiple tissues. Using our new adapter design, we successfully produce NGS results that more accurately reflect the actual concentrations of miRNAs while maintaining the ability to precisely identify small RNA ends.

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216 Selective Depletion of Abundant RNAs to Enable Transcriptome Analysis of Low Input and Highly Degraded RNA from FFPE Breast Cancer Samples

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Deep sequencing of cDNA prepared from total RNA (RNA-seq) has become the method of choice for transcript profiling, and discovery. The standard whole-transcriptome approach faces a significant challenge as the vast majority of reads map to ribosomal RNA (rRNA). One solution is to enrich the sample RNA for polyadenylated transcripts using oligo (dT)-based affinity matrices; however, this also eliminates other biologically relevant RNA species, such as microRNAs and noncoding RNAs, and relies on having a high quality and quantity RNA sample.

Here, we present a method to eliminate abundant RNAs from total RNA with different degradation levels, from intact RNA to highly degraded formalin-fixed paraffin-embedded (FFPE) samples. This method is based on hybridization of probes to the targeted abundant RNA, followed by subsequent enzymatic degradation. We applied this method to remove cytoplasmic and mitochondrial rRNA from different eukaryotic total RNA samples (human, mouse and rat), as well as degraded (1 year old) and highly degraded (10 year old) FFPE breast tumor biopsy RNA samples. We evaluated the depletion efficiency and off target effect of this method using strand specific RNA high-throughput sequencing. Ribosomal RNA depletion resulted in a minimal percentage of total reads mapping to rRNA sequences, regardless of the species, input amount (1µg or 100 ng), or degradation level. Additionally, there was very good transcript expression (FPKM) correlation (>0.93) between rRNA depleted and non-depleted libraries.

This method offers a robust and simple solution for transcriptome analysis of a variety of samples, including low quality and low quantity clinical samples such as FFPE RNA. Moreover, it is amenable to high-throughput sample preparation and robotic automation. This method is sensitive, specific, and produces increased coverage of less abundant, non-targeted transcripts in RNA-Seq studies.

217 Determining RNA and RNA•Protein conformation ensembles with Au-SAXS interferometry

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Obtaining conformational ensembles, beyond average structures, is extremely challenging but necessary for a complete understanding of the folding and functions of biological RNA, RNA•protein complexes and machines, and engineered RNA nanostructures. Recently we developed a solution-phase scattering technique that solves this problem. Small angle X-ray scattering (SAXS) interferometry provides the precise and instantaneous distance distribution, in aqueous solution, between a pair of Au-nanocrystal probes site-specifically conjugated to a biological macromolecule, thereby revealing the underlying macromolecule ensemble. We have applied SAXS interferometry to determine the conformational ensemble of a small model RNA, the HIV Tar RNA, and its response to changes in solution salt conditions, and to probe the conformational ensemble of a biologically important and recurring RNP motif, the kink-turn, and the affect of L7Ae protein binding on the kink-turn ensemble. Tar RNA exists preferentially in two regions of conformational space, with different helix-helix bend and twist angles. Kink-turn RNA is found to exist as an equilibrium between a kinked and unkinked state, and each state is itself an ensemble. Protein association pulls the equilibrium to the kinked state but does not reduce the kinked ensemble to a single conformation -i.e., the protein-bound kinked state remains a dynamic ensemble that populates a similar range of conformations as the protein-free kinked state. The dynamic and ensemble nature of RNA•protein motifs are expected to play crucial roles in the assembly and function of RNA•protein complexes and machines.

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218 RNA detection in live breast cancer cells enables FACS enrichment based on gene expression and invasion assays of the sorted populations

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Determining gene expression in cells has typically been performed in an end point assay. Cells can be lysed and their RNA extracted as is the case for qRT-PCR or the cells can be fixed and permeabilized to detect using RNA FISH probes. Both of these methods only provide a snapshot of the RNA expression profile at that given time. A better method for understanding gene expression and its effect on cells is by utlilizing detection which does not affect the cell health or viability. Through the use of live cell RNA detection probes we have successfully sorted a mixed population of breast cancer cells through FACS sorting effectively enriching for cells with higher expression of ERBB2. We then employed the same strategy for a mixed population of epithelial and mesenchymal cells utilizing both miR-221, and miR-222. Since live cell sorting was employed and the cells are not affected by the detection method we were then able to subsequently apply the positive and negative sort fractions to an invasion assay to look at the correlation of known cancer markers to the invasive ability of the cells. Here we present data showing that the cells expressing higher levels of both miR-221 and miR-222 exhibit invasive properties consistent with aggressive cancers. The ability to enrich for a specific expression profile based on gene expression in live cells and utilize the same cells for further functional analysis provides a better and more dynamic method for studying RNA biology.

219 Efficient ssDNA ligation splinted with RNA by Chlorella virus DNA ligase

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Single-stranded DNA molecules (ssDNA) annealed to an RNA splint are notoriously poor substrates for DNA ligases. Herein we report the unexpectedly efficient ligation of RNA-splinted DNA by Chlorella virus DNA ligase (PBCV-1 DNA ligase). PBCV-1 DNA ligase ligated ssDNA splinted by RNA with $k_{cat} \approx 8 \times 10^{-3}$ s^{-1} and $K_M < 1$ nM at 25°C under conditions where T4 DNA ligase produced only 5′-adenylylated DNA with a 20-fold lower k_{cat} and a $K_M \approx 300$ nM. The rate of ligation increased with addition of Mn^{2+}, but was strongly inhibited by concentrations of NaCl >100 mM. Abortive 5′-adenylylation was suppressed at low ATP concentrations (<100 μM) and pH >8, leading to increased product yields. The ligation reaction was rapid for a broad range of substrate sequences, but was relatively slower for substrates with a 5′-phosphorylated dC or dG residue on the 3′ side of the ligation junction. Nevertheless, PBCV-1 DNA ligase ligated all sequences tested with 10-fold less enzyme and 15-fold shorter incubation times than required when using T4 DNA ligase. This robust activity suggests practical applications in a variety of RNA detection by ligation methodologies. We also found another viral ligase, T4 RNA ligase 2, to ligate RNA splinted DNA nicks with relative efficiency. With the discovery of the CRISPR/Cas9 immune system in bacteria and archaea, which cleaves DNA in a specific fashion through RNA guided targeting, an intriguing possibility is that the ability of viral ligases to target RNA splinted DNA may allow viruses to counteract CRISPR immunity.

220 Abstract Withdrawn

221 Transcriptome analysis reveals thousands of targets of nonsense-mediated mRNA decay that offer clues to the mechanism in different species

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Many alternatively spliced isoforms contain a premature termination codon that targets them for degradation by the nonsense-mediated mRNA decay RNA surveillance system (NMD). Some such unproductive splicing events have a regulatory function, whereby alternative splicing and NMD act together to impact protein expression. Numerous RNA-binding proteins are regulated by alternative splicing coupled to NMD, in conjunction with ultra-conserved elements. The "50nt rule" is the prevailing model for how premature termination codons are defined in mammals, and requires a splice junction downstream of the stop codon. There is evidence that this rule holds in *Arabidopsis* but not in other eukaryotes including *Drosophila*. There is also evidence that a longer 3′ UTR triggers NMD in yeast, plants, flies, and mammals.

To survey the targets of NMD genome-wide in human, zebrafish, and fly, we performed RNA-Seq analysis on cells where NMD has been inhibited via knockdown of UPF1, a critical protein in NMD. We found that thousands of genes produce alternative isoforms degraded by NMD in the three species, including 20% of the genes alternatively spliced in human HeLa cells. These genes are involved in many functional categories and, in human and fly, are significantly enriched for RNA splice factors. We also found a significant enrichment for ultraconserved elements in the human NMD targets usually overlapping a poison cassette exon.

We were able to gain 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 has an effect in zebrafish and, surprisingly, in fly. In contrast, we found little correlation between the likelihood of degradation by NMD and 3′ UTR length in any of the three species. In fly, we see no enrichment for longer 3′ UTRs in isoforms degraded by NMD, unless they have an intron. We also found that thousands of transcripts have uORFs that affect their likelihood of degradation.

Ultimately, our findings demonstrate that gene expression regulation through NMD is widespread in human, zebrafish, and fly, and that NMD is strongly predicted by the 50nt rule but not by 3′ UTR length.

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222 Network of Splice Factor Regulation by Alternative Splicing Coupled with Nonsense Mediated mRNA Decay

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Nonsense-mediated mRNA decay (NMD) is an RNA surveillance pathway that degrades aberrant transcripts harboring premature termination codons. However, this pathway also has physiological targets: many genes produce alternative isoforms containing premature termination codons. In this mode of regulation, a splicing factor can induce splicing of an alternative isoform with an early stop codon. These isoforms will be degraded, resulting in lower protein expression. Regulation of alternative splicing involves complex interactions between many splice factors, and so splice factor levels must be carefully regulated. Splicing coupled to NMD allows for an additional level of post-transcriptional regulation for these genes. For example, splicing factors such as SRSF1, SRSR2, SRSF3, and SRSF7 are known to regulate their own expression and expression of other splice factors by coupling alternative splicing and NMD. hnRNP L / hnRNP LL and PTB / nPTB are regulated in the same manner. After an extensive literature search, we generated a splicing factor regulatory network that encompasses all current knowledge of splice factor regulatory interactions including the known extent of NMD regulation coupled with alternative splicing. The currently available data shows that majority of the SR proteins and a few hnRNP splicing factors are known to be regulated via alternative splicing coupled with NMD. Since all the SR proteins and many hnRNP splicing factors produce isoforms degraded by NMD, we expect that this mode of regulation is prevalent among all splicing factors. In addition, CLIP-seq data reveals yet more extensive splicing factor-mRNA interactions, providing an additional hint that many more splicing factors might be regulated via alternative splicing coupled with NMD by other splice factors themselves.

223 A feedback loop couples MSI1 activity to omega-9 fatty acid biosynthesis

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Gene expression and metabolism are coupled at numerous levels. Cells must sense and accurately respond to the nutrients in their environment, and specialized cells must synthesize the metabolic products required for their function. Pluripotent stem cells have the ability to differentiate into a wide variety of specialized cells. Exactly how metabolic state contributes to stem cell differentiation is the subject of intense investigation. We have shown that the RNA-binding activity of the stem cell translational regulator Musashi-1 (MSI1) is allosterically inhibited by nonesterified 18-22 carbon cis ω-9 monounsaturated fatty acids. Inhibition is direct and specific. The fatty acid binds to the N-terminal RNA Recognition Motif (RRM) and induces a conformational change that prevents RNA association. Musashi-family proteins are critical for development of the brain, blood, and epithelial lineages, and play an important role in maintaining the viability of oligodendrocyte progenitor cells (OPCs). We identify the enzyme that produces oleic acid, stearoyl-CoA desaturase 1 (SCD), as a new MSI1 target, and show that downstream lipid products of SCD are upregulated in response to MSI1 expression. This feedback loop between ω-9 fatty acid biosynthesis and MSI1 activity provides a novel link between metabolic state and post-transcriptional regulation by an RNA-binding protein in eukaryotes. We propose that other RRM proteins could also act as metabolite sensors to couple gene expression changes to physiological state.

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224 Transcriptome sequencing of mouse Polyomavirus infection

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Murine Polyoma virus lytically infects mouse cells, transforms rat cells in culture and is highly oncogenic in rodents. We have used deep sequencing to follow polyoma virus infection of mouse NIH3T6 cells. We carried out RNA-seq at various times after infection and analyzed both the cellular and viral transcriptomes. Alignment of sequencing reads to the host genome revealed over 400 upregulated and 800 downregulated host transcripts by the late phase of infection. Gene ontology analysis indicated transcripts involved in translation, metabolism, RNA processing, DNA methylation, and protein turnover were upregulated while transcripts involved in extracellular adhesion, cytoskeleton, zinc finger binding, SH3 domain, and GTPase activation were downregulated. Noncoding RNAs such as NEAT1 and MALAT1, involved in paraspeckles and splicing speckles respectively, were noticeably downregulated, while several other abundant noncoding RNAs were strongly upregulated. Alignment of sequences to the viral genome illustrated the transcriptional profile of the early late switch with both early and late RNA being transcribed at early time points with an increase in read-through of the polyadenylation site at late times. We will discuss these results in light of what is currently known about the polyoma life cycle and its effects on host cell growth and metabolism.

225 Linking the C-Terminal Domain Code of RNA polymerase II to Modulating Chromatin States in *Schizosaccharomyces pombe*

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Regulation of gene expression is essential for all living organisms. One critical step in modulating gene expression is altering the ability of the transcriptional enzyme, RNA Polymerase II (RNAPII), to access DNA by manipulating chromatin states. The carboxy-terminal domain (CTD) of RNAPII, is believed to play a critical role in chromatin remodeling through its recruitment of factors that modify histones. Conserved throughout evolution, the RNAPII CTD contains a repeated $Y_1S_2P_3T_4S_5P_6S_7$ heptapeptide sequence that undergoes dynamic posttranslational modifications. The capability of each serine in the sequence to undergo phosphorylation and dephosphorylation creates a readable 'code' for recruiting factors that can influence when processing events such as chromatin remodeling occur. In order to characterize how specific phosphorylation marks in the CTD affect gene expression, mutants of fission yeast *Schizosaccharomyces pombe* were rendered defective for phosphorylation by substituting a nonphosphorylatable alanine in place of each serine in position 2 in the heptad sequence (S2A), each position 7 serine (S7A), or all serines in position 2 and position 7 in combination (S2A/S7A). In addition, a fourth mutant was created in which the position 7 serines were substituted for the phosphomimetic glutamic acid (S7E). We have performed microarray experiments with these mutants to study the genome-wide effects of eliminating and altering these phosphorylation events. While others have observed defects in snRNA levels with these mutants in human cells, we do not see a similar decrease with our *S. pombe* mutants, nor do we see any large changes in global splicing efficiency. Interestingly, analyses of our microarray data reveals an upregulation of positionally related clusters of genes, specifically at both ends of chromosomes one and two, but not chromosome three. Further quantitative PCR analysis of genes in these subtelomeric regions confirm a significant upregulation of gene expression in these regions spanning approximately 50-100kb. Our microarray analyses and subsequent qPCR validation suggest a role for the dynamic phosphorylation and dephosphorylation of serines within the CTD code in modulating the chromatin states in large subtelomeric regions of *S. pombe*.

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226 U2 snRNP is required for transcription elongation in a gene specific manner

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Pre-mRNA in eukaryotes is subjected to mRNA processing, which includes capping, polyadenylation, and splicing. Transcription and mRNA processing are coupled, and this coupling stimulates mRNA processing; however, the effects of mRNA processing on transcription are not fully understood. In this study, we found that inhibition of U2 snRNP by a potent splicing inhibitor, spliceostatin A (SSA), or by an antisense oligonucleotide to U2 snRNA, caused gene-specific 3′-end down-regulation. Removal of SSA from the culture media restored expression of the 3′ ends of genes, suggesting that U2 snRNP is required for expression of the 3′ end of genes. Finally, we found that SSA treatment caused accumulation of Pol II near the 5′ end of genes showing 3′-end down-regulation, indicating that SSA treatment led to transcription elongation arrest on these genes. These findings suggest that U2 snRNP is important for production of full length mRNA probably through regulation of transcription elongation, and that a novel checkpoint mechanism prevents pre-mRNA from accumulating as a result of splicing deficiencies, and thereby prevents production of aberrant proteins that might be translated from pre-mRNAs through the arrest of transcription elongation.

227 The NuA4 and Swr1 chromatin modification complexes are important for RNA splicing

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Efficient and precise gene expression depends, in part, on effective coordination between RNA splicing and transcription[1], yet the mechanistic details that underlie this coordination have remained relatively unexplored. Recent high throughput genetic interaction studies using Saccharomyces cerevisiae revealed that splicing factors interact with factors that are important for regulating transcription[2-4]. We have utilized a targeted genetic screen to identify novel interactions between splicing factors and factors that modify chromatin to modulate transcription. Using both qualitative and quantitative growth assays we identified negative genetic interactions between genes encoding splicing factors and both SWR1 and VPS72, components of the Swr1 chromatin remodeling complex[5]. In addition, our screen revealed novel negative genetic interactions between splicing factors and both EAF3 and ESA1, components of the NuA4 histone acetyltransferase complex[5]. Notably, both the NuA4 and Swr1 complexes function together to regulate transcription[6]. Using quantitative RT-PCR we have shown that mutation of individual components of the NuA4 or Swr1 complexes causes a modest block in RNA splicing and exacerbates the splicing defects observed in a yeast strain lacking a splicing factor. Taken together, these data support a model in which the NuA4 and Swr1 chromatin modification complexes interact with the splicing machinery to coordinate transcription and splicing. We are currently testing whether mutation of the NuA4 or Swr1 complexes impacts the recruitment of splicing factors during transcription.

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228 To define the role of Sam68 in p53-mediated functions

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Allelic loss and mutations in the *P53* (*TP53*) gene are frequently associated with human cancers. Elevated expression of Sam68, a KH-type RNA binding protein, has been observed in many cancers. KH-containing RNA binding proteins such as RPS3 and hnRNP K can interact with p53 and function as transcriptional coactivators. We therefore hypothesize that Sam68 may serve as a coactivator of p53 in response to DNA damage. To define the role of Sam68 in the p53 pathway, we have ablated the *SAM68* (*KHDRBS1*) gene in both HCT116 $p53^{+/+}$ and $p53^{-/-}$ cell lines using the CRISPR/Cas9 genome editing method, as this would provide us with isogenic cell lines. Disruption of *KHDRBS1* was verified via genomic PCR and sequencing, and the absence of protein expression was confirmed using immunoblotting. To study the effect of Sam68 on p53 targets at the transcription level, RT-qPCR was performed to measure expression of p53 targets including *P21, MDM2, GADD45A, PUMA,* and *NOXA*. Following DNA damage, specifically the induction of *P21* and *MDM2* was attenuated in $p53^{+/+}$;$Sam68^{-/-}$ cells compared to $p53^{+/+}$;$Sam68^{+/+}$ cells, while there was no significant difference between $p53^{-/-}$;$Sam68^{+/+}$ and $p53^{-/-}$;$Sam68^{-/-}$ cells, consistent with the need for p53. Immunoblots of p21 further confirmed its diminished induction in $p53^{+/+}$;$Sam68^{-/-}$ cells compared to the wildtype counterpart. In addition, flow cytometry analyses showed that $p53^{+/+}$;$Sam68^{-/-}$ cells were defective in DNA damage-induced G_1/S arrest compared to wildtype as more cells accumulated in S-phase, correlating with decreased p21 expression. Moreover, co-immunoprecipitation data demonstrated that Sam68 associates with p53 after DNA damage. These results suggest that Sam68 functions as a gene-selective transcriptional coactivator of p53 in response to DNA damage. This work is supported by a grant from CIHR.

229 Exosome identification in *Lithobates catesbeianus*

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The exosome is a 3′-5′ exoribonucleolytic complex present in many organisms, from archaea to higher eukaryotes. Although the exosome has been shown to be conserved throughout evolution, this complex has not yet been described in all organisms. In this work, we investigated the exossome in male adult *Lithobates catesbeianus*, an amphibian species, analyzing the expression levels of some of its core subunits during the reproductive (summer) and quiescent (winter) periods. As the complete genome sequence of this animal is not yet available, we obtained a partial sequence of LcRRP40, and from that, we cloned the full length gene and sequenced it. LcRRP40 gene sequence encodes a protein with 79% and 65% identity to RRP40 from *Xenopus laevis* and *Homo sapiens*, respectively, which corroborate the exosome conservation throughout the eukaryotic evolution. Our preliminary data show that the expression levels of LcRRP40 and LcRR46 are higher in the testis when comparing with others tissues. Interestingly, there were significant changes in the expression levels of these proteins between the reproductive and quiescent periods of *L. catesbeianus*. In addition, the expression of LcRR40 in the testis was higher in the summer as compared to winter. These results could suggest that the exossome can participate in the control of gene expression during the seasonal spermatogenesis of *L. catesbeianus*.

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230 Autoregulatory association of a cyanobacterial DEAD-box RNA helicase with polycistronic message processing

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DEAD-box RNA helicases are associated with all aspects of RNA metabolism, functioning as molecular motors to catalyze the ATP-dependent unwinding and annealing of RNA duplexes and protein displacement, in all kingdoms of life. Prokaryotic RNA helicases are frequently associated with cellular response to abiotic stress, participating in RNA degradation, translation initiation or ribosome maturation.

Here we show that the *Synechocystis* 6803 DEAD-box RNA helicase CrhR (slr0083) is required for the post-transcriptional processing of its own dicistronic message (slr0082-slr0083). In wild type cells, processing of this operon generates a number of transcripts with different fates. The half-life of the slr0083 transcript is significantly extended compared to slr0082 and, although rapidly degraded, the slr0082 transcript generates two shorter, stable sRNAs, originating from the 5′ UTR and the ORF. In a *crhR* mutant, processing occurs at a much slower rate and differential transcript stability is not observed. The CrhR-dependent processing is therefore required for the differential transcript stability. This autoregulatory process will be placed in context with our previously identified autoregulation associated with CrhR in response to temperature shift. The data provide unique insights into how a RNA helicase can influence the regulation of gene expression in response to temperature shift, in the absence of traditional transcription factor or two-component signal transduction pathways.

231 Defining functions for DZF domain-containing proteins in *C. elegans*

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RNA binding proteins containing DZF (dsRNA-binding motif (dsRBM) and zinc-finger-associated) domains are conserved throughout metazoa and mediate diverse cellular functions. Mammalian DZF protein family members, including Nuclear Factor 90 (NF90) and its binding partner Nuclear Factor 45 (NF45), have been implicated in control of translation, RNA nucleocytoplasmic shuttling, DNA repair, cell division, and embryonic development. While mechanisms have been proposed to explain several of these diverse roles, a detailed understanding of DZF family functions is lacking.

Vertebrate DZF gene families contain five members, while non-chordate metazoans such as *Drosophila melanogaster* and *Caenorhabditis elegans* encode two DZF proteins: a small NF45-like protein and another protein containing a DZF domain and three widely-spaced C2H2 zinc-fingers. These zinc-fingers resemble those in dsRNA-binding zinc finger proteins and are highly conserved along with the DZF domain in mammalian homologues. We hypothesize that these two DZF proteins represent an ancestral form of this gene family and predict that the mechanisms by which these proteins function will be applicable to mammalian DZF proteins. To study DZF proteins, we have used CRISPR/Cas9 gene targeting to generate mutations in the *C. elegans* DZF family members *Y95B8A.8* and *R11H6.5*, which we designate *dzf-1* and *dzf-2* respectively. Nonsense mutations in early codons of each gene cause no measurable lethality or sterility. We are beginning to investigate roles for these genes in dsRNA-dependent processes including RNA interference and RNA editing to provide insight into their functions.

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232 RNA transcription in Trypanosoma brucei mitochondria

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The unicellular parasite Trypanosoma brucei is typified by the presence of a kinetoplast, a compact nucleoprotein structure that contains mitochondrial DNA (kDNA). The mitochondrial genome is composed of two types of catenated circular DNA molecules: the maxicircles encoding ribosomal RNAs and messenger RNAs, and the minicircles, encoding guide RNAs (gRNAs) and gRNA-like molecules. Guide RNAs direct the U-insertion/deletion editing which creates open reading frames for 12 out 18 mitochondrial mRNAs. Both mRNAs and gRNAs are transcribed as polycistronic precursors and processed by nucleolytic degradation. Previous studies from our laboratory identified antisense gRNA transcripts that may be involved in the processing of gRNA precursors by impeding their 3′-5′ exonucleolytic degradation. We hypothesized that in overlapping sense-antisense transcription units the mature 3′ end of the gRNA is defined by the position of the antisense RNA's 5′ end. It follows that the precise initiation of mitochondrial transcription is essential for processing of mitochondrial mRNAs. Furthermore, it is also possible that sense and antisense gRNA transcription events are coupled, in order to ensure the processing of the sense gRNA. The mitochondrial RNA polymerase (mtRNAP) has been identified but neither promoters nor transcription factors have been characterized. In this study, we generated a transgenic T. brucei cell line expressing a TAP-tagged mtRNAP and purified associated proteins. We will present data on the composition mitochondrial transcription complex and functionality of its components.

233 Alternative splicing and nonsense-mediated decay in a null mutant of *UPF1* in Arabidopsis

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We previously showed that around 18% of genes in Arabidopsis potentially undergo AS/NMD using a high resolution RT-PCR platform and mutants in the NMD protein genes, *UPF1* and *UPF3* (Kalyna et al., 2012). The *upf1-5* and *upf3-1* mutants are viable but the *upf1-3* mutant is seedling lethal. Disruption of the UPF1 gene in *upf1-3* causes changes in gene expression and AS profiles, increased salicylic acid levels and expression of PR genes giving a hypersensitive defence response (HR) (Riehs-Kearnan et al., 2012) suggesting that one function of NMD is regulation of plant defence responses. By combining a mutation in the *PAD4* gene involved in the salicylic acid pathogen signalling pathway, the lethality of *upf1-3* has been overcome (Riehs-Kearnan et al., 2012). The *upf1-3 pad4* double mutant now allows the real extent of NMD on transcripts to be determined in a null background. We have analysed alternative splicing/NMD in the *upf1-3 pad4* mutant and a related double mutant, *smg7-1 pad4,* by high resolution RT-PCR using primers targeted to known NMD-sensitive transcripts to investigate the effect of the mutants on NMD. We found large significant changes in the level of NMD-sensitive alternatively spliced products in the mutants and particularly in the *upf1-3 pad4* mutant background. The increases were often far greater than observed in the weaker *upf1-5* and *upf3-1* mutant alleles tested previously suggesting that substantial proportions of transcripts from some genes are degraded and effectively turned over by NMD. We also identified novel NMD-sensitive transcripts and further demonstrated that many transcripts with unspliced introns are not turned over by NMD. We are currently examining whether stabilised NMD-sensitive transcripts in the mutants are translated using a novel plant SILAC method (Lewandowska et al., 2013).

Kalyna et al., (2012). Nucleic Acids Res 40:2454-2469; Riehs-Kearnan et al (2012) Nucleic Acids Research 40:5615-24; Lewandowska et al (2013) PLoS ONE 8:e72207.

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234 Splicing of a specific intron is required for *in vivo* translation of a reporter gene containing a neurofilament 3′ untranslated region in *Xenopus laevis* tadpoles

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Neurons use post-transcriptional control of gene expression to coordinate the supply of critical cytoskeletal proteins, such as the medium neurofilament (Nefm), with axonal growth dynamics in response to extracellular cues encountered by the growing axons. Thus, a thorough understanding of how such post-transcriptional control is regulated through the interactions of RNA binding proteins with their targeted *cis*-regulatory elements within the *nefm* pre- and mature mRNA requires studying them within the context of the intact, developing nervous system. We have established a model system for rapidly evaluating the expression of reporter genes bearing mutated *cis*-regulatory domains *in vivo* through injection of modified plasmid DNA into early stage *Xenopus* embryos. In using this method to study *cis*-regulatory elements of the *nefm* gene's 3′ untranslated region (3′UTR), we discovered that splicing of a specific intron was required for robust transgene protein expression, regardless of promoter or cell type. Transgenes utilizing the *nefm* 3′UTR but substituting other introns expressed little or no protein, indicating that the required *cis*-elements were specific to this intron. Surprisingly, all constructs bearing different introns yielded comparable levels of fully spliced, virtually identical mature message. This finding demonstrated that although splicing was required, it alone was insufficient for robust reporter protein expression, and further supported the conclusion that poor protein expression resulted from defects in mRNA translation as opposed to transcription or splicing *per se.* Furthermore, results from co-immunoprecipitation experiments with heterogeneous nuclear ribonucleoprotein K, which is required for *nefm* translation, indicated that the *nefm* intron promoted this RNA binding protein's association with its mature target mRNA. In summary, we have utilized a simple *in vivo* system to demonstrate in an intact, developing vertebrate nervous system that splicing of a specific intron, rather than splicing in general, was required for translational regulation by an RNA binding protein associated with the final spliced message. *Supported by NSF IOS-1257449.*

235 Abstract Withdrawn

236 Modifications induced by HIV-1-encoded RRE and TAR RNAs on RNA interference activity and siRNA/miRNA binding to TRBP

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Virus replication results in reciprocal activities on cell and viral functions. RNA interference (RNAi) is a mechanism by which small double-stranded RNAs called micro(mi) or small interfering(si) RNAs bind messenger RNAs (mRNAs) to inhibit their expression. The mechanism involves the RNA-induced silencing complex (RISC) composed of Dicer, TRBP and Ago2 proteins in which TRBP loads miRNAs into the active complex. Several mammalian viruses interfere with RNAi activity. The human immunodeficiency virus type 1 (HIV-1) does not shut-off the RNAi pathway but subtle changes have been observed in HIV-infected cells and in patients. Alterations of the miRNA composition and mRNA expression suggest that specific modifications in the RNAi pathway could bring clues to the mechanism leading to these changes. To try to elucidate part of the relationship between RNAi and HIV-1, we investigated the ability of the HIV-1-encoded RNAs Rev-Response Element (RRE) and the Trans-Activation Response (TAR) to suppress RNAi. We also explored the consequences of HIV-1 infection on the miRNA pathway.

We used a model based on miRNA Let7 activity on a reporter gene (RL or EGFP) linked to a complementary sequence (cLet7) to measure RNAi activity or its suppression. In this context, we observed that RRE and TAR act as RNAi suppressors without modifying the endogenous RISC.

Using RNA-immunoprecipitation (IP) and gel mobility shift assays, we compared TRBP binding to TAR, RRE or siRNAs. We found that TAR and RRE RNA displace siRNAs from TRBP, which suggests a change in miRNA incorporation into the RISC.

In the context of a lentiviral vector expressing TAR and RRE, we observed a suppression of RNAi, but this function was abolished when the RNA binding proteins, Rev or GagPol, were present.

TAR and RRE are RNAi suppressors that act by competition with siRNA and miRNA for binding to TRBP and therefore incorporation into the RISC. This likely occurs in a limited window of time in the cell and could, in part, explain the altered expression of certain genes in patients with long-term HIV-1 infection.

237 Characterization of Terminal Uridylyl Transferase 7 (TUT7) and Its Substrate Requirements in Pre-miRNA Mono-uridylation

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Uridylation plays an integral role in the maturation of let-7 microRNAs (miRNAs) in vertebrates. Unlike canonical group I pre-miRNAs that contain 3′ 2nt overhang, group II pre-miRNAs (which account for the majority of let-7) acquire a 3′ 1nt overhang from Drosha processing and require 3′-end mono-uridylation for Dicer processing. We previously reported that Terminal uridylyl transferase 7 (TUT7), TUT4, and TUT2 are responsible for pre-miRNA mono-uridylation. However, it was unclear how TUT7/4/2 recognize and uridylate their RNA substrates. Here, using TUT7 and pre-let-7a-1 as a model, we delineate the minimal requirements for pre-miRNA mono-uridylation. Deletional mutagenesis indicates that the C-terminal half of TUT7 is sufficient for pre-miRNA mono-uridylation. Mutational analyses of pre-let-7a-1 reveal that the end structure and the terminal loop of pre-miRNA are important for the selective recognition by TUT7. Using single molecule fluorescence measurement, we find that TUT7 discriminates RNA substrates based on binding rate rather than dissociation rate. Our study reveals the molecular basis of the specific interaction between terminal uridylyl transferase and group II pre-let-7.

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238 Building an interaction network of Argonaute2 in the nucleus

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RNA interference (RNAi) is an endogenous mechanism for regulating gene expression and can be manipulated for experimental or therapeutic purposes. In the mammalian cell cytoplasm, small RNAs direct protein machinery to cleave mRNA targets. Argonaute 2 (Ago2) is the core catalytic protein of RNAi that possesses target cleavage function. Recently, Ago2 has been shown to use promoter-targeted duplex RNAs to induce transcriptional silencing and activation through the RNAi pathway (Chu et al. 2010, Matsui et al. 2013). Ago2 was also shown to direct pre-mRNA-targeted duplex RNAs to redirect alternative splicing (Liu et al. 2012). Although these nuclear RNAi processes have been extensively studied in mammalian cells, the mechanisms are not well understood.

Thus, our goal was to build a protein interaction network of nuclear Ago2 by utilizing semi-quantitative mass spectrometry analysis. To do this, we prepared nuclear cell extracts, performed large-scale immunoprecipitations for Ago2, performed mass spectrometry analysis of the protein complexes. We then compiled a list of proteins that pulled out consistently and significantly in each of our samples. Up to date, we have observed nuclear Ago2 associating with known RNAi factors, transcription factors, histone modifiers, and splicing factors. We are performing co-immunoprecipitations to verify each of these potential interacting factors. The future direction on this project is to study interactions more in-depth to provide a clear understanding of the mechanisms by which Ago2 functions in transcriptional regulation and splicing. These studies will provide the framework for improving RNAi therapeutics and experimental techniques.

239 Sensing viral nucleic acids by Dicer-2 in *Drosophila melanogaster*

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RNA interference plays a central role in antiviral innate immunity in flies. Indeed, flies mutant for the three key components of the small interfering (si)RNA pathway, namely Dicer-2, R2D2 and Argonaute (AGO) 2 are highly sensitive to a wide range of viruses (1). Dicer-2 produces virus derived-siRNAs from viral RNAs throughout its RNaseIII activity. The Dicer-2/R2D2 heterodimer then loads these siRNAs onto AGO2 in the RNA-induced silencing complex, RISC. The RISC complex is then able to target viral RNAs, thus impairing the ability of the virus to successfully replicate.

Although *in vitro* and *in vivo* experiments clearly indicate that Dicer-2 can process long double stranded RNA, the exact nature of the viral RNAs sensed *in vivo* in infected cells remains mysterious. We are interested in understanding how Dicer-2 senses viral RNAs, with a particular focus on the contribution of the N-terminal DExD/C helicase domain, which is conserved in mammalian RIG-I like receptors. Indeed, *in vitro* experiments have revealed a critical role of this domain in both processivity of the enzyme and discrimination of the extremities of the template RNA (2,3). To address this question, we take advantage of a combination of approaches including *Drosophila* genetics, next-generation sequencing technologies and cutting edge bioinformatics analysis.

We have compared the resistance to viral infection of wild type, and Dicer-2 null or helicase mutant flies. These flies contain an inverted repeat silencing the *white* gene in the eye, allowing us to monitor the activity of Dicer-2 against a perfect long RNA hairpin. Surprisingly, we observed that the helicase domain, which is required for processing the synthetic hairpin, contributes differently to resistance to two different RNA viruses, Drosophila C virus (DCV) and Sindbis virus (SINV). Comparative analysis of the siRNA profiles from wild-type and Dicer-2 mutants confirmed this finding, and revealed striking differences between the two viruses. Altogether, our data indicate that sensing of viral RNAs involve other features besides double-strandedness.

(1) Kemp *et al*., J. Immunol. 2013
(2) Cenik *et al.*, Mol Cell. 2011
(3) Welker *et al.*, Mol Cell. 2011

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240 Regulation of human Dicer by GCN5 and MBIP, two members of the ATAC complex

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The ribonuclease Dicer mediates the formation of mature microRNAs, which are known to regulate expression of ~60% of the genes in human, mainly through repression of messenger RNA (mRNA) translation. Dicer itself may thus need to be tightly regulated in order to maintain microRNA levels and cellular homeostasis. In some pathological conditions, such as cancer, there is a generalized decrease in microRNA levels associated with deregulation of Dicer expression.

To gain further insights into the mechanisms regulating Dicer, we aimed to expand the known protein interactions network of Dicer by performing a yeast two-hybrid screen using human Dicer as bait. This approach identified two members of the Ada2A Containing (ATAC) complex, namely General-Control of Amino-acid Synthesis 5 (GCN5) and Mitogen-activated protein kinase upstream kinase-binding inhibitory protein (MBIP). Besides its role as a transcriptional co-activator complex, ATAC has been implicated in cell cycle progression via acetylation of cyclin A and cdc6 proteins.

Here, we show that Dicer is acetylated by GCN5 *in vitro* and *in vivo* during the G2/M phase of the cell cycle. Overexpression of GCN5 led to a decrease in Dicer protein levels, suggesting that the acetylation process may reduce Dicer levels during mitosis, when Dicer can interact with GCN5 and MBIP. Exhibiting a cytoplasmic localization when co-expressed with its cofactor TAR RNA Binding Protein 2 (TRBP2), Dicer localized to the nucleus upon co-expression of MBIP. Inactivation of MBIP C-terminal nuclear localization signal (NLS), by arginine substitution of the K301 residue (K301R), induced a loss of MBIP nuclear staining and impaired the ability of MBIP to mediate nuclear localization of the MBIP-interacting Dicer N-terminal domain.

Our results suggest that GCN5 and MBIP proteins may regulate Dicer protein levels in human cells during mitosis and mediate the transit of Dicer between the cytoplasm and the nucleus, where it may play important functions.

241 In vivo coupling between group IIC intron insertion and transcriptional termination

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Group II introns are a class of retroelements consisting of a catalytic RNA and an intron-encoded protein (IEP), which is a reverse transcriptase (RT). Group II ribozymes are classified into three subgroups, IIA, IIB and IIC. IIC introns have the novel mobility property of inserting directly after transcriptional terminator motifs rather than into homing sites of defined sequence. Previous experiments with the IIC intron *B.h.*I1 demonstrated that the intron's RNP (intron lariat bound by IEP) inserts intron sequence into DNA in vitro; however, the substrate must be single-stranded and contain a DNA stem-loop that is analogous to the RNA stem-loop formed during transcriptional termination (1). The RNP also recognizes the DNA target through the **i**ntron and **e**xon **b**inding **s**equences IBS1-EBS1 (4 bp) and IBS3-EBS3 (1 bp). Because the RNP cannot utilize a double-stranded DNA substrate in vitro, the DNA target in vivo must be unwound by other means. Here we use in vitro and in vivo experiments to test the hypothesis that the RNP recognizes its unwound DNA target during transcription termination. In vitro experiments argue against RNP recognition of a cruciform structure. In contrast, in vivo mobility experiments support a link with termination because a series of mutations in the target's terminator motif had equivalent effects on both transcriptional termination and the frequency of intron insertion. In another set of experiments, target DNAs were constructed in which the native terminator motif was replaced with sequences from the *E. coli* tryptophan attenuator or the *B. subtilis* adenine riboswitch, which allowed control of termination in vivo by manipulating metabolite concentrations in the growth medium. Again, the levels of homing in vivo correlated well with levels of termination. Together the data support a model for in vivo mobility by which the RNP gains access to its unwound DNA target during the pause of transcriptional termination, allowing it to recognize the DNA stem-loop structure and the IBS1 and IBS3 sequences.

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242 Control of messenger interacting mRNAs (mimRNAs) fate by the 5′-3′ exoribonuclease Xrn1 in S. cerevisiae

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We recently demonstrated that an interesting connection exists between the ability to maintain levels of specific proteins and the cytoplasmic capacity of Xrn1 to degrade RNAs in the 5′-3′ direction (Sinturel et al., 2012). Here, we analyse in detail the expression of the mitochondrial porin gene, POR1, for which the accumulation of the corresponding protein is one of the most affected in xrn1 mutant strain. Remarkably, POR1 mRNAs are capped but apparently not functional for translation in xrn1 mutant strains. By examining the POR1 locus, we noticed that transcription of POR1 and OCA2 genes are convergent, and we verified that OCA2 and POR1 transcripts overlap. We observed that OCA2 RNAs accumulate in an xrn1 mutant strain and that OCA2 mRNAs expressed in trans affect POR1 expression at a post-transcriptional level. We therefore hypothesize that in Xrn1 deficient cells, stabilization of these overlapping mRNAs leads to mRNA-mRNA interactions deleterious for POR1 mRNA translation. Indeed we show that these mRNA interactions can activate No Go Decay of POR1 mRNAs presumably by triggering ribosome pausing. According to this hypothesis, this No Go mRNA Decay signal can be suppressed in a dom34 mutant strain, similarly to what it has been observed for mRNAs containing an internal stem loop structure. Other pairs of converging genes are currently under study and show that our observation on the POR1-OCA2 system is apparently not unique. At the genomic scale, we have used a synthetic RNAi system to show that a large portion of mRNAs overlap in their 3′UTR regions and form double stranded RNA structures. This provides a potential pool of several hundred regulatory messenger interacting mRNAs (mimRNAs) that may be controlled by Xrn1. This is an exciting proposal considering that interaction between these pairs of sense and antisense mRNAs has not been addressed so far, either at the mechanistic or physiological level.

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243 Human circular RNAs: expression and circRNP formation

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Mammalian circRNAs were discovered last year as a new class of non-coding RNAs (Hansen et al., 2013; Jeck et al., 2013; Memczak et al., 2013). They are apparently generated by alternative splicing of pre-mRNAs, whereby single or multiple adjacent and spliced exons are released in a circular form. Single cases of circular RNAs had been known for decades, starting with the viroid RNAs (Sänger et al., 1976), but only systematic transcriptome-wide studies revealed their wide abundance in diverse eukaryotes. Functionally, they are largely unexplored, except for few cases of circular miRNA sponges.

We have selected and studied a set of 15 abundant human circRNAs, focussing on their expression, processing, and RNP formation. Comparing HeLa, HEK293, and BJ-T cells, they are expressed in a cell-type specific manner, based on monitoring their linear versus circular splicing modes as well as their cytoplasmic versus nuclear localization. Since RT-PCR assays can only suggest, but not prove, circular splicing, we combined Northern blot hybridization and RNase H cleavage assays to unequivocally demonstrate for two human circular RNAs their circular configuration. In the search for novel functions of circular RNAs, we investigated the assembly of RNA-protein complexes, based on our set of 15 circRNAs and comparing cytoplasmic and nuclear extracts. CircRNPs of distinct sizes were identified. Bioinformatic screening for protein-binding motifs suggested the IGF2BP3 (IMP3) protein as a putative circRNA-binding protein, with enriched binding motifs in the circularized exon, validated by iCLIP analysis and co-immunoprecipitation.

Memczak et al. (2013) Nature 495: 333-38.
Jeck et al. (2013) RNA 19: 141-57.
Sänger et al. (1976) Proc Natl Acad Sci USA 73: 3852-56.
Hansen et al. (2013) Nature 495: 384-88.

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244 Divergent antisense transcription and R loop formation promotes transcriptional activation at the Vimentin locus

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Global transcriptomic analyses have revealed a much richer RNA landscape than previously anticipated, and a variety of regulatory roles for noncoding RNAs are emerging. Vimentin is a member of the intermediate filament family that is involved in cell and tissue integrity and that is deregulated in different types of cancer. Vimentin mRNA levels are positively correlated with the expression of a previously uncharacterized noncoding transcript originating from divergent transcription, both transcripts being silenced in colon primary tumors concomitant with promoter hypermethylation. Furthermore, antisense transcription promotes formation of a stable DNA:RNA hybrid called R loop that can be disfavored *in vitro* and *in vivo* by RNaseH1 overexpression, resulting in Vimentin down-regulation. Antisense knock-down and R loop destabilization both result in chromatin compaction around the Vimentin promoter, as seen by nucleosome occupancy assays, and a reduction in the binding of transcriptional activators of the NF-*k*B pathway. These results are the first reported example of R loop-mediated enhancement of gene expression involving divergent antisense transcription at a cancer-related locus. Our findings highlight the role of noncoding RNAs as central components of regulatory chromatin structures.

245 Reductive evolution of non-coding RNAs in bacteria

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Genomes of bacteria that live as parasites or endosymbionts within cells of eukaryotic hosts, such as of Buchnera, Mycoplasma and Rickettsia, are typically smaller when compared to their free-living counterparts. The process of genome reduction, triggered by the conversion from free-living to host-restricted lifestyle, has been extensively studied for protein-coding genes. This process, however, was never systematically studied for non-coding RNA (ncRNA).

We applied a comparative genomics approach to study changes in ncRNAs repertoire triggered by the genome reduction process. We mapped the presence of ncRNA families onto the bacterial phylogenetic tree and investigated patterns of their distribution. We found that in different taxonomic groups the transition from free-living to host-restricted life style is accompanied by a massive loss of ncRNAs. The cis-regulatory RNAs are typically lost together with the regulated protein-coding genes, however sometimes only ncRNA is lost and its genomic surrounding remains unaffected. Such cases allowed us to identify sequences of reduced genomes that do not contain structured RNAs, but are homologous to ncRNAs from non-reduced genomes. By comparing these relict sequences to their functional counterparts, we demonstrate that rapid deletions and reduction of GC content are responsible for the ncRNA loss process. Our study reveals that host-restricted bacteria of different taxa have independently reduced their ncRNA repertoire in a short time frame, presumably at the early stages of the genome reduction process, and we suggest a molecular mechanism responsible for that loss.

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246 The IGF2 mRNA binding protein 1 (IGF2BP1) modulates the action of tumor-suppressive microRNAs

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IGF2BP1 (insulin-like growth factor 2 mRNA binding protein) is an oncofetal RNA-binding protein which becomes de novo synthesized in plethora of distinct cancers. IGF2BP1 expression in cancer was correlated with an overall poor prognosis supporting in vitro evidence suggesting the protein to act as an oncogenic factor. Consistently, IGF2BP1 promotes tumor cell survival, migration and pro-mesenchymal gene expression of tumor cells in vitro. These multiple regulatory roles of IGF2BP1 essentially rely on preventing the degradation of various target mRNAs including MYC, LEF1 and BTRC.

Previous studies have shown that IGF2BP1 itself is targeted by putative tumor-suppressive miRNAs of the let-7 family. Moreover, the IGF2BP1 3′UTRs comprises various in silico-predicted poly-adenylation (APA) sites suggesting that IGF2BP1 is subjected to extensive control by APA and miRNA targeting. Surprisingly, however, the longest IGF2BP1 transcript comprising a 7kb long 3′UTR with five validated let-7 targeting sites was observed in various tumor-derived cells expressing the protein at exceedingly high levels. This suggested that the upregulation of IGF2BP1 in aggressive cancers is largely independent by the APA-dependent shortening of its 3′UTR. Therefore, we aimed at analyzing miRNA-dependent regulation of IGF2BP1 expression in further detail. To this end, we recently established miTRAP, a method allowing the rapid identification of regulatory miRNAs. MiTRAP suggested various novel miRNAs targeting the IGF2BP1 3′UTR. The role of putative tumor-suppressive miRNAs in controlling IGF2BP1 expression and thus IGF2BP1-dependent control of tumor cell fate will be presented.

247 Stress-induced siRNAs target the intron sequences that regulate splice site selection in Brachypodium

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In plants, modulation of gene expression by non-coding small RNAs (smRNAs) in response to environmental conditions acts as an important mechanism regulating cellular networks. Regulation of gene expression by smRNAs also plays crucial roles in defining cellular identity, coordinating developmental programs, and implementing stress responses and disease resistance. However, examples of smRNAs in regulation of gene expression in response to stresses remain largely limited to their role in mRNA stability and translation, as well as in epigenetic regulation, through DNA methylation and histone modifications. Here, we report that in Brachypodium distachyon, exposure to different abiotic stresses specifically induces a group of novel, endogenous small interfering RNAs (stress-induced, UTR-derived siRNAs, or sutr-siRNAs) that originate from the 3′ UTRs of a subset of coding genes. Our bioinformatics analyses also predicted that sutr-siRNAs have regulatory potential and that over 90% of sutr-siRNAs target intronic regions of many mRNAs in trans. Importantly, a subgroup of these sutr-siRNAs target the regulatory regions within introns that affect splice-site selection, indicating that these sutr-siRNAs might affect splicing or alternative splicing of the target mRNAs. We hypothesize that in Brachypodium, the sutr-siRNAs may affect splicing to mediate gene expression in response to stresses and this may serve as a general mechanism for regulation of gene expression in plants.

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248 Involvement of centromeric non-coding RNA in regulation of chromosome segregation

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Chromosome segregation is regulated strictly to inherit genetic information into daughter cells properly. Centromeres are important chromosome regions for assembly of kinetochores and consist of repetitive sequences from which Satellite I non-coding RNAs are transcribed. To investigate functions of Satellite I RNA in chromosome segregation, we performed knockdown of Satellite I RNA using antisense oligonucleotides (ASOs) in human HeLa cells. Depletion of Satellite I RNA caused abnormal chromosome segregation and generation of nuclei with a grape shape phenotype. Similar grape shape phenotype was also observed when mouse satellite non-coding RNA (major and minor RNAs) were knocked down by ASOs in mouse C2C12 cells, suggesting that roles of centromeric non-coding RNAs in chromosome segregation are conserved among mammals. Interestingly, co-immunoprecipitation experiment showed that Satellite I RNA associates with Aurora B kinase, a component of the chromosome passenger complex (CPC) regulating proper attachment of microtubules to kinetochores, in mitotic HeLa cells. In addition, depletion of Satellite I RNA resulted in up-regulation of kinase activity of Aurora B kinase and delocalization of Aurora B kinase from centromeres during mitosis. These results suggest that centromeric non-coding RNA is involved in chromosome segregation through controlling activity and centromeric localization of Aurora B kinase.

249 Interrogation of functional aspects of transcription at heterochromatic loci

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Heterochromatin is essential for the organization of DNA within eukaryotic genomes and plays an important role in protecting genome integrity, centromere formation and nuclear organization. Traditionally, it was assumed the more condensed structure of heterochromatin prevented transcription within heterochromatic regions of the genome by preventing RNA pol II machinery from accessing DNA. Recently, it has been shown that transcription within heterochromatic regions plays an important role in the establishment and maintenance of heterochromatin at three major loci, namely pericentromic, mating-type and subtelomeric. However, it is still not well understood how transcription is accommodated in heterochromatic regions. Furthermore, differences in the mechanisms by which heterochromatin is maintained have been uncovered at each of the three major heterochromatic regions mentioned above. The aims of this study are to expand current knowledge of transcription within heterochromatic loci. Biochemical purification of RNA transcripts arising from heterochromatin regions of the S. pombe genome will be used to identify protein factors involved in silencing of heterochromatic loci and expose possible differences in the mechanisms by which silencing occurs at each of the three major heterochromatic loci. This will be particularly important given the challenges placed on the RNA pol II machinery at condensed and crowded heterochromatic regions. This study will seek to uncover previously unappreciated aspects of transcription at heterochromatic loci.

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250 iSHiRLoC Shines Light on Cancer-Linked microRNA-21 Activity Differences

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MicroRNAs (miRNAs) are ubiquitous, short (~22-nucleotide) non-coding RNA molecules that cells utilize to modulate their protein production. miRNAs bind target messenger RNAs (mRNAs) with partially complementary sequences and recruit the RNA Induced Silencing Complex (RISC), leading to translation initiation inhibition and eventual degradation of the target. The majority of miRNAs have an unusually long half-life (~24 h), which is hypothesized to result from their binding to RISC protein Argonaute 2 (Ago2) that protects them from the cellular degradation machinery. It was originally assumed that the cellular concentration of a particular miRNA is closely correlated with its extent of Ago binding and thus its potential for RISC activity. Recent studies, however, have demonstrated that miRNAs are vastly overexpressed relative to Ago2, but the fraction of each miRNA loaded into Ago2 varies depending on cell type and state.

In 2012, we discovered that disease linked miRNA-21 (miR-21) exhibits starkly reduced target binding and degradation activity in healthy mouse liver relative to a cancer cell-line (HeLa), despite being highly expressed in both cell types. These findings contrasted with other highly abundant miRNAs endogenous to these cell types, such as miR-122 and let-7. Our results correlate well with the refractory response of these healthy cells to anti-miR21 therapeutics, unlike their cancer equivalent. We are currently testing the hypothesis that these differences in miR-21 activity between diseased and non-diseased cells is attributable to the extent of RISC loading of miR-21 in the two cell types.

To this end, we are utilizing our newly developed intracellular single-molecule, high-resolution localization and counting (iSHiRLoC) technique to monitor the spatiotemporal distributions of microinjected, fluorescently labeled duplex miRNAs in cellulo. Here, using a combination of live cell particle tracking and fixed-cell photobleaching analysis, the cellular localization and assembly of miRNA containing particles (such as Processing (P-)Bodies) is monitored over time. Through measuring the spatiotemporal behaviors and cellular localizations of fluorescent miR-21 particles, relative to let-7, we aim to determine the cause of the disease-linked difference. Our current understanding of this difference based on the use of the unique iSHiRLoC assay will be presented.

251 Exploring the evolution of autogenous RNA regulators for ribosomal protein S15

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One of the first proteins recruited during bacterial ribosome assembly, S15 plays a pivotal role in the assembly of the small ribosomal subunit. In addition, S15 regulates its own synthesis in many bacterial species by interacting with a structured portion of the mRNA transcript to prevent further translation. The sequence and structure of this small (12 kD) globular protein and its rRNA binding site are well-conserved among prokaryotes, yet the four mRNA regulatory structures reported are very different, having few or no obvious similarities. While cross-species interaction between S15 and these mRNA structures occurs for some pairings, the mRNA structure found in gammaproteobacteria, such as *E. coli* (Eco-RNA), does not interact with S15 from firmicutes, e.g. *G. kaustophilus* (Gka-S15). This diversity of functionally-equivalent, but distinct, RNA structures suggests that individual binding sites and regulatory mechanisms emerged independently in different bacterial phyla. In order to explore how readily S15-interacting RNA structures maybe formed and better understand the evolutionary processes that lead to such a diversity of RNA regulators, we assessed the potential RNA-binding pool of GK-S15. Our starting RNA pool randomized 30 nucleotides within the core of the Eco-RNA structure, and 11 rounds of Systematic Evolution Ligands by Exponential Enrichment (SELEX) were performed to select for RNAs that are able to interact with Gka-S15. Our preliminary analysis of this population isolated several synthetic RNAs that bind Gka-S15 with nanomolar affinity. Using *in vitro* structural probing assays we determined that the secondary structure of one such RNA is distinct from previously described structures. Further analysis of this population with high-throughput sequencing suggests that S15 may specifically interact with many RNA structures, thus leading us to conclude that the independent evolution of such regulators is relatively facile.

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252 Interaction of the 3′ end of the non-coding RNA 7SK, a regulator of human transcription elongation, with its stabilizing partner LaRP7

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The non-coding 7SK snRNA regulates transcription by RNA polymerase II in human nucleus. This stable RNA sequesters and inhibits the transcription elongation factor P-TEFb which, by phosphorylation of polII and the pausing factors NELF and DSIF relieves pauses of transcription (Peterlin et al. 2012). This regulation process depends on the association between 7SK and protein HEXIM, neither isolated partner being able to inhibit P-TEFb alone. LaRP7, a protein with a La domain, has been shown to bind 7SK specifically to ensure its stability (He et al. 2008). Indeed, several reports of deficiencies in patients have been related to mutations in LaRP7 and loss of LaRP7 leading to 7SK degradation (Alazami et al. 2012). Thus, 7SK and LaRP7 work as a pair to regulate P-TEFb.

The specific recognition of 7SK by LaRP7 has been studied by a mixed approach. The crystal structure of the homology domain at the N-terminus was solved in the presence of RNA, of which only a small stretch is clearly seen in the map. It shows that the uridine triplet at the 3′-end of 7SK RNA is bound exactly at the same place and in the same way as the U-triplet in the homologous La protein (Kotik-Kogan et al. 2008). On the whole, the RNA folds like a fish-hook, with U-2 wedged into a cleft between the two LAM and RRM domains, and U-1 and U-3 stacking on each other. Comparison of La and LaRP7 protein surfaces reveals that most differences are gathered on one face of the RRM side. Some sequences characteristic of each protein indicate different potential RNA-binding surfaces. In order to highlight which elements of 7SK are responsible for the specificity of the interaction, we performed binding (EMSA) and footprinting assays with several RNA and protein constructs, showing that specificity is provided by a C-terminal domain. Our model will be presented.

253 The Prader-Willi critical region generates non-canonical snoRNAs that regulate genes involved in lipid metabolism

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Prader-Willi syndrome (PWS) is a congenital disease characterized by hyperphagia and the resulting obesity. A crucial contributor to PWS is the loss of expression of two small non-coding RNAs (SNORD115 and SNORD116). SNORD115/116 have all the hallmarks of C/D box small nucleolar RNAs, but their expressing units consisting of a hosting intron and two flanking exons also generate smaller RNAs that we termed psnoRNAs for processed snoRNAs (1).

C/D-box small nucleolar RNAs (snoRNAs) are small, non-protein coding RNAs that have been mainly implicated in 2′-O-methylation of pre-rRNAs in nucleoli. SNORD115 and SNORD116 do not posses binding sites for rRNA, which raises the question of their physiological role.

To characterize SNORD115/116 we fractionated cell extracts in 10-40% glycerol gradients. The sedimentation of SNORD115/116 is different from canonical snoRNAs, such as SNORD14a, that is involved in rRNA processing. Importantly, *fibrillarin* and *NOP58*, essential components of canonical C/D box snoRNAs, are absent from SNORD115/116 fractions. Deep sequencing of nuclear extract fractionated in a similar way showed numerous snoRNAs present in fractions lacking *fibrillarin* and *NOP58*, but containing RNA processing factors. This indicates that numerous reported snoRNA fragments are metabolically stable and likely have non-canonical functions.

Using RNA pull-down, we found *hnRNPU* as major component of SNORD116 complexes, further suggesting a non-canonical function. Genome-wide exon junction arrays showed that SNORD115/116 regulates the mRNA level of multiple genes acting in energy metabolism. We identified clusters of putative SNORD115/116 binding sites in target genes that could be validated by pull-down of chromatin containing these genes, as well as functional assays. For example, *FIT-2* a gene that promotes lipid droplet formation is up-regulated by SNORD115/116 and both SNORDs increase the number of lipid droplets, similar to the over-expression of FIT-2 cDNA.

Our findings suggest that numerous snoRNA hosting genes give rise to non-canonical snoRNAs that similar to SNORD115/116 regulate mRNAs, indicating that they form a new class of non-coding RNA regulating biological programs. These ncRNAs likely regulate pre-mRNA processing by bringing regulatory proteins to nascent RNA.

(1) Shen *et al*, 2011, *NAR* 39, 9720-9730.

(2) Falaleeva *et al*, 2013, *BioEssays*, 35, 46-54.

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254 Deciphering RNA regulatory elements using co-expression graphs

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High-throughput gene expression data and its associated computational approaches have been used widely to identify RNA regulatory elements. However, the lack of comprehensive transcriptome data has hampered their application in non-model organisms.

Here, we present a simple but efficient graph-based approach, GRAFFER, which systematically identifies regulatory elements from co-expression graphs, permitting the integration of a wide range of independent datasets.

To demonstrate the potential and versatility of GRAFFER, we applied it to four differently structured co-expression graphs. Our results indicated that 55% of motifs that were predicted based on human dataset, matched with previously known RREs, proving the accuracy of our approach. Besides, application of our approach to a set of organisms known as Kinetoplastids, led to the identification of experimentally established RNA regulatory elements, indicating the applicability of our approach on non-model organisms.

255 Spatiotemporal dissection of cytoplasmic and nuclear miRNA function

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Endogenous microRNA (miRNA) genes are transcribed in the nucleus as primary miRNA transcripts (pri-miRNA) and processed via multiple steps to generate mature miRNAs in the cytoplasm. These small non-coding RNA (ncRNAs) associate with components of the RNA-induced silencing complex (RISC) and engage mRNA targets and regulate gene repression via translational inhibition and/or mRNA degradation. Despite rapid advances in our understanding of miRNA biogenesis and mechanism, the intracellular dynamics and assembly of miRNA-associated complexes and the spatiotemporal modulation of miRNA-regulated gene expression are still unclear. To uniquely probe intracellular RNA silencing pathways, our lab has developed a method termed intracellular Single-molecule High-Resolution Localization and Counting (iSHiRLoC) to determine the localization, diffusion constant and assembly state of single miRNA complexes inside living human cells at 30 nm spatial and 100 ms temporal resolution. We have extended the capabilities of iSHiRLoC to simultaneously detect two colors and examined the diffusion and co-localization of microinjected fluorophore-labeled miRNAs and fluorescent protein labeled P-bodies, sub-cellular foci enriched in RNA degrading enzymes. Our data indicate that there are at least two types of temporarily increasing miRNAs that reside in P-bodies for ~1.18 s and >10 s, representing transient and tight association with P-bodies, respectively, wherein the latter likely reflects miRNA-directed mRNA degradation. In addition, we have used iSHiRLoC to probe nuclear functions of miRNAs. We found that a significant fraction of microinjected mature let-7-a1 (~20-30%) localizes to the nucleus, in contrast to a control cxcr4 miRNA whose whereas nuclear localization was minimal (~5-10%). Inhibition of transcription reduced nuclear localization of let-7-a1 to ~5-10%, the level of cxcr4 in the absence or presence of transcription inhibitor, strongly suggesting that only let-7-a1 binds RNA targets in the nucleus. These observations are consistent with a recent report that describes autoregulation of let-7-a1 biogenesis in the nucleus by mature let-7-a1. We are currently pursuing time course experiments, subcellular fractionation followed by northern blotting and knock down of miRNA-associated proteins to better understand the import mechanism and target engagement of nuclear miRNAs. Together, these studies highlight the versatility of iSHiRLoC by providing biological insight regarding assembly and localization of intracellular miRNAs.

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256 Structural Studies of Intact Long Noncoding RNAs in Plants and Mammals.

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Long non-coding RNAs (lncRNAs) have emerged as key players in development, cancer and plant biology. Few structural studies of lncRNAs have been performed to date [Puerta-Fernandez, PNAS, 2006; Novikova, NAR, 2012; Wan, Mol. Cell 2012; Ilik, Mol. Cell 2013; Davidovich, NSMB, 2013]. Fundamental questions regarding structure have yet to be addressed, including: (1) do lncRNAs have a well-defined structure?, and (2) are lncRNAs organized into modular sub-domains? A third issue that remains poorly understood is the extent of conservation of lncRNAs, which is difficult to ascertain without knowledge of secondary structure. We developed a new experimental strategy called Shotgun Secondary Structure (3S) determination [Novikova, NAR, 2012; Novikova, Methods, 2013]. 3S helps eliminate large numbers of possible secondary folds corresponding to a single chemical probing profile, allowing us to produce the secondary fold of a lncRNA with little need for computational predictions. The technique enabled us to produce the first experimentally derived secondary structure of an intact mammalian lncRNA, the steroid receptor RNA activator, revealing it to be modularly organized into 4 sub-domains with many helices, internal loops and junctions [Novikova, NAR, 2012]. We have also applied 3S to the Coolair, Braveheart and Gas5 lncRNA systems. While Coolair and Braveheart are structured, Gas5 is a chain of stem loops separated by unstructured regions. We use the experimentally derived structures to find a given lncRNA in other species. Using multiple sequence analysis, we then validate helices by identifying covariant base pairs. Our results demonstrate that lncRNAs have a diverse range of structural architectures.

257 Structural insight into the molecular pathway that links miRNA target recognition to silencing

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Structural insight into the molecular pathway that links miRNA target recognition to silencing

Animal miRNAs silence the expression of mRNA targets through translational repression, deadenylation and subsequent mRNA degradation. Silencing requires association of miRNAs with an Argonaute protein (AGO) and a GW182 family protein. In turn, GW182 proteins interact with PABPC and the PAN2-PAN3 and CCR4-NOT deadenylase complexes. These interactions are required for the translational repression, deadenylation and decay of miRNA targets. GW182 proteins are characterized by their richness in tryptophan (W) residues, which are usually located in a sequence environment of predicted structural disorder. These W-containing motifs are either flanked by glycine (GW or WG, termed GW motifs) or by serine/threonine (S/TW or WS/T, termed S/TW motifs). The tryptophan residues in these sequence contexts have been shown to mediate the interactions of GW182 proteins with the Argonaute proteins, and the PAN2-PAN3 and CCR4-NOT deadenylase complexes via cumulative avidity effects. In molecular terms, it has been speculated that the tryptophan residues are accommodated in hydrophobic pockets of the protein partners and that several such pockets and their spatial arrangement could confer increased affinity and specificity. However, the molecular details of these interactions have remained unclear. We have combined cellular, biochemical and structural approaches to investigate how the GW182 proteins specifically interact with their partners to mediate silencing. Our studies uncovered the presence of W-binding pockets in PAN3 and the CNOT9 subunit of the CCR4-NOT complex, revealing the structural basis for the recruitment of deadenylase complexes to miRNA targets. We further show that a MIF4G domain in the CNOT1 subunit of the CCR4-NOT complex interacts with the RNA helicase DDX6, a translational repressor and decapping activator. The crystal structure of this complex demonstrates striking similarity to the eIF4G-eIF4A complex. Together, our data provide the missing physical links in a molecular pathway that connects miRNA target recognition with translational repression, deadenylation and decapping.

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258 Domain dissection of steroid receptor RNA activator (SRA) for coactivation of steroid receptor-mediated transcription

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It has been well known that diverse non-coding RNA (ncRNA) participates in a wide range of cellular processes and plays a key role in cancer development or other disease progression. The steroid receptor RNA activator (SRA) is an RNA regulator that activates steroid receptor-mediated transcription as a putative coactivator in the estrogen receptor signaling pathway. It functions as an RNA molecule itself, but its isoform can encode a protein. SRA RNA levels are elevated in the majority of tumors. Regulatory actions of non-coding SRA core RNA vary depending on the type of estrogen receptors, the presence or absence of ligand, and even reporter systems. SRA RNA can be dissected into 4 domains, but the contribution of each domain to its coactivation ability remains obscure. In this study, we dissected SRA RNA to find structural elements essential for the RNA function. We found that special combinations among the domains as well as specific helices within the domains were important for the coactivation. This result may give an insight into structural basis for interaction of SRA RNA with the estrogen-estrogen receptor complex or other SRA-binding proteins.

259 Rbfox3 Controls the Biogenesis of a Subset of MicroRNAs

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RNA-binding proteins (RBPs) regulate numerous aspects of gene expression, thus identification of endogenous targets of RBPs is important for understanding their functions in cells. Here we identified transcriptome-wide targets of Rbfox3 in neuronally differentiated P19 cells and mouse brain by using Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP). Although Rbfox3 is known to regulate pre-mRNA splicing through binding to the UGCAUG motif, PAR-CLIP analysis revealed diverse Rbfox3 targets including primary-microRNAs (pri-miRNAs) which lack the UGCAUG motif. Induced expression and depletion of Rbfox3 led to changes in the expression levels of a subset of PAR-CLIP-detected miRNAs. In vitro analyses revealed that Rbfox3 functions as a positive and a negative regulator at the stage of pri-miRNA processing to precursor-miRNA. Rbfox3 binds directly to pri-miRNAs and regulates the recruitment of the microprocessor complex to pri-miRNAs. Our study proposes a novel function for Rbfox3 in miRNA biogenesis.

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260 Promoter elements required for expression of BC200 RNA in HeLa cells

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Brain cytoplasmic 200 RNA (BC200 RNA) is a 200 nucleotide-long noncoding RNA in human and shares homologous sequences with 7SL RNA at the 5′ domain. Since BC200 RNA was not observed in somatic cells other than neurons, it was regarded as a neural-specific RNA. However, this neural-speific BC200 RNA is also highly expressed in a number of tumors that are of non-neuronal origin. Therefore, it is conceivable that there is a regulatory circuit for BC200 RNA expression in cancer cells. It was previously shown that RNA polymerase III transcribes BC200, but the promoter elements have not been identified yet. As an initial step to find the regulatory circuit, we set to analyze elements required for efficient expression of BC200 RNA in HeLa cells. We found that the upstream sequences as well as the downstream sequences are important for BC200 expression. Extragenic factors involved in BC200 gene transcription were also characterized. The results may provide mechanistic links between aberrant BC200 expression and specific signaling pathways related to tumorigenesis.

261 Small RNAs derived from lncRNA RNase MRP have gene-silencing activity relevant to human cartilage–hair hypoplasia

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Argonaute proteins of the RNA-Induced Silencing Complex (RISC) have been shown to interact with a diverse pool of RNAs. The post-transcriptional processing of some long noncoding RNAs has been shown to be the source of miRNAs. Here we report that the post-transcriptional processing of a long non-coding RNA, the RNA component of mitochondrial RNA processing endoribonuclease (RNase MRP), produces two short RNAs that associate with Ago2. These short RNAs, approximately 20 nucleotides in length, function as miRNAs and regulate post-transcriptional levels of many genes. Point mutations in RNase MRP are known to be the cause of human cartilage-hair hypoplasia (CHH), a development disease with a spectrum of phenotypes that vary in severity including metaphyseal osteochondrodysplasia, mild-to-severe immunological deficiencies, anemia, gastrointestinal malabsoportion and increased incidence of malignancies. Several of these disease causing mutations map to the two short RNAs identified, designated RMRP-S1 and RMRP-S2. These mutations alter secondary structure as SHAPE chemical probing was used to identify two alternative conformations. RMRP-S1 and -S2 are both significantly reduced in two fibroblast cell lines and one B-cell line derived from CHH patients. The gene regulatory activity of RMRP-S1 and -S2 was tested and showed significant regulation of over 900 genes, 75% of these were down-regulated and 90% contained target sites with seed complementarity to RMRP-S1 or -S2. Pathway analysis identified regulated genes are linked to major CHH phenotypes including genes that function in skeletal development, hair development and hematopoietic cell differentiation including PTCH2 and SOX4 among others. Furthermore, genes associated with splicing of minor introns, cell proliferation and differentiation were highly targeted. Therefore, alterations RMRP-S1 and -S2, caused by point mutations in RMRP, are strongly implicated in the molecular mechanism of CHH.

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262 Antisense RNA from the Histone Gene Clusters : Novel Regulator or Noisy Transcription?

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Bidirectional transcription produces *cis* natural sense-antisense (SA) transcript pairs. Those can form double-stranded (ds) RNA through base-pairing. In Eukaryotes, dsRNA activates RNAi pathways, regulating gene expression through targeting of complementary transcripts for degradation and/or chromatin remodeling. As much as 20% of transcripts form SA pairs in fly, mouse and human, supporting the view that antisense regulation represents a major, conserved layer of expression regulation.

Interestingly, we have documented SA transcript pairs of all five canonical *histone* genes (H1, H2A, H2B, H3, H4) in *Drosophila* ovarian, embryonic tissues and cultured cell lines by Northern blotting (NB) and RNA FISH. Antisense *histone* RNA populations appear as chromatin-bound *foci*, accumulating in early syncitial embryos as a result of precocious zygotic transcription. It reflects a rare expression event, since this phenotype is noted well before the midblastula transition (MBT) and global zygotic transcriptional activation. Moreover, our NB analysis suggests bidirectional expression of discrete polycistronic long transcripts, encompassing the four canonical core *histones* and spacer regions. This piece of data is especially intriguing in the light of emerging regulatory functions of long non-coding (lnc) RNA.

Indeed, the histone-related non-coding and heavier-than-mRNA transcripts could exert crucial and diverse regulatory roles in *histone* gene expression. Importantly, the *Drosophila histone* locus contains a large complex of tandemly arrayed units of the five *histones*. Expression of these genes is replication-dependent, extensively relies on a set of specific factors and requires the assembly of a *histone* locus body (HLB). This unique transcriptional landscape may involve RNA effectors, which could act as scaffolding devices, guide chromatin-binding factors to genomic regions or block mRNA expression in a cell cycle-dependent fashion. Here, we pursue a detailed characterization of the *histone*-related transcriptome relying on hybridization-mediated target enrichment and long-read, paired-end deep-sequencing. This effort provides valuable insights into the transcriptional regulation of canonical *histone* gene expression and will likely uncover its RNA-mediated facet.

263 Computational model of the dynamics of fourU and ROSE RNA thermometers at elevated temperatures

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RNA thermometers are short non-coding RNAs responsible for cell's response to heat or cold shock conditions (1). Thermometers are built of RNA sequences (up to 120 nucleotides long) and typically positioned in the 5′ UTR region of mRNA. Two important examples of thermometers are fourU (2) and repression of heat shock gene expression (ROSE) element. Both work by forming a hairpin that includes the Shine-Dalgarno sequence of mRNA. Upon heating the hairpin gradually unwinds, which increases the translation of a gene adjacent to the RNA thermometer. Such thermosensing sequences may be used as translation control method but a reliable method to design sequences with such properties has to be developed.

We have been developing a coarse-grained molecular dynamics (3) approach to model the dynamic behavior of a 3D structure of an RNA thermometer during temperature rise. To parameterize the model (4) we perform full-atomistic molecular dynamics simulations of these thermometers, biophysical experiments (UV-monitored thermal melting, FRET measurements) and use previous experiments of other groups, e.g., (1,2).

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264 RNA-Directed Regulation of pri-miRNA Processing by a Long Noncoding RNA Transcribed from an Ultraconserved Region

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A rich landscape of short and long regulatory noncoding RNAs (ncRNAs) is becoming apparent. In addition to controlling cellular programs by affecting protein-coding genes, evidence increasingly points to their involvement in a network of ncRNA-ncRNA interactions. Here, we investigate the role of a long ncRNA transcribed from an ultraconserved region (T-UCR) in the control of post-transcriptional pri-miRNA processing. The regulation is based on complementarity between the lower stem region in pri-miR-195 transcript and the ultraconserved sequence in Uc.283+A, which prevents pri-miRNA cleavage by Drosha. Mutation of the site in either RNA molecule uncouples regulation in vivo and in vitro. We propose a model in which lower-stem strand invasion by Uc.283+A impairs microprocessor recognition and efficient pri-miRNA cropping. In addition to providing the first reported case of RNA-directed regulation of miRNA biogenesis, our study reveals new regulatory networks involving different ncRNA classes of importance in cancer.

265 Nuclear localization control of the long non-coding roX RNA involved in dosage compensation in fly

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Long, non-coding RNAs participate in a wide range of cellular processes. A particularly instructive example is provided by the dosage compensation system of *Drosophila melanogaster*, which involves the transcriptional activation of most genes on the single X chromosome in male flies to match their expression levels to those in females, where both X chromosomes are transcribed. The dosage compensation process requires the activity of a ribonucleoprotein assembly called dosage compensation complex (MSL-DCC). The MSL-DCC consists of five male-specific lethal (MSL) proteins and two long, non-coding RNA, r*oX1* and r*oX2* (RNA on the X). These RNAs are transcribed by RNA polymerase II, capped, spliced and polyadenylated. However, despite their common features with messenger RNAs, the *roX* RNAs localize exclusively to the nucleus, where they regulate the association of the MSL-DCC with the X chromosome. Up to now, the mechanism controlling the *roX* RNA localization is poorly understood. Here, we report on the results of our recent efforts to identify and characterize factors controlling nuclear retention of *roX* RNAs.

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266 Widespread accumulation of circRNAs during aging

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While most cellular RNA is believed to be linear, RNA can also exist in circular form (circRNAs). Such species can be accessed by performing total RNA-sequencing on rRNA-depleted samples. Although rare circRNAs can titrate miRNA activity, the functions of these recently-appreciated non-coding RNAs, if any, are largely unknown. Here, we mined billions of total RNA-seq reads from *Drosophila* cell lines, developmental stages and tissues to annotate ~3000 circRNAs using stringent criteria. These circRNAs are comprised predominantly of protein-coding exons, and are biased to arise from early exons flanked by large introns. Accumulation of circRNAs was higher in the nervous system compared to other tissues; moreover, circRNAs increased relative to their host genes across an aging timecourse of heads. Our observations support future investigations into potential roles for circRNAs in age-related neurodegenerative diseases and/or as aging biomarkers.

267 3′ end processing affects the ability of noncoding RNAs to act as platforms for chromatin modification

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Targeting of nascent noncoding pericentromeric transcripts by an siRNA-programmed Argonaute complex leads to recruitment of histone H3 lysine 9 (H3K9) methylation and heterochromatin formation in fission yeast. However, siRNAs cannot promote heterochromatin formation at complementary euchromatic regions, suggesting that they cannot use mRNA as a scaffold to recruit H3K9 methylation. We recently showed that signals within the mRNA 3′ untranslated region antagonized siRNA-mediated heterochromatin formation. I will report on our progress in identifying the 3′ end pathways that process centromeric noncoding RNAs. These pathways play key roles in determining which RNAs can become targets of RNAi and furthermore which RNAs can act as scaffolds for siRNA complexes that promote histone methylation and heterochromatin formation.

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268 Expression of the vault RNA protects cells from undergoing apoptosis

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Non-protein-coding RNAs are a functionally versatile class of transcripts found in all domains of life exerting their biological role at the RNA level. Recently, we demonstrated that the vault-associated RNAs (vtRNAs) were significantly up-regulated in human B cells upon Epstein-Barr virus (EBV) infection [1,2].

vtRNAs are an integral part of the vault complex, a huge and evolutionarily conserved cytoplasmic ribonucleoprotein complex. The major vault protein (MVP) is the main structural component of the complex while vtRNA accounts for only 5% of its mass. Very little is known about the function(s) of the vtRNAs or the vault complex. In particular the role and significance of the previously observed vtRNA up-regulation upon EBV infection remained unclear. We individually expressed EBV-encoded genes in B cells and found the latent membrane protein 1 (LMP1) as trigger for vtRNA up-regulation. To unravel a putative functional interconnection between vtRNA expression and EBV infection, we ectopically expressed vtRNA1-1 in human B cells and observed an improved viral establishment. Furthermore, expression of vtRNA1-1 but not of the other vtRNA paralogs protected cells from undergoing apoptosis. Knock-down of MVP had no effect on these phenotypes thus revealing the vtRNA and not the vault complex to contribute to the enhanced EBV establishment and apoptosis resistance. Mutational analysis highlighted the central domain of the vtRNA to be involved in the anti-apoptotic effect. Ongoing research aims at characterizing the target of vtRNA1-1 in the apoptotic pathway. In summary, our data reveal a crucial cellular function for the so far elusive RNA biology of the vtRNAs.

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269 Are viroid-specific small RNAs instructive and mediate a pathogenesis network?

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Viruses and subviral particles including viroids afflict and exploit hosts to ensure informational integrity. The enigmatic Potato Spindle Tuber Viroid (PSTVd), composed of a non-coding, circular, unencapsidated and single-stranded RNA of ~360 nucleotides (nt), infects plants as an obligate parasite of the host transcription machinery. Depending on the sequence variant, PSTVd induces mild to severe symptoms of dwarfism, epinasty, stunting and necrosis on the PSTVd model-plant tomato (*Solanum lycopersicum*) and leads to significant global crop losses (for review see Matoušek *et al.*, 2012). Based on the proposed pathogenicity theorem (Diermann *et al.*, 2010), we hypothesize that viroids encode viroid-specific instructive small RNAs (vsiRNA) (19-24 nt) to modulate a viroid-favorable environment by interfering with the host expression machinery similar to microRNA (miRNA) or small interfering RNA (siRNA) pathways. Subsequently, vsiRNA-guided cleavage converts host transcripts into pathogen-intended trans-acting small RNAs (tasiRNA) that initiate and propagate a regulatory cascade through the transcriptome and reciprocally on the viroid, establishing a complex bidirectional pathogenesis network. The broad host range of viroids (Matoušek *et al.*, 2013) implicates an adaptive evolution of a modular network. Recently, Navarro *et al.* (2012) showed in support of the pathogenicity theorem that Peach Latent Mosaic Viroid (PLMVd)-derived vsiRNAs mediate host transcript degradation resulting in a severe albinism. We developed a bioinformatical framework for vsiRNA target prediction to identify host targets and explore this network. Subsequently, we essayed experimentally these *in silico* relations and show distinct target transcription alterations. We conclude from computational and experimental results that PSTVd expresses vsiRNAs to adaptively modulate the respective host through signals that enter a self-amplifying circuit converting endogenous transcripts resulting in a massive extension of the viroid instruction set.

Acknowledgments

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270 An mRNA-derived ncRNA targets and regulates the ribosome

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Small non-protein-coding RNA (ncRNA) molecules are key players in controlling gene expression at multiple steps in all domains of life. While the list of validated ncRNAs that regulate crucial cellular processes grows steadily (such as micro RNAs and small-interfering RNAs), not a single ncRNA has been identified that directly interacts and regulates the ribosome during protein biosynthesis (with the notable exceptions of 7SL RNA and tmRNA).This is unexpected, given the central position the ribosome plays during gene expression.

To investigate whether such a class of regulatory ncRNAs does exist we performed genomic screens for small ribosome-associated RNAs in various model organisms of all three domains [1,2]. Here we show that an mRNA-derived 18 nucleotide long ncRNA is capable of down-regulating translation in *Saccharomyces cerevisiae* by directly targeting the ribosome [3]. This 18-mer ncRNA binds to polysomes upon salt stress and is crucial for efficient growth under hyperosmotic conditions. Although the 18-mer RNA originates from the *TRM10* locus, which encodes a tRNA methyltransferase, genetic analyses revealed the 18-mer RNA nucleotide sequence, rather than the mRNA-encoded enzyme, as the translation regulator under these stress conditions. Our data reveal the ribosome as a target for small regulatory ncRNAs and unveil the existence of a novel mechanism of translation regulation.

Analogous genomic screens in organisms spanning all three domains of life demonstrate the existence of thousands of ncRNA candidates putatively regulating the ribosome. We therefore anticipate that ribosome-bound ncRNAs are capable of fine tuning translation and might represent a so far largely unexplored class of regulatory ncRNAs.

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271 Functional analysis of miR-202 and its role in zebrafish sexual development

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MicroRNAs (miRNAs) are short regulatory RNAs which mainly suppress translation of their target genes. In recent years, high throughput sequencing has identified conserved and novel miRNAs in a variety of species. However, while target prediction software is improving in its accuracy, there is still a need for functional studies to determine the biological role of a specific miRNA. In the present study, we examine the function of miR-202 in zebrafish (*Danio rerio*). miR-202 is an evolutionarily conserved miRNA notable for its gonad-specific expression and suspected role in promoting early testis development through down-regulation of ovarian transcription factors. Aside from its function in testis, there also remains questions about its function in ovary and during embryogenesis. The current study aims to expand knowledge on miR-202 through a variety of functional studies including: quantitative real-time PCR during embryogenesis and sexual maturation, whole-mount and tissue-specific *in situ* hybridization, as well as targeted genetic knock-down and knock-out trials. Through these studies we hope to determine the crucial interactions of miR-202 in teleost fishes while developing zebrafish as a robust model for functional studies of other conserved miRNAs

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272 Distant SAM Riboswitch Variants in Betaproteobacteria

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Riboswitches are structured RNA elements within mRNAs that control gene expression through selective binding of their ligand. Their aptamer domain is responsible of binding their cognate metabolite ligand, which exert gene control following a conformational rearrangement of the expression platform domain. S-adenosylmethionine (SAM) is currently the metabolite for which there is the largest number of different classes of riboswitches reported, at least four different classes, and up to seven, depending on the classification used.

Recent bioinformatics searches in our lab revealed new structured motifs upstream of *metK* which were found to be capable of binding SAM by in-line probing assays. Thorough analysis of the alignments showed us that this putatively new riboswitch was in fact a variant of class II SAM riboswitches harboring an additional stem that apparently competes with the SAM II binding pocket. These variant riboswitches are conserved in close to a hundred species of Betaproteobacteria, including many pathogenic bacteria in the *Burkholderia* and *Bordetella* genuses. The selectivity for SAM is orders of magnitude greater than for S-adenosylmethionine, even if it is somewhat lower than with other SAM-riboswitches.

273 P53 alternatively regulates the expression of GADD45a by miR-138 / AGO2 / miR-130b pathway in human lung cancer cells

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P53 alternatively regulates the expression of GADD45a by miR-138/ AGO2/miR-130b pathway in human lung cancer cells

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In past decades, p53 is considered as the core molecule in the complicated regulatory molecular network related to cancer and stress. Until recent years, people found that the components of p53 regulation network was not only proteins and coding genes, noncoding RNAs such as microRNAs were emerging as new molecules involved in the p53 regulation network. More and more evidences showed that p53 as a trancriptional factor was directly or indirectly involved in the transcriptional regulation of microRNAs. Here we showed that p53 could active the transcription of miR-138 in human lung cancer H1299 and H460 cells. The highly expressed miR-138 could target AGO2 and result in the downregulation of miR-130b. We found that the decresed expression of miR-130b could increase the expression of GADD45a which was confirmed as one of the target of miR-130b. As the results of p53/miR-138/AGO2/miR-130b/GADD45a regulation pathway, the cell cycle was arrested in mainly in G2/M phase. Our results showed for the first time that p53 could alternatively regulate the expression of GADD45a through miR-138/ AGO2/miR-130b pathway in human lung cancer cells except for its classical p53 - GADD45A direct ineraction regulation pathway.

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274 Characterization of Selenoprotein P 3′UTR and its role in translation

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Selenoproteins constitute an important family of proteins that have a variety of redox-related essential functions. Selenoproteins are unique as they incorporate amino acid selenocysteine (Sec) which is encoded by an in frame stop codon (UGA) located within the coding sequence. Sec incorporation is highly specific and requires a Sec insertion sequence (SECIS) element in the 3′ UTR of all eukaryotic selenoprotein mRNAs. Three trans-acting protein factors are also required, namely the SECIS binding protein 2 (SBP2), a dedicated eukaryotic elongation factor eEFSec and a unique selenocysteyl tRNA. These factors are known to be sufficient for Sec incorporation *in vitro,* albeit with an efficiency of only ~3% of total translation. But *in vivo,* this process is both specific but efficient. Among the known selenoproteins, Selenoprotein P (SelP), known to be responsible for selenium transport, is the only selenoprotein that contains multiple Sec residues in its primary structure and 2 SECIS elements in its 3′UTR. The mechanism of Sec incorporation within this protein is further complicated by its presumably processive nature. Interestingly, this protein has a long 3′UTR which leads to our postulation that either a potential *cis*-element located within the SelP 3′UTR or a *trans* protein factor that possibly binds the SelP 3′UTR is responsible for efficient and processive Sec incorporation *in vivo*. In our study, we have characterized the SelP 3′UTR using deletion mutagenesis and attempted to analyze the interplay between processivity from efficiency. We found that SECIS elements are intrinsically processive and is more efficient when put in context to its own coding region. In addition, *in vitro* translation using mammalian cell lysate yielded processive full length SelP and absence of early termination products. However, reconstitution of known Sec incorporation factors *in vitro* in wheat germ lysate allowed translation of a reporter construct containing single selenocysteine residue but was not sufficient for full length SelP synthesis thus suggesting the role of yet unidentified mammalian factors for processive Sec incorporation. We are currently investigating potential candidates that could govern SelP synthesis either as an efficiency factor or by enhancing RNA stability.

275 Translation of small open reading frames within unannotated RNA transcripts in *Saccharomyces cerevisiae*

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High-throughput gene expression analysis has revealed that eukaryotic cells express many previously unidentified and largely uncharacterized transcripts. In most cases, these transcripts are bioinformatically predicted to lack protein-coding capacity. Here, we investigate >1100 unannotated transcripts in yeast predicted to be non-coding. We show that a majority of these RNAs associate with polyribosomes to an extent more similar to mRNAs than classical, well-characterized non-coding RNAs. Furthermore, ribosome profiling demonstrates that many of these RNAs are directly bound by ribosomes, with a number demonstrating periodicity of ribosome footprints indicative of *bona fide* translation. Location of ribosome footprints facilitated the identification of putative open-reading frames 10-96 codons in length within a number of unannotated RNAs. We directly demonstrate translation of a subset of these open-reading frames through detection of the predicted polypeptide, and provide evidence for potential conservation of polypeptide sequences across yeast species. Consistent with their translation, many unannotated RNAs are targeted for degradation by the translation-dependent, nonsense-mediated RNA decay (NMD) pathway. Based upon ribosome profiling, we predict these RNAs are targets for NMD due to long 3′ ribosome-free regions. Inspection of mammalian lncRNAs reveals that a subset are also sensitive to NMD, indicating translation of non-coding transcripts in higher eukaryotes. These data demonstrate that transcripts considered to lack coding potential do, in fact, engage translating ribosomes. An intriguing possibility is that this translation serves either as a mechanism to regulate the metabolism or function of these RNAs or to produce short proteins, potentially expanding the proteome of yeast and other eukaryotes.

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276 SINE-mediated repression of gene expression

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Alu elements are conserved, ~300 nucleotide long sequence repeats that belong to the SINE family of retrotransposons found abundantly in primate genomes. Alu elements are enriched in gene-rich regions, where they are located within non-coding segments of transcripts, such as introns and untranslated regions. SINEs can have a dramatic impact on the transcriptome by several means such as repressing global transcription by impairing polymerase II activity, affecting folding and splicing, or by triggering Staufen mediated decay. In addition, SINE elements in inverted orientation can basepair and have been reported to control gene expression. As underlying mechanisms, RNA-editing, sequence specific degradation, or translational control has been discussed.

Here we aim at determining the impact of inverted, base-paired SINEs located in 3′ UTRs on gene expression. So far we demonstrate that the presence of inverted SINEs in 3′ UTRs can repress gene expression and reduce mRNA levels. Using knock-out cells we show that the reduced gene expression does not rely on known double-stranded RNA-dependent pathways mediated by Dicer, Staufen, PKR, or ADAR, and is sequence independent.

The reduced RNA levels measured for RNAs containing base-paired double-stranded SINEs, could not be correlated with an increase in mRNA decay. In addition the length or position of poly-adenylation, which could alter translational efficiency or mRNA stability, has not been affected. Our preliminary chromatin immunoprecipitation (CHIP) data show that the distribution of Pol-II is different in iSINE containing mRNA and control mRNA. It therefore appears that inverted SINEs can control the rate of RNA synthesis. Thus, besides the previously reported nuclear retention and translational repression induced by inverted SINEs, transcription may be regulated by a third Alu-triggered mechanism.

277 Circular Intronic Sequences in the Cytoplasm of *Xenopus* Oocytes

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We previously demonstrated that oocyte nuclei (germinal vesicles or GVs) of *Xenopus tropicalis* store stable intronic sequence (sis) RNAs. To determine if sisRNAs also occur in the cytoplasm, we sequenced RNA from purified cytoplasmic samples. Because the GV can be removed intact from the oocyte by simple manual dissection, it is possible to obtain a cytoplasmic fraction that is completely free of nuclear contamination. To our surprise, a small number of reads mapped to 10% of all introns. We showed that these intronic reads correspond to small cytoplasmic sisRNAs, mostly 200 to 500 nt in length. To assess the molecular nature of cytoplasmic sisRNAs, we treated the cytoplasmic RNA fraction with RNase R, a processive 3′ to 5′ exonuclease. Most mRNAs were degraded, whereas cytoplasmic sisRNAs were enriched 25 fold. Further analysis demonstrated that these sequences are stored as circular molecules (lariats without tails). Lariats are normally produced as an intermediate in splicing and are rapidly degraded in the nucleus by the debranching enzyme (Dbr1) and exonucleases. We show that this degradation pathway is present in the nucleus, as expected, but cytoplasmic sisRNAs are not degraded. Our data suggest that specific intronic sequences somehow escape the debranching pathway and are exported intact to the cytoplasm, where they are stable. Cytoplasmic sisRNAs are specific to late oogenesis and are transmitted to the early embryo.

We also found numerous circular sisRNAs in the cytoplasm of *Xenopus laevis* oocytes. There is no conservation of the genes or introns in which these sequences occur, perhaps reflecting the 50 million year evolutionary separation between *X. tropicalis* and *X. laevis*.

Taken together, the data suggest that cytoplasmic sisRNAs may function in late oogenesis or early embryogenesis. Translation of mRNAs is tightly regulated during these periods and one possibility is that sisRNAs are exported to the cytoplasm to play a role in protein synthesis.

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278 Quality control of telomerase RNA biogenesis

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Telomerase is a key enzyme that maintains and replenishes telomeric DNA by using part of an RNA subunit as a template for reverse transcription. Previously we have identified telomerase RNA (TER1) from fission yeast and revealed the presence of an Sm binding site immediately followed by an intron near the 3′ end of the RNA. We showed that, unlike other intron-containing RNAs, the TER1 precursor undergoes only the first transesterification reaction but escapes exon ligation. After this cleavage reaction, the Sm proteins are replaced by a complex comprised of Lsm2 to Lsm8. Lsm2-8 proteins protect the mature 3′ end of TER1 from degradation and facilitate the assembly of telomerase holoenzyme. Both precursor and spliced forms of TER1 RNA fail to support telomere maintenance and the accumulation of these inactive products may cause dominant-negative effects. To characterize the stability and processing of the different forms, we synthesized the TER1 precursor, spliced, and mature forms in vitro, and individually incubated them with *S. pombe* extracts. We found that the precursor and spliced forms were highly unstable and rapidly degraded. A series of deletions of TER1 RNA reveals an RNA motif that is critical for destabilizing the precursor and spliced forms of TER1 RNA. Interestingly, when the mature form of TER1 was incubated in the presence of ATP, the TER1 RNA was polyadenylated and destabilized. The efficiency of polyadenylation was dramatically enhanced when the association of Lsm2-8 complex with the mature form of TER1 but was abolished by mutating or deleting the sm-binding site. Our results provide insights into the molecular mechanism that underlie the quality control in TER1 RNA biogenesis that eliminates inactive forms of the RNA.

279 The DRBD13 RNA binding protein is involved in the developmental regulation of mRNA stability in Trypanosoma brucei

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Trypanosoma brucei, a unicellular parasite, is the causative agent of human sleeping sickness. In this organism, unlike most eukaryotes, regulation of gene expression occurs mainly at the post-transcriptional level. In this process, RNA binding proteins (RBPs) through recognition of specific sequence motifs or secondary structures in the transcripts, mainly in the 3′ untranslated regions (3′-UTRs), affect mRNA maturation, localization, stability, and translation rate. Previous application of our novel computational framework led to the identification of a highly conserved AU-rich element (ARE) in 3′-UTR of many T. brucei transcripts with a major role in the developmental regulation of target mRNAs. Further experimental and computational analyses uncovered DRBD13 as an RBP contributing to the stability of ARE-containing mRNAs. DRBD13 immunoprecipitation followed by RNA sequencing verified the association of DRBD13 with ARE-containing transcripts, including the developmentally critical TbRBP6. Here, we explored the regulatory effect of RNAi-mediated inhibition as well as ectopic overexpression of DRBD13 on three ARE-containing target mRNAs that it acts on. Using quantitative PCR, we validated a correlation between the presence of DRBD13 and the mRNA abundances of these three genes. In addition, a CAT reporter construct, containing the RBP6 3′-UTR showed that the CAT expression was affected by the levels of DRBD13 expression, suggesting that the regulatory effect of DRBD13 on the targets is mediated by their 3′-UTRs.

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280 Regulation of microRNA Turnover in Mammalian Spermatogenesis

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Mammalian spermatogenesis is a complex cell differentiation process that begins at birth and continues throughout life. Mouse testicular germ cells undergo sequential morphological and biochemical changes to develop into mature spermatozoa. Regulation of the molecular events required for spermatogenesis is essential for fertility. Small RNAs play a key role in this process. MicroRNAs (miRNAs) compose the largest class in mouse testes and are known for their function in posttranscriptional gene regulation. A number of miRNAs are enriched in the testis. In addition, conditional *Drosha* and *Dicer* knock-out mice show spermatogenic defects[1,2]. The findings imply a requirement for miRNAs for normal spermatogenesis. To understand the miRNA expression during spermatogenesis, we measured the miRNA abundance from 0.5 – 42.5 dpp mouse testes across 11 developmental time stages. The analysis shows that 198 miRNAs contribute to the majority of all miRNA reads. Their temporal expression patterns are distinct. Non-templated nucleotide addition—"tailing"—of miRNAs has been proposed to influence their stability, regulatory efficiency, and loading into Argonaute proteins. How miRNAs are targeted for tailing remains largely unknown. We analyzed the 3′ ends of the 198 miRNAs and found that many of them exist as isoforms bearing non-templated nucleotides added to their 3′ ends. An increase in the isoform abundance often precedes a decrease in the expression level of a miRNA. PIWI-interacting RNAs (piRNAs) represent another important class of small RNAs in spermatogenesis. The transcription factor, A-MYB, initiates transcription of the precursors of a sub-class of piRNAs, pachytene piRNAs[3]. We identified miRNAs that are regulated by A-MYB. This suggests possible coordination of the miRNA and piRNA pathways by A-MYB to ensure successful spermatogenesis. We aim to understand three key aspects of miRNAs in mouse germ cell development: (1) miRNA tailing as a potential major mechanism of regulating miRNA turnover, (2) the cellular environment as a determinant of miRNA tailing and abundance, and (3) interconnection of the miRNA and piRNA networks as a requirement for normal spermatogenesis.

281 Species-spedific alternative splicing leads to unique expression of *sno-lncRNA*s

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Intron-derived long noncoding RNAs with snoRNA ends (*sno-lncRNA*s) are highly expressed from the imprinted Prader-Willi syndrome (PWS) region on human chromosome 15. However, *sno-lncRNAs* from other regions of the human genome or from other genomes have not yet been documented. By exploring non-polyadenylated transcriptomes from human, rhesus and mouse, we have systematically annotated *sno-lncRNA*s expressed in all three species. In total, using available data from a limited set of cell lines, 19 *sno-lncRNA*s have been identified with tissue- and species-specific expression patterns. Although primary sequence analysis revealed that snoRNAs themselves are conserved from human to mouse, *sno-lncRNAs* are not. PWS region *sno-lncRNA*s are highly expressed in human and rhesus monkey, but are undetectable in mouse. Importantly, the absence of PWS region *sno-lncRNA*s in mouse suggested a possible reason why current mouse models fail to fully recapitulate pathological features of human PWS. In addition, a *RPL13A* region *sno-lncRNA* was specifically revealed in mouse embryonic stem cells, and its snoRNA ends were reported to influence lipid metabolism. Interestingly, the *RPL13A* region *sno-lncRNA* is barely detectable in human. We further demonstrated that the formation of *sno-lncRNA*s is often associated with alternative splicing of exons within their parent genes, and species-specific alternative splicing leads to unique expression pattern of *sno-lncRNA*s in different animals. This study demonstrates a complex regulatory network of coding and noncoding parts of the mammalian genome.

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282 Long non coding RNAs in Epithelial to Mesenchymal Transition

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The Epithelial to Mesenchymal Transition (EMT) is a biological process that occurs during normal embryonic development and tissue regeneration. EMT is thought to be one of the mechanisms of metastatic process during carcinogenesis. Different mRNAs and microRNAs are strongly misregulated in EMT and identified as markers of EMT [1]. With the expansion of long non-coding RNAs catalogs these last few years, we address the question of their potential implication in EMT, and their value as EMT markers.

For this purpose, we performed strand-specific RNAseq in a stable cellular model of EMT in Human Epithelial Kidney (HEK) cells [2]: before transition with normal epithelial traits (HEK-Epi) and after transition with mesenchymal traits (HEK-Mes).

Computational analysis of RNAseq data confirmed the differential expression of known Epithelial and Mesenchymal mRNAs markers. In addition, we identified a set of long non-coding RNAs (88 annotated and 10 novel intergenic ones) that are significantly misregulated between Epithelial and Mesenchymal cells. Thanks to the strand-specific data generated by RNAseq, we succeeded to identify annotated antisense RNAs (18) and a new class of antisense transcripts (195) that are misregulated in EMT. We confirmed a selection of RNA-seq candidates (mRNAs, lncRNAs and antisense RNAs) for differential expression using RT-qPCR in HEK-EPI and HEK-Mes (14 mRNAs candidates, 3 lncRNAs and 9 antisense RNAs).

Interestingly, we identified Hotair, a known antisense lncRNA, as highly expressed in mesenchymal cells compared to epithelial cells, and having a putative role in invasiveness and motility of cells. Hotair is involved in poor prognosis in several human cancers by regulating the expression of several mRNAs targets [3].

Finally, to assign potential roles to misregulated non-coding RNAs in EMT, we measured their correlation with all expressed mRNAs among different cell lines and tissues. This approach identifies links/targets or co-expression players potentially implicated in EMT and in carcinogenesis.

283 A knockout mouse model to define the roles of the Epithelial splicing regulatory proteins in development and epithelial cell function

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The Epithelial splicing regulatory proteins 1 and 2 (Esrp1 and Esrp2) coordinate a splicing regulatory network (SRN) of transcripts associated with processes necessary for epithelial differentiation. Esrp targets include regulators of cell polarity, adhesion, cytoskeletal organization, and motility such as FGFR2, CD44, and p120-Catenin. Esrp expression is epithelial cell-type-specific and switch-like changes of several known Esrp regulated alternative splicing targets are crucial during embryonic development. While signaling and transcriptional regulation of epithelial-mesenchymal interactions have been extensively evaluated during development, the contribution of tissue-specific splicing regulatory factors is limited. We propose the Esrps regulate a SRN converging on pathways crucial for normal epithelial cell differentiation and epithelial-mesenchymal interaction during embryonic development. To evaluate the requirement of the Esrps in these fundamental processes, we generated mouse knockout alleles for Esrp1 and Esrp2. Consistent with in vitro studies which indicate Esrp1 as the primary functional splicing factor, ubiquitous knockout of Esrp1 alone leads to neonatal lethality. Esrp1 knockout mice present with an array of developmental defects analogous to perturbation in FGF signaling, including cleft lip and palate, with increasingly severe defects when Esrp2 is also ablated. Moreover, Esrp knockout mice provide the first evidence for a functional requirement of the Esrps in normal development, and provide a genetic tool to evaluate the complex regulation of epithelial-mesenchymal interaction at the level of alternative splicing. Splicing analysis in Esrp knockout mouse tissues indicates transcript specific susceptibility to alteration in splicing of Esrp targets, with changes in splicing ranging from complete switches in Esrp1 KO tissues while others require loss of both Esrp1 and Esrp2. This analysis of transcript specificity will provide insight into the complex SRN regulated by the Esrps in normal epithelial development and correlate specific alternative splicing events with the phenotypes observed upon ablation of Esrp1 alone or Esrp1/Esrp2 double KO. Furthermore, we have generated conditional knockout alleles for Esrp1 and Esrp2 that will afford the identification of in vivo Esrp targets in a variety of epithelial populations from various tissues, as well as evaluation of the functional consequences of specific alternative splicing events during development and in primary cells.

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284 Homologous *trans*-editing factors with broad substrate specificity prevent global mistranslation

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Aminoacyl-tRNA synthetases (aaRSs) are responsible for covalent attachment of the correct amino acid to their cognate tRNA substrates. This process is inherently error prone due to the isosteric nature of amino acids thus quality control mechanisms have evolved to maintain translational fidelity. In the case of bacterial prolyl-tRNA synthetase (ProRS), a triple-sieve editing mechanism is generally employed, which consists of the ProRS active site that discriminates amino acids based primarily on volume and size, a *cis*-editing domain (INS) that hydrolyzes Ala-tRNA^{Pro}, and a *trans*-editing factor, YbaK, which clears Cys-tRNA^{Pro}. A global bioinformatics analysis has uncovered five distinct *trans*-editing domains related to the INS domain. While a subset of these factors correct ProRS-dependent errors, recent studies have revealed distinct substrate specificities and tRNA recognition capabilities that extend beyond Ala- and Cys-tRNA^{Pro}. Most known editing mechanisms clear standard non-cognate amino acids; how non-protein amino acids are prevented from misincorporation is unclear. Non-protein amino acids are found in many foods and have the potential to adversely affect human health. The non-protein amino acid aminobutyrate (Abu) is a secondary metabolite involved in various cellular processes. Due to Abu's similarity to Ala and Val, it is recognized and misactivated by several aaRSs. The metabolically versatile bacterium *Rhodopseudomonas palustris* (Rp) encodes for a ProRS containing a catalytically inactive, truncated INS domain, in addition to two distinct INS homologs: YbaK, which deacylates Cys-tRNA^{Pro}, and ProXp-x of unknown function. Comparison of known crystal structures reveals that the catalytic pocket of ProXp-x is larger than that of INS, which suggests substrates larger than Ala are preferred. Indeed, ProXp-x weakly deacylates tRNAs charged with Ile and Val, but robustly edits Abu mischarged onto tRNA^{Pro}, tRNA^{Val}, and tRNA^{Ile}. Semi-promiscuous editing may offer advantages to cells and our data suggest ProXp-x may act as a general Abu-tRNA deacylase. *Rp* ProRS specificity for activation of Pro over Abu is only about 1,000:1, which strongly suggests that editing of Abu-tRNA^{Pro} is required *in vivo*. Taken together, these data suggest that Abu-tRNA editing by the *trans*-editing factor ProXp-x, now renamed ProXp-abu, is likely to be a critical checkpoint to ensure high fidelity in codon translation.

285 The DEAH-box helicase Ecm16 unwinds U3-pre-rRNA duplexes but not other substrates

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A central question in ribosome biogenesis is how RNA-RNA and RNA-protein structural rearrangements are coordinated. In eukaryotes, the highly conserved U3 snoRNA forms base-pairs with the pre-rRNA to coordinate early cleavages and folding events within pre-40S particles. U3 forms base-pairs with two sites in the 5′ ETS region of pre-rRNA (U3-ETS and U3-ETS2 duplexes) and at least one site in the 18S region of the pre-rRNA (U3-18S duplex). Formation of these duplexes is essential for pre-rRNA cleavages at sites A0, A1 and A2 that produce the large and small subunit precursors. A helicase is expected to unwind these interactions because studies by us and others indicate that the dissociation rate of these U3-pre-rRNA interactions, especially the U3-ETS2 duplex, is too slow in the absence of accessory factors to support the rates of ribosome assembly observed *in vivo*. Genetic and physical evidence that the DEAH-box protein Ecm16 (Dhr1) is the helicase that dislodges U3 and its associated proteins from the pre-RNA is presented in the abstract by Sardana et al. Here, we show that Ecm16 is an NTP-dependent RNA helicase. As expected for a DEAH-box protein that lacks the contacts to discriminate between the four nucleobases, Ecm16 hydrolyzes ATP, CTP, GTP and UTP equally well. Its ATPase activity is stimulated by poly(A) RNA and less so by U3 snoRNA and pre-rRNA fragments with active site mutants inhibiting activity. Ecm16 unwinds mimics of the U3-ETS2 and the U3-18S duplexes. Unexpectedly, unwinding activity is not observed with other substrates, suggesting that Ecm16 has intrinsic specificity for its substrate. Helicase activity is observed in the presence of ATP or its nonhydrolyzable analog AMP-PNP but is not observed in the presence of ADP or the absence of ATP. Thus, activity depends on ATP binding but not hydrolysis. Previous work indicated that assembly of the U3-pre-rRNA complex requires annealing factors Imp3 and Imp4. Now disassembly of this complex is shown to require the helicase Ecm16. Together, these proteins promote the transient association and subsequent dissociation of U3 snoRNA from the pre rRNA.

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286 Thermodynamic landscape of the bacterial 30 S translation initiation complex assembly

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In bacteria, translation initiation involves the binding of the messenger RNA start site and the fMet-tRNAfMet to the 30S ribosomal subunit. The interaction is under control of the initiation factors IF1, IF2 and IF3, resulting in the formation of the 30S initiation complex. The 50S ribosomal subunit then binds to this initiation complex and, after ejection of IFs, leads to the transition to the elongation phase. In spite of a wealth of detailed structural views of ribosome structures obtained in the past 10 years by many crystal and cryo-EM structures, direct thermodynamic data on the ribosome machinery are still sparse.

We have recently developed new methods extending the possibilities of Isothermal Titration Calorimetry (ITC): (1) incremental ITC was designed to study step by step successive chemical reactions, and (2) kinITC was designed to obtain kinetic information in addition to thermodynamic information usually obtained from ITC. Taking advantage of these innovative approaches, we have investigated the E coli 30 S initiation complex assembly and obtained a thermodynamic (and partially kinetic) view of alternative pathways differing in the order of addition of the substrates. In particular, we showed that messenger-RNA binding is strongly dependent on the presence of initiation factors on the 30S. Our thermodynamic and kinetic data thus leads to the identification of a preferred assembly pathway of 30S preinitiation complex.

287 Elongation Factor G Undergoes an Extensive Structural Rearrangement during Ribosomal Translocation

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Translocation of mRNA and tRNAs through the ribosome is catalyzed by the universally conserved elongation factor (EF-G in prokaryotes and EF-2 in eukaryotes). It has been hypothesized that EF-G undergoes structural rearrangements during translocation. Here, we test this hypothesis by following the movement of domain IV of EF-G, which is essential for the catalysis of translocation, relative to protein S12 of the small ribosomal subunit using single-molecule Förster resonance energy transfer (smFRET). We show that ribosome-bound EF-G adopts distinct conformations corresponding to the pre and posttranslocation states of the ribosome. Upon ribosomal translocation, domain IV of EF-G moves toward the A site of the small ribosomal subunit and propels the movement of peptidyl-tRNA from the A to the P site. We found no evidences of direct coupling between structural rearrangements of EF-G and GTP hydrolysis. Our results also suggest that the pretranslocation conformation of EF-G is significantly less stable than the posttranslocation conformation. Hence, the structural rearrangement of EF-G makes a considerable energetic contribution to promoting tRNA translocation.

288 Abstract Withdrawn

289 The architecture of the large subunit of the mammalian mitochondrial ribosome

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Mitochondrial ribosomes (mitoribosomes) perform protein synthesis inside mitochondria, the organelles responsible for energy conversion in eukaryotic cells. Mammalian mitoribosomes have become highly specialized towards the synthesis of the membrane protein components of mitochondrial respiratory chain complexes. Concomitantly, they have undergone extensive structural and compositional changes throughout evolutionary, including acquisition of mitochondrial-specific ribosomal proteins and shortening of the ribosomal RNA (rRNA).

We have determined the structure of the large ribosomal subunit from porcine mitochondria at 4.9 Å resolution using cryo-electron microscopy (cryo-EM) [1]. Our cryo-EM structure at near-atomic resolution combined with data from chemical crosslinking and mass spectrometry experiments enables a detailed interpretation of the architecture of the 39S subunit. We have been able to localize several mitoribosomal-specific proteins and provide a detailed model of the highly reduced mitoribosomal 16S rRNA. Additionally, our cryo-EM density indicates the presence of a second rRNA molecule in the 39S subunit. The identification of mitochondrial ribosomal protein L45, a membrane-binding protein, near the highly remodelled polypeptide tunnel exit provides a mechanistic explanation for the permanent membrane attachment of mitochondrial ribosomes, which likely facilitates the insertion of newly synthesized membrane proteins into the mitochondrial inner membrane. Furthermore, our structure provides evidence for a step-wise replacement of rRNA contacts by protein extensions during rRNA reduction.

[1] Greber BJ, Boehringer D, Leitner A, Bieri P, Voigts-Hoffman F, Erzberger JP, Leibundgut M, Aebersold R, Ban N. (2013). Architecture of the large subunit of the mammalian mitochondrial ribosome. Nature 505(7484): 515-9. Epub 2013 Dec 22.

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290 The telomerase inhibitor Gno1p/PINX1 activates the helicase Prp43p during ribosome biogenesis

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We provide evidence that a central player in ribosome synthesis, the RNA helicase Prp43p, can be activated by yeast Gno1p and its human orthologue, the telomerase inhibitor PINX1. Gno1p and PINX1 expressed in yeast interact with Prp43p and the integrity of their G patch domain is required for this interaction. Moreover, PINX1 interacts with human PRP43 (DHX15) in HeLa cells. PINX1 directly binds to yeast Prp43p and stimulates its ATPase activity, while alterations of the G patch abolish formation of the PINX1/Prp43p complex and the stimulation of Prp43p. In yeast, lack of Gno1p leads to a decrease in the levels of pre-40S and intermediate pre-60S pre-ribosomal particles, defects that can be corrected by PINX1 expression. We show that Gno1p associates with 90S and early pre-60S pre-ribosomal particles and is released from intermediate pre-60S particles. G patch alterations in Gno1p or PINX1 that inhibit their interactions with Prp43p completely abolish their function in yeast ribosome biogenesis. Altogether, our results suggest that activation of Prp43p by Gno1p/PINX1 within early pre-ribosomal particles is crucial for their subsequent maturation.

291 RPG and snOPY: Databases for ribosomal protein genes and small nucleolar RNA genes

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The ribosome is a universal and essential catalyst of protein synthesis in all organisms. Because of the fundamental role played by the ribosome, its structure and function have been significantly conserved during evolution. In eukaryotes, the ribosome is composed of four RNA molecules (rRNAs) and about 80 different proteins (RPs). The genes encoding rRNAs are clustered at a few sites in the eukaryotic genome, whereas the genes encoding RPs are widely dispersed. In archaea and eukaryotes, rRNAs undergo intense modifications such as 2′-*O*-methylations and pseudouridylations. These modifications are catalyzed by an assemblage of small RNAs and proteins termed the small nucleolar ribonucleoprotein (snoRNP) particle. The small nucleolar RNAs (snoRNAs), which are a component of the snoRNP, guide these modifications. Although the rRNA modification sites are highly conserved during evolution, the functional significance of these modifications remains unknown.

It was long assumed that ribosomes were considered critical for cellular survival. However, it has now become clear that defects in ribosome biogenesis cause pleiotropic effects that may manifest as specific disease conditions in humans, called ribosomopathies. To investigate the pathogenic mechanisms of ribosomopathies, detailed information about the ribosomal components are essential. Here we introduce a ribosomal protein gene database (RPG) and a snoRNA orthological gene database (snOPY) as valuable resources for biological research and tools for studying ribosomopathies. Currently, RPG provides information on 4,819 RP genes from 47 species and snOPY provides information on 13,770 snoRNA genes from 34 species. All these data are freely available on the web described below.

RPG: http://ribosome.med.miyazaki-u.ac.jp/

snOPY: http://snoopy.med.miyazaki-u.ac.jp/

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292 The eukaryotic ribosome in motion: integrating X-ray, cryo-EM and SHAPE into a coherent picture of dynamics

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The recent explosion of structural data of eukaryotic ribosomes has had a significant impact on our understanding of eukaryotic protein synthesis. These new studies provide a structural framework for investigations of eukaryotic ribosome dynamics. High-throughput selective 2′-hydroxyl acylation analyzed by primer extension (hSHAPE) probing measures the mobility of each rRNA nucleotide, yielding information on the flexibility of the ribosome in solution at nucleotide resolution. We developed a novel method to integrate hSHAPE data to molecular simulations to accurately capture the mobility of the ribosome, providing a three-dimensional visualization of the hSHAPE measurements. Here we report results from the molecular simulations of eukaryotic ribosomes. Our novel procedure generates molecular dynamics simulations consistent with structural data and hSHAPE probing experiments. Using data from X-ray crystallography and cryo-electron microscopy, we construct all atom models of various functional ribosome complexes in the elongation cycle. These models are then used as beginning and end points for simulations of large-scale conformational changes, allowing us to examine changes in interactions in a manner consistent with chemical probing data. Using this method, we study the conformational dynamics of large-scale conformational changes from pre- to post- state.

293 Atomic mutagenesis of the ribosomal Sarcin-Ricin-loop to study EF-G GTPase activation

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Translocation factor EF-G, possesses a low basal GTPase activity, which is stimulated by the ribosome. One potential region of the ribosome that triggers GTPase activity of EF-G is the Sarcin-Ricin-Loop (SRL) (helix 95) in domain VI of the 23S rRNA. Structural data showed that the tip of the SRL closely approaches GTP in the active center of EF-G, structural probing data confirmed that EF-G interacts with nucleotides G2655, A2660, G2661 and A2662.[1-3]The exocyclic group of adenine at A2660 is required for stimulation of EF-G GTPase activity by the ribosome as demonstrated using atomic mutagenesis.[4]Recent crystal structures of EF-G on the ribosome, gave more insights into the molecular mechanism of EF-G GTPase activity.[5]Based on the structure of EF-Tu on the ribosome[1], the following mechanism of GTPase activation was proposed: upon binding of EF-G to the ribosome, the conserved His92 (*E.coli*) changes its position, pointing to the γ-phosphate of GTP. In this activated state, the phosphate of residue A2662 of the SRL positions the catalytic His in its active conformation. It was further proposed that the phosphate oxygen of A2662 is involved in a charge-relay system, enabling GTP hydrolysis. In order to test this mechanism, we use the atomic mutagenesis approach, which allows introducing non-natural modifications in the SRL, in the context of the complete 70S ribosome. Therefore, we replaced one of the non-bridging oxygens of A2662 by a methyl group. A methylphosphonat is not able to position or activate a histidine, as it has no free electrons and therefore no proton acceptor function. These modified ribosomes were then tested for stimulation of EF-G GTPase activity. First experiments show that one of the two stereoisomers incorporated into ribosomes does not stimulate GTPase activity of EF-G, whereas the other is active. From this we conclude that indeed the non-bridging phosphate oxygen of A2662 is involved in EF-G GTPase activation by the ribosome. Ongoing experiments aim at revealing the contribution of this non-bridging oxygen at A2662 to the mechanism of EF-G GTPase activation at the atomic level.

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294 The proto-ribosome: a prebiotic RNA bonding machine functioning within the contemporary ribosome

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The ribosome is a fundamental cellular machine common to all life forms. The primary function of the ribosome is to catalyze peptide bond formation at the peptidyl transferase center (PTC) in the large ribosomal subunit. In all the existing ribosome 3D structures the PTC lays within a pseudo 2-fold symmetrical region composed solely of RNA. This symmetrical region possesses remarkably high sequence conservation in all domains of life. We suggest that this region is a vestige of an entity we termed the 'proto-ribosome'. The proto-ribosome is hypothesized to be an RNA entity capable of catalyzing peptide bond formation in the RNA world.

In attempt to experimentally prove our suggestion, RNA constructs, based mainly on the sequence of the contemporary symmetrical region were prepared, aiming at reconstructing the proto-ribosome. These RNA constructs are being structurally characterized and subjected to various activity and binding assays.

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295 Eukaryote-specific Extensions of Ribosomal Proteins are Necessary for 60S Subunit Assembly

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Eukaryotic ribosomes are 40% larger than their prokaryotic counterparts, reflecting the increased complexity of their function. High-resolution crystal structures of the large ribosomal subunit from prokaryotes and eukaryotes reveal that the core of the ribosome is conserved. In addition, there are eukaryote-specific elements (ESEs), including extensions of conserved ribosomal proteins and expansions of rRNA, as well as some ribosomal proteins. ESEs might enable more complex regulation of translation in eukaryotes. However, they are not adjacent to the peptidyl transferase center; rather, they are clustered at the solvent-exposed side of the 60S subunit. Thus, these elements may function via long-range interactions to regulate translation. Alternatively, they may have a role in ribosome assembly, e.g. by stabilization of pre-ribosomes through formation of additional rRNA-protein and protein-protein interactions.

To investigate the roles of eukaryotic extensions in ribosome assembly, we sequentially deleted them from five ribosomal proteins (L6, L7, L8, L25 and L35). In addition, we constructed mutations to disrupt interactions of extensions with rRNA and other ribosomal proteins. Initially, we confirmed that the majority of these extensions are essential for cell viability. Next, we assayed the incorporation of mutant proteins into pre-ribosomes. We assessed the effects of the mutations by investigating the landmarks of ribosome assembly, i.e. pre-rRNA processing. By these methods, we identified several eukaryote-specific extensions that are involved in maturation of the 60S subunit. Interestingly, truncations of L8 and substitution mutations in L7 and L35 resulted in pre-rRNA processing defects different than the ones observed when the entire protein was depleted from cells. These findings demonstrate that eukaryotic extensions of ribosomal proteins may be involved in steps different than their globular domains during 60S subunit assembly.

Most eukaryotic extensions are predicted to contain intrinsically disordered domains, although their possible functions are yet to be determined. Nevertheless, the flexibility and folding-upon-binding properties of these domains strongly hint that they might function as chaperones. Additionally, due to their positioning on the surface of the ribosomes, they might recruit translation or assembly factors.

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296 PDCD2L is an RPS2-associated shuttling protein that interacts with the late small ribosomal subunit precursor

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The ribosomal protein S2 (RPS2) forms a stable complex with the protein arginine methyltransferase 3 (PRMT3), an association that is evolutionarily conserved. Mouse embryos deficient for PRMT3 expression are smaller than control littermates; yet, the molecular basis for this developmental defect remains unclear. To elucidate the cellular role of the PRMT3-RPS2 complex, we used a quantitative proteomic approach, SILAC, to identify PRMT3-associated proteins. Using SILAC, we identified Programmed Cell Death 2-like (PDCD2L), a protein of unknown function, as a novel PRMT3-associated protein. Immunopurification experiments revealed that PRMT3, RPS2, and PDCD2L form a stable complex. We also show that RPS2 directly interacts with both PRMT3 and PDCD2L by two-hybrid assays, suggesting that RPS2 forms a bridge between PRMT3 and PDCD2L. Consistent with this model, PRMT3 and PDCD2L failed to associate in cells depleted of RPS2. Interestingly, results from a SILAC analysis indicated that PDCD2L interacts with several proteins involved in the late processing of the small ribosomal subunit. Immunopurification of PDCD2L showed specific enrichment for a known 3′-extended precursor of the 18S rRNA (18S-E), consistent with the fact that PDCD2L associates to the late 40S precursor. We also examined PDCD2L localization by fluorescence microscopy, as 18S-E-associated 40S precursors are found in the nucleus and the cytoplasm. Although PDCD2L localized in the cytoplasm and at the nuclear periphery at steady state, we found that PDCD2L is a shuttling protein that is exported from the nucleus in a CRM1-dependent manner via a functional leucine-rich nuclear export signal (NES). Notably, we also showed that PDCD2L transits through the nucleolus before nuclear export. Our findings indicate that PDCD2L is a NES-containing protein that associates with the late 40S precursor, suggesting that PDCD2L could represent a novel adaptor for small ribosomal subunit export by CRM1.

297 Bcp1 is a novel 60S ribosome transacting factor which works as a chaperone of Rpl23

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Assembly of ribosome requires about 200 transacting factors and is regulated in response to growth signals. Ribosomal proteins are translated in the cytoplasm and required to be imported for further assembly with rRNAs. It has been shown that chaperones or karyopherins could maintain solubility of ribosomal proteins, by neutralizing the unfavorable positive charges, and facilitate the transport. Bcp1 was identified as a factor required for the export of Mss4, a PI4P 5-kinase. Here we identified Bcp1 is also required for maturation of 60S ribosomal subunits. Cells with mutation of Bcp1 are lethal and have deficient 60S levels. To further characterize its functional role, we carry a high-copy suppressor screen and identify Rpl23 as a suppressor of bcp1 mutant. Bcp1 is shown to have physical interaction with Rpl23 and is required for stabilization of Rpl23. In conclusion, we identify that Bcp1 is a novel 60S biogenesis transacting factor which works as a chaperone of Rpl23.

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298 Senescence as a result of impaired ribosome biogenesis

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Senescence is a stable arrest of cell proliferation in which the cells remain viable and metabolically active but display a constitutive activation of the DNA damage response and of the tumor suppressors p53 and RB. The senescent phenotype can be induced by multiple stresses including short telomeres and oncogenes. We have shown that senescence, involves the ERK-dependent degradation of selective proteins involved in cell cycle progression and tumorigenesis. We call this process senescence associated protein degradation (SAPD) and it involves many nucleolar proteins that play a role in ribosome biogenesis. Using tritium pulse labelling we found a strong decrease of rRNA synthesis in senescent cells indicating that the degradation of nucleolar proteins is functionally relevant. Because we know how exactly the human 47S precursor rRNA is processed, it was possible to design primers on both sides of some processing sites and study their maturation by QPCR. In this way we showed defects in the processing of rRNA in senescent cells. Knocking down some of the nucleolar proteins degraded in senescence was sufficient to trigger the process indicating that a decrease in ribosome biogenesis is causal to cellular senescence. Mechanistically, the degradation of nucleolar proteins during senescence involves the ubiquitin-proteasome system suggesting that E3 ligases link the oncogenic stress that trigger senescence to nucleolar proteins degradation. We will discuss ongoing efforts to identify these enzymes.

299 Investigating the functional roles of RPS3 in RNA damage

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Damaging agents, such as UV rays and endogenous reactive oxygen species (ROS), compromise both DNA and RNA molecules. Yet while cellular response to DNA damage has received considerable attention, the extent to which RNA damage is problematic, and how cells process it, remains poorly understood. Nonetheless, there is evidence that cells are capable of recognizing damaged RNA. For one, UV irradiation of enucleated HEK cells activates the NF-kB pathway[1], suggesting that induction of the DNA damage response (DDR) is initiated by something other than damage to genomic DNA. Furthermore, UV irradiation of mammalian cells can trigger a ribotoxic stress response, which is characterized by the presence of damaged ribosomal RNAs, activation of stress kinases, and inhibition of protein synthesis[2]. Last, UV-irradiated U1 snRNA molecules sufficiently induce production of cytokines from non-irradiated human skin cells[3].

No-Go mRNA Decay (NGD) and 18S Nonfunctional rRNA decay (18S NRD) are mechanistically related quality control mechanisms that recognize stalled translation complexes and stimulate degradation of their associated defective RNAs. Since damaged bases, particularly within mRNA coding regions, result in stalled translation complexes, we wondered if a translation-dependent surveillance pathway might also exist for damaged mRNA molecules. Ribosomal protein S3 (RPS3), which forms part of the mRNA entrance channel, has been implicated as an endonuclease for abasic and UV-irradiated dsDNA[4]. To investigate whether RPS3 plays any role in detection and/or process of damaged RNA, we are taking a site-directed mutagenesis approach with *S. cerevisiae* RPS3. To date, we have identified alleles without growth defects under normal growth conditions that exhibit either decreased or increased resistance to UV irradiation, alkylating agents, and UV-mimic 4-NQO. We are currently testing whether other characteristics of the yeast stress response, such as phosphorylation of eIF2-alpha, are abrogated in *rps3* mutant strains.

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300 IF2 stabilizes the ribosome in a semi-rotated conformation during a late step of translation initiation

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During translation elongation, the ribosome was shown to undergo intersubunit rotation between the classical, non-rotated and hybrid, rotated conformations. Intersubunit rearrangements of the ribosome during translation initiation are less well understood. Bacterial initiation entails the binding of the large and small ribosomal subunits to the mRNA with the aid of three initiation factors, initiation factor (IF) 1, 2, and 3,which ensure the fidelity of initiation. IF2 is a GTPase that recognizes the initiator tRNA and promotes subunit joining. Using a single-molecule (sm)FRET assay to monitor intersubunit rotation, we found that IF2, trapped on the ribosome in the presence of non-hydrolyzable analogue of GTP, GDPCP, stabilizes the ribosome in a unique, semi-rotated conformation. The stabilization of the semi-rotated state requires the presence of an aminoacylated initiator tRNA in the P site of the ribosome. Other translational GTPases, elongation factor G (EF-G) and release factor 3 (RF-3), which are involved in the elongation and termination steps of translation, respectively, and known to modulate intersubunit rotation, failed to induce the semi-rotated conformation of the ribosome in the presence of initiator tRNA. This result suggests that the semi-rotated state is uniquely induced by IF2. We also found that neither IF1 nor IF3 cooperate with IF2 to stabilize the semi-rotated state. We hypothesize that the semi-rotated conformation of the ribosome induced by IF2 facilitates subunit joining during translation initiation.

301 Structural Basis of Decoding by +1 Frameshift Suppressor tRNA^{SufA6}

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Biological fitness is critically dependent upon the accurate flow of genetic information from DNA to RNA to protein. During translation, shifts in the mRNA reading frame from standard triplets of nucleotides result in abnormal or nonsense proteins that are targeted for degradation. However, it became evident in recent years that nature utilizes induced/programmed frameshifting to maintain the correct reading frame in some cases. Departures from the normal three-base genetic code were first discovered in the *Salmonella typhimurium* histidine operon where four site-specific bases in the genome were decoded by the ribosome as a single amino acid. External suppressors of these mutations were mapped to separate loci and most alleles encoded base insertions or mutations in tRNA genes. These **su**ppressors of **f**rameshift (*suf*) tRNAs therefore did not cause errors but rather 'suppressed' the base insertion/deletion in the genetic code by altering the number of bases decoded by the ribosome to restore the correct reading frame. Here, we aim to understand the decoding mechanism of suppressor tRNAs that result in an altered genetic code using X-ray crystallography. We have solved structures of anticodon stem-loop (ASL) of tRNA^{SufA6} bound to the *Thermus thermophilus* 70S ribosome in the A site with both suppressible and canonical codons at resolutions of 3.2-3.8Å. tRNA^{SufA6} is a tRNA^{Pro} derivative that induces +1 frameshift by decoding a four nucleotide codon to suppress a single base insertion in the mRNA. As in tRNA^{Pro}, tRNA^{SufA6} contains a modified nucleoside, 1-methyl-guanosine (m^1G) at position 37, 3′ adjacent to the anticodon. In addition, tRNA^{SufA6} has an extra G inserted between m^1G37 and A38. Control structures of *Tth* 70S with ASL of tRNA^{Pro} in the A site were also solved at resolutions of 2.9-3.5Å, which enable a direct structural comparison between these two tRNA species. Together, these structural data give us a new understanding of how non-canonical decoding takes place within the A site.

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302 Ribosomal protein S1 unfolds structured mRNAs on the ribosome for translation initiation in *Escherichia coli*

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Structures of mRNA have for a long time been described as key elements in the regulation of gene expression. Many bacterial mRNAs adopt structures in their 5′ untranslated regions that modulate the accessibility of the 30S ribosomal subunit. Structured mRNAs interact with the 30S in a two-step pathway where the docking of a folded mRNA precedes an accommodation step. Recently, we demonstrated (1) that ribosomal protein S1 endows the 30S with an RNA chaperone activity that is essential for the docking and unfolding of structured mRNAs, and the correct positioning of the initiation codon inside the decoding channel. The rate of the S1-induced RNA melting is slow, suggesting that this step is rate-limiting in the initiation process of structured mRNAs. The first three OB-fold domains of S1 retain all the activities of the protein on the 30S subunit, while the function of the last two remains to be addressed. However, S1 is not required for all mRNAs and acts differently on mRNAs according to the signals present at their 5′ ends. Interestingly, the action of S1 on the ribosome is countered by repressor proteins to prevent translation. All in all, S1 confers activities to the ribosome in such a way that the initiation of translation is selectively adapted for unstructured and structured mRNAs.

(1) *Escherichia coli* ribosomal protein S1 unfolds structured mRNAs onto the ribosome for active translation initiation, (2013) PloS Biology 11(12):1-15, Mélodie Duval, Alexey Korepanov, Olivier Fuchsbauer, Pierre Fechter, Andrea Haller, Attilio Fabbretti, Laurence Choulier, Ronald Micura,Bruno Klaholz, Pascale Romby, Mathias Springer and Stefano Marzi

303 Ribopuromycylation reveals the presence of translating ribosomes at transcription sites in Schizosaccharomyces pombe

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A growing body of evidence is again challenging the consensus that translation is a strictly cytoplasmic process and suggests that it may also occur in the eukaryotic nucleus. The most striking recent developments came from two studies in human cells and *Drosophila*. In human cells, puromycin, a classic antibiotic that is an analogue of the adenosine-amino acid end of charged tRNAs, and which terminates translation by incorporation at the C-terminus of nascent peptides, was revealed by immunostaining to be readily incorporated at nuclear sites, particularly in the nucleolus. Key to this study was that puromycylation was conducted in the presence of translation elongation inhibitors that appear to prevent the release of nascent peptides from ribosomes, indicating that nuclear puromycin incorporation is likely reporting ribosome-mRNA-bound nascent peptides. The study in *Drosophila* revealed the presence of 80S ribosomes in the nucleolus and other nuclear sites by a novel technque involving visualization of the joining of ribosomal subunits via the application of bimolecular fluorescence complementation, in which ribosomal proteins of the small and large subunits were tagged with complementary halves of YFP. Remarkably, the *Drosophila* study also reported a pattern of puromycylation at polytene chromosomes, indicating translation of nascent RNAs. We are using this ribopuromycylation method to further investigate the potential for nuclear translation in the fission yeast *Schizosaccharomyces pombe*. Since wild-type yeast are resistant to puromycin, we have first screened a library of deletion mutants and identified a number of puromycin sensitive strains, one of which consistently incorporates puromycin into nascent peptides in the presence of elongation inhibitors. This strain has been used to perform Chromatin Immunoprecipitation (ChIP) with an anti-puromycin antibody and quantitative PCR analysis has revealed that a number of gene loci are enriched with ribopuromycylated peptides and that this enrichment is RNase sensitive. We aim to use high-throughput methods to provide a genome wide analysis of genes which are cotranscriptionally translated and will report our latest results at this meeting.

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304 Click Fluorescent Labeling and Enzymatic Mapping of RNA Targets of Platinum (II) Anticancer Therapeutics

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Cis-diamminedichloroplatinum (II), or cisplatin, is a universally prescribed antitumor drug that binds to a range of biomolecular targets including nucleic acids, proteins, and small molecule metabolites. Irreparable intrastrand crosslink formation on DNA is the primary effector in the induction of programmed cell death in several eukaryotic models, yet less than 10% of Pt (in the case of cisplatin) accumulates within genomic DNA. We are interested in non-genomic contributions to cisplatin's therapeutic profile, with a focus on interactions with noncoding and coding RNAs. In particular, cisplatin treatment can disrupt RNA-based processes such as splicing and translation. The contribution of cisplatin-mediated RNA damage to the overall cytotoxicity of the drug has not been directly investigated. Also of interest is the potential for Pt compounds to report on RNA structure *in vivo*.

To better understand the range of cellular interactions available to Pt compounds, robust methods to track and analyze Pt-bound species are needed. Our lab has designed and synthesized a family of azide-containing Pt(II) derivatives capable of undergoing bioorthogonal cycloaddition 'click' reactions with functionalized alkynes. Using cyclic ring-strained alkynes for Cu-free click reactions, we demonstrate significant fluorescent in-gel labeling of Pt-bound ribosomal RNA and transfer RNA from *S. cerevisiae* treated with Pt-azide *in vivo*, revealing the first evidence that cellular tRNA is a Pt(II) substrate.

Within ribosomal RNA, we have enzymatically mapped specific Pt(II) lesions in the universally conserved sarcin-ricin loop (SRL) and the peptidyl transferase center (PTC), two regions critical to translation. Platinum demonstrates specificity towards solvent-accessible purines and conserved metal ion binding pockets, while protection from Pt(II) binding is observed in regions occluded by hydrogen-bonding networks or steric constraints. Current work is focused on enriching Pt-bound species for sequencing using a biotin-alkyne pulldown assay. Taken together, our results indicate a potential ribotoxic mechanism for cisplatin cytotoxicity and broadly describe a convenient click chemistry methodology that can be applied to identify other metal or covalent modification-based small molecule RNA targets.

305 Centers of Motion in the Bacterial Ribosome

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The ribosome is a dynamic molecular machine, driven in large part by induced intersubunit ratcheting. During the ratcheting process, ribosomal motion is created from the binding of a set of G-protein cofactors and propagated through a series of flexible elements or *pivot points*. Herein we describe a full set of large scale pivoting positions in rRNA (~3A to ~40A in size) induced by the elongation factor G (EF-G) - ribosome binding event. The observed flexible elements are typically associated with non-canonical base pairs, most commonly an A-G mismatch, in sequences adjacent to helix junctions. A total of 19 major pivot points were found; of these, 8 were observed in the 50S subunit and 11 in the 30S. With respect to functionality, two groups of pivoting positions are described, those associated with global intersubunit ratcheting and 16S head swiveling processes as well as those responsible for tRNA translocation through the decoding center. Overall these results present the ribosome as a machine of interconnected pivoting positions the sum function of which is the forward translation of mRNA.

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306 tRNA-derived fragments target the small ribosomal subunit to fine-tune translation

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Post-transcriptional cleavage of RNA molecules to generate smaller fragments is a widespread mechanism that enlarges the structural and functional complexity of cellular RNomes. In particular, fragments deriving from both precursor and mature tRNAs represent one of the rapidly growing classes of post-transcriptional RNA pieces. Importantly, these tRNA-derived fragments (tRFs) possess distinct expression patterns, abundance, cellular localizations, or biological roles compared with their parental tRNA molecules (1).

Here we present evidence that tRFs from the archaeon *Haloferax volcanii* directly bind to ribosomes. In a previous genomic screen for ribosome-associated small RNAs we have identified a 26 residue long fragment originating from the 5′ part of valine tRNA (Val-tRF) to be by far the most abundant tRF in *H. volcanii* (2). The Val-tRF is processed in a stress-dependent manner and was found to primarily target the small ribosomal subunit *in vitro* and *in vivo*. Translational activity was markedly reduced in the presence of Val-tRF, while control RNA fragments of similar length did not show inhibitory effects on protein synthesis. Crosslinking experiments and subsequent primer extension analyses revealed the Val-tRF interaction site to surround the mRNA path in the 30S subunit. In support of this, binding experiments demonstrated that Val-tRF does compete with mRNAs. Therefore this tRF represents a ribosome-bound non-protein-coding RNA (ncRNA) capable of regulating gene expression in *H. volcanii* under environmental stress conditions probably by fine-tuning the rate of protein production (1).

Our data reveal the ribosome as a target for a small regulatory ncRNA and demonstrate the existence of a yet unknown mechanism of translation regulation. Ribosome-targeted small ncRNAs are found in all domains of life and represent a prevalent but so far largely unexplored class of regulatory molecules (3). Future work on the small ncRNA interactomes of ribosomes in a variety of model systems will allow deeper insight into the conservation and functional repertoire of this emerging class of regulatory ncRNA molecules.

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307 NSUN4 is a dual function mitochondrial protein required for both methylation of 12S rRNA and coordination of mitoribosomal assembly

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Biogenesis of mammalian mitochondrial ribosomes requires a concerted maturation of both the small (SSU) and large subunit (LSU). We demonstrate here that the m^5C methyltransferase NSUN4, which forms a complex with MTERF4, is essential in mitochondrial ribosomal biogenesis as mitochondrial translation is abolished in conditional *Nsun4* mouse knockouts. Deep sequencing of bisulfite-treated RNA shows that NSUN4 methylates cytosine 911 in 12S rRNA (m5C911) of the SSU. Surprisingly, NSUN4 does not need MTERF4 to generate this modification. Instead, the NSUN4/MTERF4 complex is required to assemble the SSU and LSU to form a monosome. NSUN4 is thus a dual function protein, which on the one hand is needed for 12S rRNA methylation and, on the other hand interacts with MTERF4 to facilitate monosome assembly. The presented data suggest that NSUN4 has a key role in controlling a final step in ribosome biogenesis to ensure that only the mature SSU and LSU are assembled.

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308 Insight into mitoribosome maturation by structural methods

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Mitochondrial ribosomes (mitoribosomes) are essential for translating 13 mitochondrially-encoded proteins, crucial for ATP generation by the oxidative phosphorylation pathway. The mammalian mitoribosome (55S) is composed of a large (39S) and a small (28S) subunit, with a richer protein component than its bacterial counterpart. In addition, chemical and spatial differences were revealed from the cytoplasmic and bacterial homologues by cryo-electron microscopy. Nearly all mitoribosome protein components are transcribed in the nucleus and transported into the mitochondria, where ribosomal assembly takes place in the inner membrane. Studies in bacteria and yeast mitochondria have shown that specific post-transcriptional modifications are essential for ribosomal activity. Despite these findings, little is known about the human mitoribosome assembly and rRNA modifications. Our aim is to characterize the mitoribosome biogenesis pathway using structural methods. For this purpose, putative assembly and modifying factors are recombinantly expressed, purified and complexed with their substrates to determine their role in mitoribosome maturation.

309 Deciphering roles for small ribosomal subunit assembly factors in blocking premature translation initiation in *E.coli*

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Ribosome biogenesis is a complex and highly coordinated process which involves processing and modification of ribosomal RNAs (rRNAs), modification and binding of ribosomal proteins (r-proteins), and the transient interactions of many assembly factors. In addition to roles in ribosome maturation, some small ribosomal subunit (SSU) assembly factors have been proposed to block premature translation initiation. In *E.coli*, the bacterial SSU rRNA methyltransferase and biogenesis factor KsgA, has been shown to compete with translation initiation factor 3 (IF3) and the large ribosomal subunit (LSU) for SSU association (Connolly et al., 2008; Xu et al., 2008). In the absence of KsgA overexpression of the SSU biogenesis factor RbfA causes immature SSUs to be incorporated into 70S-like complexes, suggesting that KsgA functions together with RbfA in an unknown (translation initiation-related) mechanism. Here we investigate the possibility that RbfA and other SSU assembly factors may also be involved in altering interaction of premature SSUs with the translation initiation machinery. Our data indicate that in *E. coli* strains lacking the SSU biogenesis factor RbfA overexpression of translation initiation factor 1 (IF1) is detrimental to growth and confers severe defects in ribosome biogenesis. The extent of these changes in growth are not observed in parental strains or strains lacking several other SSU biogenesis factors. Furthermore structural studies have shown that the major site of contact between RbfA and the SSU overlaps with the binding site of IF1 on SSUs. These results suggest that binding of RbfA to the SSU may preclude binding of IF1 thus preventing translation initiation. Experimental data aimed at deciphering possible roles for SSU assembly factors in blocking premature translation initiation in *E.coli* will be presented.

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310 Mechanism of regulation of GTP hydrolysis by translation elongation factors EF-Tu and EF-G as deduced from *in silico* analysis of their complexes with ribosome

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The cleft between the ribosomal subunits is the place where both elongation factors EF-Tu and EF-G associate with ribosome. Systematic comparison of available conformations of ribosome in complexes with EF-Tu and with EF-G demonstrates that the width of the cleft is reversely dependent on the angle of ratchet between the two ribosomal subunits. More specifically, when the angle of ratchet (R) raises from 0° to 5°, the width of the cleft narrows from 44 Å to 39 Å. The variation of the size of the cleft modulates the ability of ribosome to interact with both elongation factors. Thus, the size of EF-Tu (42.5 Å) allows ternary complex EF-Tu*GTP*aminoacyl-tRNA to fit into the cleft only if ribosome has a non-ratcheted conformation (R < 1.5°). The original interaction of the ternary complex with ribosome occurs when the latter has a partly ratcheted conformation (R > 3.5°). Such conformation does not allow EF-Tu to fit into the inter-subunit cleft. However, cognate codon-anticodon interaction causes rearrangements in the ribosome structure known as domain closure. These rearrangements diminish the ratchet and widen the cleft, thus allowing EF-Tu to fit in. As a result of this fitting, EF-Tu forms contacts with the sarcin-ricin loop (SRL), which promotes GTP hydrolysis. In other words, the message concerning cognateness of tRNA goes to the GTP-binding pocket in EF-Tu in the form of rearrangements in the ribosome structure. Compared to EF-Tu, EF-G is substantially slimmer (39.0 Å) and can always fit into the inter-subunit cleft regardless of the angle of ratchet. However, the interaction of EF-G with the inter-subunit cleft sharply intensifies in the row from a non-ratcheted to half-ratcheted and to a fully ratcheted ribosome, suggesting that promotion of ratchet by EF-G is an exothermic process. In all structures, EF-G interacts with SRL, which suggests that a simple contact between EF-G and SRL is not sufficient for GTP hydrolysis. However, systematic comparison of all EF-G-ribosome complexes revealed specific rearrangements in the structure of L7/L12 stalk that occur during the first and second half-ratchet and that cause particular rearrangements in the EF-G structure potentially leading to GTP hydrolysis.

311 Development of New Methods for the Study of Bacterial Ribosome Biogenesis and Characterization of its Associated Factors

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The ribosome is the central apparatus for protein translation in cells. As such, gene expression, and ultimately growth and proliferation, is dependent on a cells ribosomal content, both in terms of quantity and the translational capability of the individual ribosomes. Indeed, a linear correlation between growth rate and ribosomal content has been observed for multiple wild-type microbes. We have examined this relationship in several *Escherichia coli* mutants with impaired ribosome biogenesis. While a linear correlation between growth and ribosomal content still exists in these mutant strains, there is a significant increase in the total cellular ribosomal material in comparison to the wild-type. This phenotype is not wholly exclusive to perturbations in biogenesis. Indeed, linear correlations and elevated levels of ribosomal content are also observed for several translation mutants. The degree of elevation, however, is marginal in comparison to that seen in the biogenesis mutants. Thus, quantifying ribosomal content may provide a convenient means of identifying aberrant ribosome biogenesis. Our work explores this phenomenon and the possibility of exploiting it to identify and further characterize perturbation in the ribosome assembly process.

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312 Bcp1 is a critical checkpoint for Tif6 binding in 60S ribosome biogenesis pathway

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We have shown that Bcp1 is a chaperone of Rpl23. Here, we further show that depleting of Bcp1 causes deficient loading of Rpl23. Besides, Tif6 loses its nucleolar distribution and 60S interaction. Tif6 is a ribosome biogenesis factor of 60S subunits with dominant nuclear and nucleolar distribution; its homolog in higher eukaryotic cells is eIF6. Tif6 is bound on the 60S subunits, and the C-terminus of Rpl23 contributes as the major interaction site. The binding of Tif6 on the 60S subunits blocked association between 60S and 40S subunits by blocking the inter-subunit bridge formation. This prevents inappropriate interaction with 40S subunits before fully maturation of 60S. Interestingly, whereas Rpl23 is loaded properly in bcp1 mutant which is persistent on the 60S subunits, it also blocks Tif6 from binding. This might be due to the retention of Bcp1 which causes steric hindrance and prevents Tif6 from assessing to Rpl23. To sum up, the data above suggests that once Rpl23 is loaded properly by Bcp1, Tif6 is bound subsequently after Bcp1 is released.

313 Ribosome dysfunction and erythroid failure: Analyzing the zebrafish model of Diamond-Blackfan anemia

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Ribosome biogenesis in eukaryotes involves a coordinated participation of RNAs, ribosomal proteins (RPs), and large number of accessory factors that regulate subunit assembly and transport. Recently, mutations in genes involved in ribosome biogenesis were identified in patients with various diseases that are categorized as ribosomopathies. An impaired ribosome can cause tissue-specific abnormalities in patients via an unknown pathway. Diamond-Blackfan anemia (DBA) is one of such disorders, characterized by diminished numbers of erythroid progenitors.

The DBA has been attributed to the mutations in RP genes. To investigate the molecular mechanism underlying DBA, we have developed a zebrafish model by morpholino oligo-mediated repressing of *rps19*, which is the most mutated RP gene in the patients. The knockdown embryos (morphants) displayed a drastic reduction of red blood cells, whereas differentiation of other myeloid cells and endothelial cells seemed to be normal. The anemic phenotype was almost rescued by injecting synthesized *rps19* mRNA, but not by mRNAs with patient-type mutations. Interestingly, although many studies have suggested a critical role for Tp53 in DBA, inhibition of Tp53 activity did not alleviate the erythroid aplasia in the morphants. We also developed *rpl35* morphants, another model of DBA, and found that treatment with particular amino acids, like L-Leucine or L-Argenine that augment mRNA translation via mTOR pathway, resulted in a substantial recovery of erythroid cells. These results suggested that altered translation, not an activated apoptosis pathway, could be responsible for defective erythropoiesis in DBA. To evaluate the impact of RPS19 deficiency on translation, we carried out an RNA-Seq analysis of polysomal RNAs and found that translational efficiencies of some genes involved in specific pathways were significantly changed between control and the RPS19-deficient embryos. This study should provide an important clue to the pathogenesis of DBA.

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314 A novel regulatory network controlling translation rates under stress and apoptosis-like programmed cell death in *Leishmania*

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We have shown previously that induction of stress within the phagolysosomal macrophage compartment and more importantly of apoptosis-like programmed cell death (ALPCD) in *Leishmania* triggers fragmentation of antisense ribosomal RNA (ASrRNA), which correlates with rRNA degradation and inhibition of general translation. This newly discovered RNA regulatory network involves several RNA-binding proteins that coordinate the response to stress to allow parasite survival. Initial focus was given on a 67kDa ATP-dependent DEAD-box RNA helicase (HEL67) and a 25kDa RNA-binding protein (RBP25), shown to mutually interact in immunoprecipitation (IP) studies and to bind ribosomal RNA. Our data demonstrated an essential role of HEL67 in ALPCD by preventing ASrRNA cleavage, and hence protecting rRNA from degradation. The *L. infantum Li*HEL67$^{(-/-)}$ null mutant was indeed much more sensitive to stress and to apoptosis-inducing agents than wild type parasites. In addition, intracellular parasite growth was severely affected in the absence of HEL67. On the other hand, genomic inactivation of *RBP25* increased proliferation of extracellular promastigotes and parasite resistance to apoptosis. Stable expression of RBP25-HA into the *Li*HEL67$^{(-/-)}$ null mutant and of HEL67-HA into the *Li*RBP25$^{(-/-)}$ mutant followed by IP and mass-spectrometry studies revealed specific protein interactions to either HEL67 or RBP25. These data indicate that HEL67 interacts mainly with the 40S and 60S subunit ribosomal proteins and other ATP-dependent DEAD-box RNA helicases. On the other hand, RBP25 interacts with several translation initiation factors, proteins involved in signal transduction and antioxidant response, chaperonins, and proteasome components. HEL67 and RBP25 interact also with a distinct set of RNA-binding proteins and hypothetical proteins of unknown function. Overall, our data suggest that while HEL67 is key to the parasite protective response against intracellular stress, RBP25 seems to act as a repressor of this response. Additional studies are required, however, to better characterize the interplay between HEL67, RBP25, and their interacting partners under conditions of normal growth or stress and ALPCD in *Leishmania*.

315 Characterization of fission yeast tri-methyl guanosine synthase (tgs1)

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Tgs1 catalyzes the hyper-methylation of the mono-methylguanosine cap on a subset of polII transcripts including snRNAs, snoRNAs, and a variety of long non-codingRNAs including telomerase RNA. In higher eukaryotes, the tri-methylguanosine cap facilitates the re-import of spliceosomal snRNAs into the nucleus by providing a binding site for the import factor snurportin. In fission yeast spliceosomal snRNAs are also TMG-capped, but snurportin is absent and tgs1 deletion causes only mild phenotypes. The biological functions of cap hypermethylation in yeast are thus largely unclear.

In this study, we have examined the functions of tgs1 in the fission yeast *Schizosaccharomyces pombe*. Using immunoprecipitation and high-throughput RNA sequencing, we have cataloged TMG-capped RNAs, documented a mild splicing defect and examined the effects of tgs1 deletion on gene expression. Notably, tgs1 deletion cells exhibit a centromere silencing defect, impaired small RNA generation, and increased expression of a set of genes that are regulated by RNAi. Results from synthetic genetic arrays (SGA) further support a role for tgs1 in regulating chromatin structure and organization.

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316 Using a histone replacement system to define co-transcriptional interactions between histone modifications and elongating RNA

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Histone post-translational modifications (PTMs), including covalent additions of methyl groups to histone N-terminal tails, are thought to influence gene expression in myriad ways, most prominently by remodeling gene promoters to facilitate or prevent access of the core transcriptional machinery. In contrast, methylation at lysine 36 in the histone H3 N-terminal tail (H3K36me) is enriched in gene bodies and within constitutive exons, suggesting that this particular mark may interface with co-transcriptional RNA processing machinery. To define the requirement for H3K36me in RNA processing and maturation, we developed a genetic system for histone gene replacement in *Drosophila melanogaster* to generate *in vivo* models in which all of the replication-dependent histones are mutant at H3K36, and therefore cannot be methylated. We find that an alanine mutation (H3K36A) is lethal in late embryonic stages, indicating a significant role for H3K36me in early development. Despite this early lethality, the expression and spatial distribution of developmental patterning transcripts, including the WNT homolog *wingless*, are unaffected, suggesting that wholesale dysregulation of RNA expression is not the cause of lethality. Surprisingly, a separate allele, in which lysine 36 was mutated to arginine (H3K36R), survives robustly into larval stages, suggesting a distinct cause of lethality, perhaps related to proposed roles for H3K36me in RNA processing. To further investigate these RNA related phenotypes, we sequenced total RNA from embryos collected from wild-type controls and two independent mutant alleles, H3K36A and H3K36R, via an Illumina stranded, paired-end 100 bp library preparation. Here we report a range of RNA-related defects in H3K36 mutants that indicate the importance of H3K36me in maintenance and homeostasis of co-transcriptional RNA processing. From this data, we seek to delineate classes of RNAs disproportionately sensitive to chromatin perturbation, and to infer a potential mechanism for co-transcriptional crosstalk between histones and elongating RNAs.

317 The RNA helicase MLE facilitates association of MSL proteins with roX RNA in Drosophila

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Dosage compensation in *Drosophila* involves a global activation of genes on the male X chromosome to adjust their expression level to those of female X-linked genes. Responsible for this activation is a ribonucleoprotein complex called MSL complex (MSL-DCC), which consists of five male-specific-lethal (MSL) proteins and two long non-coding *roX* RNAs, *roX1* and *roX2*. Incorporation of the *roX* RNAs represents an essential step in the assembly and X-chromosomal targeting of the MSL-DCC. The secondary structure of *roX2* RNA revealed the presence of distinct stem loops, which are bound by the RNA helicase MLE, itself an MSL subunit. In an ATP-dependent process, MLE remodeled functionally important stem-loop structures, which in turn enabled the association of MSL2, the core subunit of the MSL-DCC, to MLE and *roX2*.

Here, we report on the results of our recent efforts to investigate the recruitment of other MSL proteins to MLE and *roX2* RNA in vitro. We found that all MSL proteins can be specifically recruited to MLE-*roX2* in an ATP-dependent manner, suggesting an essential role of MLE-dependent *roX2* remodeling for MSL-DCC assembly. Aside from MLE, the MSL proteins MSL2, MSL3, and MOF had been ascribed to have RNA-binding potential. Specific *roX* RNA binding, however, could not be demonstrated so far, raising the question how the MSL proteins and *roX* RNAs are interconnected in the MSL-DCC. Using UV crosslinking experiments, we now identified MLE, MSL3 and MSL1 as direct *roX2*-RNA binders. Intriguingly, MSL1 itself is efficiently recruited to MLE and *roX2* in an ATP-stimulated manner in the absence of other MSL subunits. Our data suggest that MSL1 is a novel direct *roX2* RNA-binding protein and may be involved - presumably together with MSL2 - in the recognition of remodeled *roX2* substrate, which then enables subsequent assembly of a functional MSL-DCC.

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318 Regulation of RNAi-mediated formation of centromeric heterochromatin through the splicing machinery in fission yeast

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prp14 is one of the pre-mRNA splicing mutants in fission yeast *Schizosaccharomyces pombe*. Interestingly, this mutant showed abnormal chromosomal segregation in mitosis and a high sensitivity to TBZ, a microtubule destabilizing drug, suggesting that the binding of microtubules to kinetochores is unstable in this mutant. It has been known that formation of centromeric heterochromatin is mediated by the RNAi system in *S. pombe*. In *prp14*, the centromeric non-coding RNAs accumulate and the amounts of siRNAs derived from them are decreased. In addition, *prp14* is defective in histone H3K9 methylation and localization of heterochromatin protein Swi6p at the centromere region. These results suggest that the product of the *prp14*$^{+}$ gene is involved in not only the splicing reaction, but also the formation of RNAi-mediated centromeric heterochromatin.

We found the mRNA-type intron in the centomeric *dg* non-coding RNA transcribed from the centromere region (Chinen et al., 2010). The IP analyses demonstrated that splicing factor Prp14p interacts with Cid12p, a subunit of RDRC (RNA-dependent RNA polymerase complex), suggesting that the spliceosome assembled on the *dg* ncRNA intron works as a platform for recruitment of RDRC.

To examine a role of the dg intron in the formation of centromeric heterochromatin, we constructed a minichromosome containing the centromere with or without the *dg* intron. Interestingly, the minichromosome without the *dg* intron showed the decreased H3K9 methylation comparing to the minichromosome with the dg intron, suggesting that the dg intron plays an important role in the methylation process of H3K9.

319 Insight into Xist RNA function and mechanism using capture hybridization analysis

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The flagship mammalian large non-coding RNA (lncRNA) is Xist, which is a master regulator of dosage compensation in mammals. Xist is transcribed from a locus on the X-chromosome and spreads across the entire chromosome, leading to the repression of most of the X-linked genes in cis. Using Capture Hybridization Analysis of RNA Targets (CHART), we have recently mapped the genomic sites where Xist lncRNA interacts with chromatin during X-chromosome inactivation, revealing that Xist spreads via a two-step mechanism. Xist first targets gene-rich regions and subsequently spreads to the rest of the chromosome. In addition to revealing the steps of Xist spreading, ongoing hybridization capture experiments are continuing to reveal new insights into Xist structure, function, and mechanism, thereby illuminating the properties of an RNA that has long been known to regulate dramatic epigenetic changes during development.

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320 Intron retention: an emerging layer of gene expression control involving epigenetic changes

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Retention of introns in mature mRNAs is emerging as a frequent event in mammalian cells under normal physiological conditions. We and others have reported the retention of introns in the mRNAs of normal cells including granulocytes(1), T lymphocytes(2) and neurons(3). Using mRNA-seq, we have discovered differential intron retention (IR) in cells at three progressive stages of mouse granulopoiesis; promyelocytes, myelocytes and granulocytes. IR affects many genes, including those specific to granulocytes and nuclear architecture. Importantly, IR was associated with the downregulation of protein measured by mass spectrometry and was conserved between human and mouse. Inhibition of nonsense-mediated decay (NMD) resulted in marked accumulation of intron retaining mRNAs, indicating that IR triggers NMD to downregulate mRNA and protein expression. Analysis of nascent RNA transcripts demonstrated that IR-mediated NMD occurred independently of transcriptional regulation. IR is essential for granulopoiesis since enforced re-expression of *Lmnb1*, which displayed the highest levels of IR, decreased granulocyte cell count, increased nuclear volume by 30% and altered nuclear morphology. Using whole genome bisulfite sequencing, we now show that IR is associated with differential DNA methylation patterns across exon-intron boundaries ($P<0.05$, Chi-square test). We focused on *Lmnb1* and *MMP8*; these genes encode significantly more transcripts that retained multiple introns in granulocytes compared to promyelocytes, as detected by mRNA-seq ($P<0.05$, Audic and Claverie test). For both genes, levels of DNA methylation across the exon-intron boundaries decreased by ~50% in granulocytes compared to promyelocytes, predominantly near the exon-intron boundaries that correspond to the first introns being retained. We have also performed nucleosome occupancy methylome sequencing (NoMe-Seq) in order to simultaneously define the nucleosome footprint and DNA methylation profile of the same DNA strand. NoMe-Seq revealed a lack of nucleosome occupancy near the preceding exon-intron boundary when an intron was retained. We conclude that IR coupled with NMD provides yet another mechanism of gene expression control during normal cell differentiation. IR may be regulated by epigenetic mechanisms involving dynamic combinations of DNA methylation and nucleosome arrangement.

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321 Dissecting eRNA mediated transcription regulation in single cells

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Enhancers are distal DNA elements that promote transcriptional activity of promoter regions. They are often the primary target of transcription factor binding to regulatory regions and, although frequently located many kilobases from promoter regions, directly interact with promoters by chromatin looping. Recent advances in sequencing technologies revealed that many enhancers are transcribed and produce RNAs with no coding potential, termed enhancer RNAs (eRNAs). eRNAs are often transcribed bidirectional and their expression correlates with the expression of their adjacent mRNA encoding gene. Inhibiting eRNA transcription was shown to specifically alter target gene expression, suggesting an active role of eRNAs in modulating activated transcription in *cis*. Furthermore, inhibition of eRNAs produced from oestrogen-regulated genes resulted in the loss of the chromatin loops between the enhancers and the target genes, implicating eRNAs in promoting enhancer promoter interaction. The mechanistic details of eRNAs function has not yet been fully established, and is it not known whether eRNA transcription is simply required for establishing chromatin rearrangement or if constant eRNA production is required to establish stable looping interactions. To shed more light on the mechanism of eRNA function, we use single molecule resolution microscopy approaches to determine the relationship between eRNA transcription, gene looping and transcriptional activity of enhancer regulated transcription. Different aspects of eRNA metabolism and their effects on mRNA expression will be discussed.

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322 Can deprotonated guanines promote activation of 2′-OH nucleophile in ribozymes: an insight from molecular dynamics simulations and hybrid QM/MM calculations

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The active participation of the active site guanine in its deprotonated form on self-cleavage of RNA backbone was suggested for hairpin ribozyme, Varkud satellite, and glmS riboswitch. The role of deprotonated guanine is the activation of 2′-OH nucleophile by accepting its proton, thus acting as a general base. Although guanine at physiological conditions is dominantly in canonical protonation form, the self-cleavage reaction might be still promoted by rarely populated deprotonated guanine, when it is sufficiently highly reactive.

Recently we used hybrid QM/MM calculations to explore the reaction mechanism of hairpin ribozyme self-cleavage with deprotonated guanine G8- acting as a general base. We indeed found that deprotonated G8- is sufficiently high reactive to promote the self-cleavage of hairpin ribozyme.[1] We used the same methodology to decipher the chemical feasibility of the self-cleavage reaction in *glmS* riboswitch with deprotonated G40- acting as a general base. Even in this ribozyme we found that the deprotonated guanine is able to activate 2′-OH nucleophile and it is sufficiently reactive to compensate the thermodynamic penalty coming from rare protonation state, which yields to reaction barrier comparable with experimentally observed rate constant.

Beside the inherent reactivity that might be examined by QM/MM calculations, the ability of deprotonated guanine to bind the 2′-OH nucleophile is also crucial for feasibility of the self-cleavage reaction. Recently we studied the conformational dynamics of hairpin ribozyme and *glmS* riboswitch with deprotonated active site guanines using molecular dynamics simulations.[2,3] We found that deprotonated guanines are prone to be repelled from the active site in both cases. It should be, however, note that the accuracy of description of deprotonated guanine might be limited in contemporary force fields. We thus reexamined our simulations, and our preliminary data suggest that although deprotonated guanines are repelled from the active sites of both ribozymes, they are able to remain temporarily bound in a reactive position for time, which might be sufficient for the catalysis.

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323 Folding and regulation mechanism of the thiC riboswitch

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Riboswitches are genetic elements that specifically recognize metabolites and regulate gene expression at various levels such as transcription and translation. The Escherichia coli thiC riboswitch is involved in the recognition of thiamin pyrophosphate (TPP) and is implicated in thiamin metabolism by regulating the expression of the thiCEFSGH operon. Previous studies have shown that TPP binding to the thiC riboswitch prevents ribosome access to the ribosome binding site, resulting in translation repression. Mostly due to its relatively large size, no mechanistic model has been proposed yet to explain how ligand binding to the thiC riboswitch is used to regulate gene expression.

We have studied the folding and regulation mechanism of the thiC riboswitch. By combining in vitro transcription assays to the well-established RNase H digestion method, we have probed nascent riboswitch transcripts in absence and in presence of TPP. By employing DNA oligonucleotides targeting the riboswitch, we have found that TPP binding to the riboswitch is significantly more efficient when performed co-transcriptionally rather than post-transcriptionally, suggesting that co-transcriptional folding is key for ligand sensing. Using site-directed mutagenesis, we have observed a strong correlation between a pause site (PsB) located in the riboswitch expression platform and riboswitch activity, suggesting that the pausing of RNA polymerase (RNAP) is important for ligand binding. To investigate the nature and role of the PsB site in TPP sensing, we have developed a novel assay in which the EcoRIQ111 roadblock protein is employed to stop elongating RNAP at the PsB site. By probing the PsB transcription complex, we have found that the affinity for TPP binding is higher than when using identical transcripts in absence of RNAP. Based on covariation analysis, we propose that paused RNAP improves ligand binding by preventing the formation of a previously uncharacterized alternative structure disrupting the P1 stem. We are currently investigating the structure of the PsB transcript using structural probing.

We report here the first structural characterization of a nascent riboswitch transcript in the context of a paused transcription complex. Our results strongly suggest that RNAP may be crucial in the folding and ligand sensing of riboswitches.

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324 Crystal structure of the Varkud Satellite Ribozyme: A peek into trans-active enzymes of the RNA World

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The **Varkud Satellite (VS)** ribozyme catalyzes RNA strand scission and ligation reactions. We have solved the first crystal structures of this ribozyme, revealed as a domain swapped dimer. Within each protomer, three-way helical junctions arrange the global geometry of the seven helices free of constraints of long range tertiary interactions. This extended fold exposes the docking site for the substrate-helix of the other protomer, resulting in the formation of two active sites in trans. The docking is facilitated by long-range kissing loop interactions between the substrate and the catalytic core, which rearranges the secondary structure of the substrate helix. This rearrangement, termed 'Shifting' is required for substrate binding and subsequent cleavage. We propose the role of the kissing interaction in 'shifting' the substrate helix and the function of shifting in substrate binding. Within each active site, adenine and guanine nucleobases abut the scissile phosphate, poised to serve as general acid and general base catalysts respectively. The VS active site bears a striking correspondence to the apparently dissimilar hairpin ribozyme and this may represent a case of convergent evolution among ribozymes.

325 Structural and thermodynamics investigation of the role of ligand binding and Mg^{2+} in the *add* adenine riboswitch folding

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Riboswitches are RNA structured elements that modulate fundamental pathways in bacteria and plants. Depending on the availability of the cognate ligand they undergo a conformational change that modulates gene expression. In the *add* adenine riboswitch from *Vibrio Vulnificus*, a translational regulator, there are three key regions for the switch between the On and the Off conformations: the P1 stem, the loop-loop interaction and the adenine binding site. Our work is based on advanced *in silico* techniques, including atomistic molecular simulations, steered molecular dynamics, umbrella sampling and Hamiltonian replica exchange combined with well-tempered metadynamics. First we investigated the P1-ligand dependent stabilization. Comparing the *holo* and the *apo* forms of the aptamer we quantified this effect in terms of free energy obtaining results in agreement with dsRNA melting experiments. Then we evaluated the separate contributions that ligand and magnesium ions give to the formation of the tertiary interaction between the loop 2 and loop 3. Our investigation clarifies how ligand binding is able to stabilize this interaction. Moreover we analysed the screening role of the Mg^{2+} and its impact on aptamer compacting. The results are in nice agreement with experimental data coming from SAXS measurements and direct measurements of excess Mg^{2+}. The simulations provide an atomistic description of metabolite stabilization to the P1 stem and to the binding site, the kissing loops tertiary-contact formation and riboswitch-Mg interaction, thus shading a new light on the adenine riboswitch aptamer folding.

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326 Investigating the impact of the local environment on RNA function through *in vitro* evolution

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RNA world theories figure prominently in many scenarios for the origin and early evolution of life. These theories posit that RNA molecules played a much larger role in ancient biology than they do now, acting both as the dominant biocatalysts and as the repository of genetic information. Strong support for an RNA world is found in the functional capabilities of RNA exhibited by modern biology. Additional support for an RNA world is found in the diversity of functions that have been demonstrated independent of biology using *in vitro* evolution. *In vitro* evolution has identified RNA molecules that bind diverse molecular targets (aptamers) and RNAs that catalyze multiple reactions (ribozymes) including phosphoryl-transfer, redox reactions, and carbon-carbon bond formation. The RNA functions exhibited by modern biology and those discovered independent of biology through *in vitro* evolution have been used to developed and constrain RNA world theories; however, the conditions under which these experiments have been conducted is relatively narrow when compared the range of conditions under which life may have emerged. The range of conditions under which random sequence space has been probed for functional RNAs is far narrower than the range of conditions under which RNA is functional in extant biology, or is presumed to have been functional in the very earliest forms of life. To better understand the impact of the local environment on the functional capacity and evolution of RNA, we have evolved several populations of self-cleaving RNAs starting from random sequences. These RNA populations were evolved in a variety of different *in vitro* environments. Evaluating these populations allows us to understand the impact of the local environment on the evolution of functional RNAs.

327 Engineering of a calcium-specific ribozyme evolved from the natural *glmS* riboswitch-ribozyme to function in gene regulation

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The *glmS* ribozyme is a catalytic RNA domain commonly found in Gram-positive bacteria. Uniquely among the 6 known classes of natural self-cleaving ribozymes, it requires a small-molecule coenzyme, glucosamine-6-phosphate (GlcN6P) to catalyze site-specific RNA cleavage through internal transesterification (*1*). This allows bacteria to sense and regulate cellular levels of GlcN6P at the mRNA level through negative feedback. To investigate the evolutionary origin of its coenzyme dependence, we previously selected *in vitro* a mutant ribozyme ($glmS^{AAA}$) that is only 3 adenine mutations away from the wildtype ribozyme ($glmS^{WT}$), and catalyzes the same cleavage reaction in the absence of GlcN6P (*2*). $glmS^{AAA}$, in contrast to $glmS^{WT}$, has a strong preference for Ca^{2+}, and requires high metal ion concentrations (>100 mM) for optimal activity. We have now evolved a third-generation *glmS* ribozyme variant, $glmS^{Ca}$. This is a calcium selective ribozyme with a higher affinity for Ca^{2+} than $glmS^{AAA}$. $glmS^{Ca}$ discriminates strongly against Mg^{2+} (>10,000-faster in 2 mM Ca^{2+} than in 2 mM Mg^{2+}), and its cleavage activity in Ca^{2+} is inhibited by Mg^{2+}. $glmS^{Ca}$ is inactive in other divalent or monovalent metal ions, except strontium, which mildly activates it. Phosphorothioate interference mapping and rescue experiments revealed a potential catalytic metal ion binding site in proximity to the active site residue A40. This site is not present in either $glmS^{WT}$ or $glmS^{AAA}$. Interestingly, this position is a guanine in both $glmS^{WT}$ and $glmS^{AAA}$, and G40A mutation in either ribozyme abolishes catalysis. In an effort to evaluate the potential of using $glmS^{Ca}$ as a calcium ion-dependent gene regulator, we have constructed a mammalian cell-based luciferase reporter system with $glmS^{Ca}$ fused with the luciferase gene, and demonstrated that $glmS^{Ca}$ is active *in vitro* under such context. Ongoing work towards introducing this system into mammalian cells will be discussed. (Supported by the Intramural Research Program of the NIH, NHLBI.)

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328 Crystal Structure of a $preQ_1$ Class 3 Riboswitch Reveals a Novel Fold with a Familiar Mode of Ligand Recognition

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The $preQ_1$-III riboswitch is the most recently discovered RNA motif that regulates bacterial gene expression in response to $preQ_1$ binding. Prior work demonstrated that the riboswitch exhibits a unique secondary structure with no sequence conservation relative to the $preQ_1$ class 1 or $preQ_1$ class 2 riboswitches. Although the basis of gene regulation is not known at present, the proximity of the aptamer domain to a downstream ribosome binding site (RBS), and absence of a rho-independent terminator helix, suggest the riboswitch governs protein translation. To gain insight into the mechanism of gene regulation by this new riboswitch class, we undertook a structure and function analysis. Isothermal titration calorimetry revealed a K_D for $preQ_1$ binding comparable to those of other $preQ_1$ riboswitch classes. We then determined an X-ray crystal structure of the $preQ_1$-III riboswitch to 2.85 Å resolution. This work revealed a novel three-dimensional fold whereby the riboswitch binds $preQ_1$ at the intersection of a three-way helical junction harboring an M-type pseudoknot. Surprisingly, the ligand binding site and adjacent U•A-U major groove base triples are spatially identical to those of the $preQ_1$-II riboswitch, although this motif does not encompass the $preQ_1$-III riboswitch RBS. Instead the RBS is located in a distal region hypothesized to base pair with a downstream stemloop as an H-type pseudoknot. This interaction is inferred from a domain swapping interaction between two $preQ_1$-III riboswitch molecules that are related by crystallographic symmetry. A model for gene regulation will be discussed.

329 The Diversity and Distribution of Riboswitches

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Riboswitches are segments of mRNAs that bind selectively to small molecules or ions. Upon binding to their ligands, riboswitches modulate gene expression, commonly at the level of transcription termination or translation initiation. Riboswitches are found mostly in bacteria, but occasionally are found in archaeal and eukaryotic species. Each riboswitch class is defined by the conserved nucleotide sequences and architecture of its representatives, or by the ligand that is selectively bound. To date, 37 classes of riboswitches or candidate riboswitches have been identified.

By examining and updating previously reported riboswitch consensus models, and by searching the expanding DNA sequence databases, we found numerous additional members of each riboswitch class. Some of the newly-found representatives exhibit sequence or structural variations that retain riboswitch function. We also identified examples wherein the variants exhibit altered ligand specificity. Additionally, we observed unusual genetic arrangements for several riboswitches. For example, numerous tandem riboswitches were identified, which indicates that some cells exploit riboswitches to form two-input Boolean logic gates or to form more "digital" gene control elements. We also observed instances in which a riboswitch aptamer resides immediately adjacent to a self-splicing RNA, and these two functional RNAs might collaborate to form an allosteric ribozyme.

We also determined the distribution of every riboswitch class down to the species level. With these data, we are able to determine phylogenetic trends that should prove useful for the discovery of new riboswitches and new genetic arrangements of riboswitches. These findings demonstrate that a great diversity of riboswitches are widely used by many bacterial lineages to control the expression of fundamental metabolic and toxicity mitigation genes.

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330 c-di-AMP recognition by its cognate riboswitch

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Second messenger signaling is critical for bacterial lifestyle changes and adaptive survival. The dinucleotides cyclic di-guanosine monophosphate (c-di-GMP) and cyclic di-adenosine monophosphate (c-di-AMP) are widespread second-messenger signaling molecules in bacteria, including in many pathogenic organisms. These small molecules are commonly used to relay information from external receptors to effectors within the cell. In particular, c-di-AMP is responsible for bacterial sporulation, host immune response, cell size, and response to cell wall stress. A recently discovered riboswitch has been shown to bind this second messenger and act as a molecular target. In order to characterize this RNA-ligand interaction, we are investigating the structural and biochemical basis of riboswitch mediated cyclic dinucleotide signaling. We have synthesized a comprehensive series of c-di-AMP analogs to probe which components of the ligand are critical for binding. Biochemical analysis, using a competitive gel shift assay, suggests a mode of ligand recognition involving A-minor interactions that are highly sensitive to perturbation. This work suggests models of how c-di-AMP is recognized by its cognate riboswitch and will help to further elucidate the molecular mechanism of action of this important signaling molecule. Understanding the chemical nature of this interaction could lead to the identification and design of antibacterial agents to a novel riboswitch target.

331 Synthetic transcriptional Riboswitches in *E. coli*

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Riboswitches are regulatory RNA elements typically located in the 5′-untranslated region of certain mRNAs and control gene expression at the level of transcription or translation. These elements consist of a sensor and an adjacent actuator domain. The sensor usually is an aptamer that specifically interacts with a ligand. The actuator contains an intrinsic terminator or a ribosomal binding site for transcriptional or translational regulation, respectively. Ligand binding leads to structural rearrangements of the riboswitch and to presentation or masking of these regulatory elements. Based on this modular organization, riboswitches are an ideal target for constructing synthetic regulatory systems for gene expression. We use an in silico pipeline for the rational design of synthetic riboswitches that regulate gene expression at the transcriptional level. Using the well-characterized theophylline-aptamer as sensor, we designed the actuator part as RNA sequences that can fold into functional intrinsic terminator structures. Several of the designed constructs show ligand-dependent control of gene expression in *E. coli*, demonstrating that it is possible to engineer riboswitches not only for translational but also for transcriptional regulation. Furthermore, these elements can be combined in tandem or tridem arrangements that strongly reduce background activity. With the tridem riboswitch, the dose-dependent activation shows an improved linear correlation, possibly allowing a quantitative read-out of the ligand concentration in the cell.

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332 Riboswitches in Deltaproteobacteria sense the second messenger c-GMP-AMP

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A riboswitch is a non-coding RNA domain typically found in the 5′-untraslated region of bacterial mRNA that is able to regulate downstream gene expression by binding small molecules or ions. C-di-GMP-I riboswitches recognize the bacterial second messenger cyclic-3′-5′-di-guanosine monophosphate and regulate a wide range of cellular processes including motility, pathogenesis, and biofilm formation. Two nucleotides in this riboswitch (G20 and C92) make important contacts with the ligand and confer binding specificity. We have discovered that a group of predicted c-di-GMP-I riboswitches contains a mutation at one of these positions that alter the specificity of the RNA. Specifically, G20A variants in Deltaproteobacteria selectively bind another cyclic dinucleotide, c-GMP-AMP. This molecule has been implicated in bacterial colonization in *Vibrio cholerae*, though roles for c-GMP-AMP in other bacteria remain unknown. These variant riboswitches are the first known receptor for c-GMP-AMP and their discovery reveals new roles for this signaling molecule, including a possible role in electron transfer processes.

333 SAM-III Riboswitch Conformational Dynamics

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Riboswitches are structured RNA elements generally found in the 5′ untranslated regions of mRNAs. They control gene expression by choosing between alternate "on" or "off" conformations in response to cellular conditions, often the concentration of a small molecule or ion. Though examples are known in all domains of life, riboswitches are particularly widespread in Eubacteria, controlling an estimated 2-4% of genes.

One of the big questions in the study of this and other riboswitches is their conformational dynamics. This includes the influence of ligand binding on the structures adopted, and thus on gene expression. Two common mechanisms by which riboswitches may bind ligands are "induced fit", in which the ligand induces any structural changes in the RNA, and "conformation capture", in which the ligand can bind and "capture" a pre-formed bound-conformation subpopulation of RNA molecules.

Here I present a study of the SAM-III riboswitch (SMK-box). SAM-III is present across the Lactobacillales, which includes a number of commensal as well as pathogenic species. It inhibits translation of S-adenosylmethione (SAM) synthetase genes in response to high SAM concentrations.

We have attempted to gain a higher spatial and temporal resolution view of the SAM-III conformational dynamics by crystallographic snapshots of intermediates. These snapshots constitute a finer-resolution view of conformational dynamics of the SAM-III riboswitch than has been established at crystallographic resolution to date. They provide evidence in support of a combination of both "conformation capture" and "induced-fit" mechanisms of SAM binding by the Enterococcus faecalis SAM-III riboswitch.

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334 Mechanistic and structural studies on the twister ribozyme

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The twister ribozyme, recently discovered in the Breaker laboratory, is a novel small nucleolytic ribozyme that is widely disseminated in the genomes of bacteria and eukarya. We recently solved the crystal structure of a twister ribozyme from *Oryza sativa* at 2.3 Å resolution (see abstract by Liu *et al.*). The RNA adopts a novel compact fold based on a double pseudoknot structure, with the active site formed by the interaction of the highly-conserved nucleosides of Loops 1 and 4. A guanine nucleobase that has its Watson-Crick edge directed towards the scissile phosphate was identified in an initial investigation of highly-conserved nucleotides as likely to participate in catalysis. Further mechanistic evidence supports a role for this nucleobase as either general base or acid in a concerted, general acid-base catalyzed reaction.

The bell-shaped dependence of cleavage rate on pH is consistent with a second nucleobase of lower pK_a also participating in catalysis. However initial investigations have not revealed a clear candidate. The crystal structure places two highly-conserved adenines close to the sissile phosphate with Watson-Crick edges free to participate in proton transfer. We will discuss plausible catalytic mechanisms for the twister ribozyme in the light of our ongoing structural and mechanistic studies that are focused on these conserved adenines.

335 Towards Optimization of a General RNA Labeling Deoxyribozyme: Analysis of Unimolecular Deoxribozyme Variants

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Using fluorescence microscopy as a tool to study biological systems has been proven to be useful in both experiments in cells and *in vitro*. However, current methods for preparing fluorescent RNAs for use *in vitro* fluorescence assays are challenging and inefficient. Recently, we have shown that deoxyribozymes can be used to site-specifically label RNA molecules with fluorescent GMP moieties. We believe that this method will prove generally useful for incorporation of fluorescent labels into different sites within an RNA molecule for use in biochemical studies. We propose that the annealing of two RNA molecules (the substrate RNA and a "helper" RNA) to the deoxyribozyme results in formation of a tertiary structure that brings key catalytic sites within close proximity in order to facilitate GMP transfer. I am currently studying a unimolecular RNA/DNA hybrid deoxyribozyme in which the substrate RNA, the helper RNA, and the DNA are all part of a single oligonucleotide. Our goal is to use this simplified construct to study conformational changes occurring in the deoxyribozyme during GMP transfer by single molecule FRET and by structural methods (X-ray crystallography, NMR). We believe that structural and conformational insights from the unimolecular construct will help us better understand the mechanism in which the deoxyribozyme labels RNAs. This will lead to development of next generation deoxyribozymes with improved function.

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336 Single-Molecule Fluorescence Using Nucleotide Analogs: A Proof-of-Principle

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Fluorescent nucleotide analogues, such as 2-aminopurine (2AP) and pyrrolo-C (PyC), have been extensively used to study nucleic acid local conformational dynamics in bulk experiments. Here we present a proof-of-principle approach using 2AP and PyC fluorescence at the single-molecule level. Our data show that RNA, ssDNA, or dsDNA, containing either 2AP and PyC can be monitored using single-molecule fluorescence. We demonstrate that this approach can be used to monitor DNA and RNA in real time. This is the first reported assay using fluorescent nucleotide analogs at the single-molecule level. We anticipate that single 2AP or PyC fluorescence will have numerous applications in studies of DNA and RNA, including protein-induced base-flipping dynamics in protein-nucleic acid complexes.

337 Molecular basis for discrimination in RNA 3′-termini binding

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RNA 2′,3′-cyclic phosphate termini play a significant role in RNA metabolism as intermediates in the chemical or enzyme-catalyzed hydrolysis of the RNA phosphodiester backbone, as substrates for the tRNA ligase RtcB, and as recognition elements present on U6 snRNA. RNA 2′,3′-cyclic phosphate termini can be synthesized from 3′-phosphate termini in an ATP-dependent reaction catalyzed by RNA 3′-phosphate cyclase (RtcA), an enzyme conserved in bacteria, archaea, and eukarya. RtcA and the tRNA ligase RtcB are the only known enzymes that activate RNA 3′-phosphate termini, with each enzyme proceeding through two similar reaction steps. The mechanism of nucleotidylation of histidine residues in RtcA and RtcB has been elucidated; however, the location of the RNA binding sites and the mechanism of RNA 3′-phosphate nucleotidylation remains unknown for each enzyme. Moreover, how RtcA and RtcB avoid binding to the abundant RNA 3′-OH termini *in cellulo* has remained an important unanswered question. Here we present a crystal structure of RtcA in complex with a 3′-phosphate terminated RNA and adenosine in the AMP-binding pocket. Our studies reveal that RtcA discriminates against 3′-hydroxyl termini by ensuring that a terminal 3′-phosphate makes a large contribution to RNA binding. Furthermore, our work elucidates the mechanism of AMP transfer to an RNA 3′-p terminus and reveals significant conformational changes upon RNA binding.

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338 The Chemical Synthesis of Long and Highly Modified RNA using 2′-ACE Chemistry

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Due to increased uses of various RNAs in understanding critical structural, functional, and regulatory roles of RNA in biology, rapid, reliable, and cost-efficient methods of RNA oligonucleotide synthesis are in demand. Traditional methods of RNA synthesis based on 2′-silyl (TBDMS or TOM) protection strategies are limited in their ability to construct oligos longer than 40 nucleotides in length (far smaller than important biologically active RNA molecules). A significant improvement in RNA synthesis technology, 5′-silyl-2′-acetoxy ethyl orthoester (2′-ACE) chemistry, results in faster coupling rates, higher yields, greater purity, and superior ease of handling. Using 2′-ACE chemistry, we have developed convenient and efficient protocols to synthesize: (1) long RNA sequences in excess of 100 bases, (2) transfer RNAs (tRNAs) with natural modifications such as pseudouridine, m2A, and m2G, (3) RNA oligonucleotides highly modified with virtually any chemical modifications; and (4) long RNA with dual-labeled fluorescent dyes. Our results clearly demonstrate that 2′-ACE chemistry is the method of choice for long RNA synthesis applications.

339 DNA-mediated synthesis of site-specifically labeled RNA

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Biochemical and biophysical investigations of RNA folding pathways, RNA-ligand, and RNA-protein interactions demand reliable access to site-specifically modified RNA. Here we present a general method for posttranscriptional labeling of RNA that capitalizes on the ability of DNA to activate ribose 2′OH groups and catalyze phosphodiester bond formation. Thereby, the deoxyribozyme combines the functions of target site selection and catalysis of the labeling reaction. Fluorescent, spin-labeled, biotinylated and crosslinker-modified guanosines were installed at specific internal nucleotides of functional in-vitro transcribed RNA.

Key to this versatile approach is the use of deoxyribozymes that synthesize 2′,5′-branched RNA and were shown to accept mononucleotides as substrates for ligation to adenosine branch-sites.[1] A binding site for guanosine triphosphate was generated at the centre of a 3-helix junction structure by a strategically positioned cytosine as Watson-Crick base-pairing partner. Ribose-modified guanosines, such as 2′-azido- or 2′-amino-GTP were well tolerated in the DNA-catalyzed reaction, and allowed the installation of bioorthogonal functional groups. The ribose modification could be further expanded, such that fluorescent or paramagnetically labeled guanosines could be directly installed into the RNA of interest in a one-step procedure. Taking advantage of our recent finding that lanthanides can act as accelerating cofactors for RNA-ligating deoxyribozymes,[2] we report beneficial effects of Tb3+ for DNA-catalyzed labeling of RNA.[3] As examples we demonstrate labeling for up to ~160-nt long RNAs, including spliceosomal U6 small nuclear RNA and a cyclic-di-AMP binding riboswitch RNA.

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340 Synthesis and design of RNA binding molecules in enthalpy-driven manner

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Discoveries of functional non-cording RNAs (ncRNAs) have attracted a lot of attentions in recent years. Small molecules that bind to specific RNA secondary structural motifs will provide valuable tools for modulating and studying RNA function. We have developed a series of naphthyridine derivatives that can bind to single nucleotide bulges in DNA complexes, and these molecules might lead the development of new molecules that modulate the functions of ncRNA.

In previous works, we synthesized the dimer with a two-atom linker that might have no intramolecular interactions between two naphthyridines because of the dimer structural restriction. In this study, we focused our attentions on naphthyridine dimers with a three-atom linker. The dimer with a three-atom linker might be flexible enough to change conformation of the dimer in which two naphthyridines are overlapped, and the dimer with overlapped conformation of two naphthyridines is likely to bind to DNA and RNA in an enthalpy-driven manner. Here we will report synthesis of new naphthyridine dimers with a three-atom linker and evaluation of their binding ability to DNA and RNA duplexes containing an unpaired regions.

341 Pseudouridylation-induced nonsense suppression is sequence context-independent

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Pseudouridine (Ψ) is the most abundant post-transcriptionally modified nucleotide in various stable RNAs of all organisms. Ψ is a uridine isomer (5-ribosyluracil) and has an extra hydrogen bond donor that brings rigidity to the RNA structure. Our lab has recently reported that pseudouridylation can be site-specifically introduced into nonsense codons of mRNA, resulting in suppression of translation termination both in vitro and in vivo. To further dissect the mechanism of nonsense suppression, we direct uridine-to-Ψ conversion at nonsense codons using box H/ACA RNA-guided pseudouridylation. The Ψ-containing nonsense codons are tested at three different positions (with different sequence contexts) of a C-terminal tagged reporter gene. The read-through efficiency is monitored by Western blot analysis. Our data indicate that, for a given Ψ-containing nonsense codon, the read-through efficiency is essentially the same in all three positions, suggesting that pseudouridylation-induced nonsense suppression is sequence-independent. Interestingly, when comparing the three stop codons, we notice that ΨAG and ΨGA have much higher read-through efficiency than ΨAA does. Thus, while the efficiency of read-through of Ψ-containing nonsense codons is independent of adjacent RNA sequence contexts, pseudouridylation at the three different nonsense codons has a different impact on read-through efficiency.

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342 Architecture of the U-insertion/deletion editosome

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The trypanosomal RNA editing core complex (RECC) catalyzes U-insertion/deletion editing of mitochondrial mRNAs whereby each enzymatic step is directed by guide RNAs. We combined proteomics, functional studies and sequencing of complex-bound RNAs to define essential accessory factors that are responsible for RNA substrate binding and coupling with pre- and post-editing processing pathways. We find that gRNAs represent only a subset of small mitochondrial RNAs, yet an inexplicably high fraction of them possesses 3′ U-tails. This bias is reflected by gRNA enrichment in a tripartite ribonucleoprotein assembly, termed the RNA editing substrate binding complex (RESC). This particle is also responsible for mRNA recruitment and interactions with RECC and polyadenylation complexes. Although both gRNAs and mRNAs are associated with RESC, their post-editing metabolic fates are distinct: gRNAs are degraded whereas edited mRNAs undergo 3′ adenylation /uridylation prior to translation. Our results indicate that RECC and RESC typify enzymatic and substrate binding constituents of the editosome.

343 *NCS2** links tRNA modification to pathogenic phenotypes in yeast

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Fungal pathogens pose a major threat to human health and biodiversity. Thus, they have gained increasing attention in recent years. However, the molecular mechanisms, which facilitate pathogenicity, are only partially understood.

Here, we characterize *NCS2**, a single nucleotide polymorphism (SNP) identified in a strain of *Saccharomyces cerevisiae* isolated from a human patient. *NCS2,* a member of the *URM1* pathway, is essential for thiolation of the wobble uridine (U_{34}) of the three cytoplasmic tRNAs tE^{UUC}, tK^{UUU} and tQ^{UUG}, a modification, which is evolutionarily well conserved in all species.

We hypothesize that increased levels of tRNA thiolation are the underlying cause of pathogenicity of *NCS2** yeast. Consistent with this, we found that *NCS2** cells grow better at higher temperatures and under stress conditions, which are linked to tRNA thiolation. Importantly, when *URM1* or *NCS6* are deleted, *NCS2** yeast loses its growth advantage, suggesting that this phenotype is facilitated through tRNA thiolation and not through a second unknown function of *NCS2**. Interestingly, we discovered that thiolation levels in wild type yeast are decreased at higher temperatures, whereas *NCS2** yeast maintains normal modification levels. To gain insights into the molecular mechanism, we tested whether the amino acid change introduced in *NCS2** is specific. Indeed, it cannot be randomly replaced by other amino acids. Finally, we observed increased interaction of Ncs2*p to its binding partner Ncs6p by yeast-two hybrid experiments. This provides a potential molecular mechanism of how increased thiolation is achieved in *NCS2** yeast.

We are currently performing experiments to examine the *in vivo* significance of our findings in mice.

Taken together, our work has uncovered tRNA modification as a potential facilitator of yeast pathogenicity, providing a novel route for clinical interventions.

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344 A pseudouridine residue in the core of the spliceosome is part of the filamentous growth program in yeast

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Pseudouridine (Ψ) is one of the ubiquitously modified nucleosides on RNA, commonly found in transfer RNAs (tRNA), small and large subunits of ribosomal RNAs (rRNA), and spliceosomal small nuclear RNAs (snRNA). Pseudouridylation creates an extra hydrogen-bond donor and, thus, has been proposed to rigidify RNA structures in its vicinity. Ψ formation is catalyzed either by site-specific enzymes, called pseudouridine synthases, or is guided by H/ACA snoRNAs. While pseudouridines on different RNAs have been shown to be critical, they are not known to have physiologic roles in cell differentiation.

We have identified a novel pseudouridine residue (U6-Ψ28) on spliceosomal U6 snRNA that is induced during filamentous growth of *Saccharomyces cerevisiae*. Filamentous growth is a morphological transition observed in some fungal species, including yeast, under nutrient limitations allowing starving cells to form filaments that scavenge for nutrients. We identified Pus1p as the pseudouridine synthase that catalyzes modification of U6-U28 and we show that it, but not other pseudouridine synthases, is up-regulated during filamentation. We also found several U6 snRNA mutants that are constitutively pseudouridylated; remarkably, these U6 mutants activate pseudo-hyphal growth, dependent upon the presence of Pus1p, arguing that U6-Ψ28 *per se* can initiate at least part of the filamentous growth program. We confirmed this conclusion using a designer snoRNA targeting U6-U28 pseudouridylation. Conversely, mutants that block U6-U28 pseudouridylation inhibit pseudo-hyphal growth and U6-Ψ28 changes the efficiency of splicing of suboptimal introns.

We propose a model for *WHI2-PUS1* mediated activation of the filamentous growth program. Similar to the general stress response wherein the transcription factor Msn2p is activated through dephosphorylation by Whi2p and its interacting partner, Psr1-phosphatase, a filamentation-specific transcription factor may similarly be activated by Whi2p-Psr1p. This activated transcription factor binds to a STRE-like (stress response elements) sequence within the promoter of *PUS1* to up-regulate it. Indeed, we identified a STRE-like element upstream of the *PUS1* promoter and observed an up-regulation of Pus1p by over-expressing Whi2p. This up-regulation consequently results in the filamentation-specific pseudouridylation at U6-U28. U6-Ψ28 likely changes the splicing efficiency of suboptimal introns; thus, filamentous growth-activated Pus1p-dependent pseudouridylation of U6 snRNA contributes to the filamentation growth program.

345 Abstract Withdrawn

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346 Dynamic RNA composition of auxiliary factors in trypanosome RNA Editing

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Mitochondrial mRNAs in trypanosomes require massive U-insertion/deletion editing. Despite the ancient origin of trypanosomes, interesting analogies have surfaced between this editing and RNA silencing in many organisms e.g., the use of Dicer-like endonucleases, hundreds of small guide RNAs (gRNAs), and dynamic interactions between ribonucleoprotein complexes (RNPs). RNA editing progresses 3′-to-5′ in small blocks, each directed by a guide RNA (gRNA), and exhibits substrate specificity during the life cycle. However, the basic regulatory mechanisms remain unsolved. We study auxiliary factors collectively known as the mitochondrial RNA-binding complex 1 (MRB1) or gRNA-binding complex (GRBC), that contain gRNA, have a dynamic protein composition, and transiently associate with RNA editing core complexes (RECC), ribosomes and other mitochondrial factors. Here, we report the first NGS (next generation sequencing) study of native subcomplexes of MRB1, immunoselected via either an RNA helicase (REH2) that binds RNA and associates with unwinding activity, or a subunit (MRB3010) that affects an early editing step. These particles contain core and variable protein subunits, and distinct RNA photo-crosslinking patterns. Among other interesting observations, we found a relative enrichment of initiating gRNAs in MRB3010-purified factors, and evidence of rapid evolution in the edited transcriptome impacting UTR and protein coding regions. Moreover, we found robust specific co-purification of edited mRNA and unedited precursors, suggesting that these particles may bind both mRNA and gRNA substrates. We propose that MRB1 subcomplexes of different RNA/protein composition may serve as a scaffold for specific assembly or usage of editing substrates and RECC, forming the editing holoenzyme. A dynamic higher-order MRB1 may modulate concerted steps in editing initiation and progression.

347 C to U RNA editing is mediated by the novel RNA Binding Protein RBM47

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The post-transcriptional modification of Cytidine (C) to Uridine (U) RNA editing is reputed to be mediated predominantly by the deaminase Apobec1 and the RNA binding protein A1CF. We have now characterised a novel RNA binding protein, RBM47 that interacts with Apobec1 and is expressed in tissues where C to U RNA editing takes place. RBM47 can substitute A1CF and is necessary and sufficient for C to U editing of *ApoB* transcripts in vitro. Editing of *ApoB* and other potential targets is impaired in *Rbm47*-deficient mutant mice. These results point to a functional partnership of RBM47 and Apobec1 in the basic machinery for C to U RNA editing.

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348 A monoclonal antibody for transcriptome-wide *N*^{6}-methyladenosine analysis

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N^6-methyladenosine (m^{6}A) has been known for many years to be the most common base modification in eukaryotic messenger RNA (mRNA) other than the 7-methylguanosine cap. With the advancement of RNA high throughput sequencing (RNA-seq) techniques and m^{6}A-RNA immunoprecipitation (m^{6}A -RIP) using polyclonal antibodies, recent studies have identified the location of m^{6}A sites in a transcriptome-wide manner in a variety of tissues. The results of these studies show that m^{6}A is most commonly associated with a sequence motif in the 3′ UTR of mRNAs near stop codons and the level of m^{6}A at particular sites is dependent upon the expression of particular methylases/demethylases. To further advance our understanding of m^{6}A in RNA, it is important to continue improving the tools needed for m^{6}A research. Here we present the generation of a new m^{6}A-specific rabbit monoclonal antibody and its use in m^{6}A-RIP-seq experiments using mouse brain polyA selected mRNA. In contrast to most published m^{6}A-RIP data, our libraries were constructed using a strand-specific sequencing approach. Our data confirms the findings of previous m^{6}A-RIP experiments and, in addition, the strand-specific information also suggests new and interesting patterns of m^{6}A sites in mouse brain mRNA.

349 The Biology of Pus3 and of Ψ38 and Ψ39 Modifications in Yeast tRNAs

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Pseudouridine (Ψ) is the most prevalent post-transcriptional modification in RNA, and is found in many classes of RNA including ribosomal RNA, transfer RNA, small nuclear RNA and small nucleolar RNA. Previous studies have shown that Ψ exerts a rigidifying influence on the sugar-phosphate backbone and enhances local base stacking. However, in a number of cases, the detailed function of Ψ in a natural biological context remains unknown.

In tRNAs, Ψ is the most abundant modified nucleoside, mainly occurring at Ψ55 in the T-loop, in the D stem, and in the anticodon stem-loop. The occurrence of Ψ at residues 38 and 39 in the anticodon stem-loop is conserved in all three domains of life. Among 472 characterized non-organellar tRNAs in the transfer RNA database, 37 of 56 U38 residues are modified to Ψ (26 of 28 in eukaryotes), while 163 of 178 U39 residues are turned into Ψ (108 of 116 in eukaryotes). The formation of Ψ at U38 and U39 is catalyzed by Pus3, a highly conserved pseudouridine synthase widely found in all three kingdoms. In the yeast Saccharomyces cerevisiae, Ψ occurs in all 5 characterized tRNAs with U38 and all 14 characterized tRNAs with U39, with 6 other uncharacterized species bearing U38 or U39. Disruption of *PUS3* results in a significant growth defect at high temperature. Using yeast, we are investigating the causes of the defect in a *pus3-Δ* mutant and looking into the biological function of Ψ38 and Ψ39 in tRNAs. Results will be presented showing that the growth defect at high temperature is primarily due to the loss of function of a specific tRNA species, and that there is a synthetic genetic interaction between *PUS3* and other tRNA modifying genes that also work on the same tRNA.

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350 Regulation of A-to-I editing in noncoding regions of mRNA by *C. elegans* ADR-1

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Adenosine deaminases that act on RNA (ADARs) are a family of proteins that catalyze adenosine (A) to inosine (I) RNA editing in double-stranded RNA (dsRNA). A-to-I editing is the most prevalent type of RNA editing in metazoa with more than 1 million editing sites identified in noncoding regions of human mRNAs. Inadequate A-to-I RNA editing occurs in many neuropathological diseases and cancers, but is often uncorrelated with ADAR levels, implying that other mechanisms exist to regulate A-to-I editing *in vivo*.

In *Caenorhabditis elegans*, two ADAR family members, ADR-1 and ADR-2, are encoded in the genome. The initial characterization of ADR-1 by Brenda Bass's lab revealed that although ADR-1 does not possess deaminase activity, it alters *in vivo* editing levels. We have recently proposed a model in which ADR-1 regulates *in vivo* editing levels by utilizing two conserved dsRNA binding domains (dsRBDs) to bind to editing targets and alter accessibility of ADR-2 to dsRNA. Using an RNA immunoprecipitation (RIP) assay, we determined that ADR-1 is capable of binding known edited mRNAs, both in the presence and absence of ADR-2 and editing. Furthermore, mutation of the dsRBDs of ADR-1 both abolish binding to mRNA and result in similar *in vivo* editing levels as deletion of *adr-1*, indicating that ADR-1 regulates editing via a direct interaction with dsRNA.

To understand the impact of ADR-1 on the editing transcriptome, we conducted high-throughput sequencing of wild-type and *adr-1* mutant worms and developed a bioinformatics pipeline to both identify and quantitate levels of A-to-I editing. Using this approach, we were able to both expand the number of known *C. elegans* edited mRNAs by 5-fold and demonstrate that ADR-1 regulates editing of the majority of these transcripts. To understand the detailed mechanism of this regulatory function, current biochemical experiments are aimed at testing whether ADR-1 binding to dsRNA alters ADR-2 binding to target mRNAs and/or deaminase activity of ADR-2 at specific sites. In addition, we are in the process of analyzing ultra-high throughput sequencing of noncoding targets from wild-type and *adr-1* mutant worms to gain insight into the *in vivo* regulation of noncoding A-to-I editing.

351 Adenosine deamination type RNA editing in Filamin A mRNA affects smooth muscle contraction

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RNA-editing by adenosine deaminases acting on RNA (ADARs) converts adenosines to inosines in RNAs. As inosines are interpreted as guanosines during translation the nucleotide modification can change codons in mRNAs, leading to the translation of proteins that differ from the genomically encoded versions. A prominent editing event that is conserved from birds to mammals affects the mRNA encoding the actin- crosslinking protein Filamin alpha (FLNA). *Flna* mRNA-editing leads to a glutamine (Q) to arginine (R) exchange at amino acid position 2.341 in the encoded protein. The amino acid exchange lies in a region that interacts with several cellular components ranging from signaling molecules like Rock kinase to transmembrane proteins like integrin beta. Filamin RNA-editing levels vary largely amongst tissues and during development. Highest Filamin RNA editing levels are observed in the smooth musculature of stomach, large intestine, and aortae, where editing levels reach between 60 and 90%.

To determine the consequences of *Flna* RNA editing we have generated a mouse deficient in *Flna* RNA-editing. The resulting homozygous $Flna^{\Delta ECS}$ mice show defects in aortic smooth muscle contraction and blood pressure control. This phenotype is assumed to be associated with reduced aortic elasticity. At the cellular level, reduced cell migration on integrin-interacting matrices, increased adhesion foci, altered actin organization, and consequently altered myosin-dependent cell contraction is observed in $Flna^{\Delta ECS}$ cells.

Thus *Flna* RNA-editing affects cellular motility, acto-myosin mediated force generation, and blood pressure control

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352 Substrate binding by pseudouridine synthase TruB occurs in two steps and is critical for bacterial fitness

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Pseudouridine (Ψ) is the most common post-transcriptional RNA modification. The enzymes responsible, pseudouridine synthases, share a conserved catalytic fold, but differ in their specific interaction with their target RNA. To understand the mechanism and function of RNA binding, we focus on the model E. coli enzyme TruB which forms the universally conserved Ψ55 in the TΨC arm of all elongator tRNAs. While a TruB knockout strain grows normally, it has a fitness disadvantage when grown in competition with wild type E. coli. Expression of wild type as well as catalytically inactive TruB rescues the knockout strain. Since inactive TruB cannot form pseudouridine, this raises the question: what function of TruB is critical for cellular fitness? We hypothesize that TruB's most important cellular function is the interaction with, but not the modification of tRNA.

First, we studied tRNA binding by TruB in vitro using 2-aminopurine-labelled tRNA in stopped-flow rapid mixing experiments. This revealed a two-step binding process where fast initial binding is followed by a slow conformational change, most likely within the tRNA. We also investigated product release and substrate dissociation by TruB. This system provides for the first time insight into the mechanism of RNA binding by a pseudouridine synthase and can be adjusted to analyze other tRNA modification enzymes.

Next, we examined the role of tRNA binding by TruB for the cellular fitness of E. coli. Individual residues, predicted to be part of the tRNA binding surface, were substituted with glutamate in order to hinder tRNA binding by TruB. Likewise, the C-terminal PUA domain was deleted to assess whether it interacts with the tRNA. In vitro characterization confirmed the predicted defects in tRNA binding. Subsequent in vivo competition assays with the wild-type strain indicate that TruB's ability to bind tRNA is critical for cellular fitness.

In conclusion, we show that tRNA binds to TruB before undergoing a conformational change and these steps are important for cellular fitness. Together, our results support the hypothesis that TruB acts as an RNA chaperone by locally unfolding tRNA upon binding which could provide a second chance at correct folding for the tRNA.

353 Coordination between DeaD RNA helicase and rRNA modification enzymes in processing of *Escherichia coli* 23S rRNA

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DeaD, one of DEAD-box RNA helicases in *Escherichia coli*, is required to survive under cold stress. In the absence of DeaD, 23S rRNA, a component of 50S ribosome, is defective in processing and the growth rate was decreased. 23S rRNA has 36 post-transcriptional modifications including methylation and pseudouridylation, each of which is generated mostly by a one-to-one rRNA modification enzyme (rRME) and plays an important role in cell growth, antibiotic resistance phenotypes, and ribosome assembly. Since rRNA modification provides driving force to associate ribosomal RNAs by local structural rearrangements of RNA helices, it seems to be related to RNA helicase functions. However, correlation between rRMEs and RNA helicases is not known. In this study, we examined how DeaD would be related to rRMEs in rRNA processing. We found that DeaD RNA helicase was coordinated with rRNA modification enzymes for 23S rRNA processing.

354 Abstract Withdrawn

355 Wobble uridine modification defects cause sensitivity to stress through perturbed protein homeostasis

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Chemical tRNA modifications are found in all domains of life and thought to modulate all aspects of tRNA biology. Importantly, defects in tRNA modification lead to increased sensitivity to stress in many organisms and have been linked to human diseases. However, the underlying molecular mechanisms that lead to these phenotypes are still unclear.

To better understand how stress sensitivity is caused by modification defects of wobble uridine (U_{34}), we used ribosome profiling to quantitatively analyze transcriptome-wide ribosome occupancy patterns in wild-type yeast and strains deficient in U_{34} modification. We found that U_{34} hypomodification leads to codon-specific translational slowdown on endogenous transcripts *in vivo*. Strikingly, CAA and AAA, and, to a lesser extent, GAA codons were selectively enriched in the A site of ribosome footprints from the mutants. This effect was conserved in *Caenorhabditis elegans* underscoring the importance of U_{34} modifications for decoding efficiency in yeast and nematodes, and possibly all eukaryotes. Interestingly, the extent of slowdown was not increased under stress conditions, suggesting that phenotypes are not caused by aggravation of translational defects during stress.

Surprisingly, we found that protein quality control pathways are significantly upregulated in yeast strains with aberrantly modified U_{34}. Furthermore, these strains accumulate protein aggregates, providing evidence that U_{34} hypomodification perturbs protein homeostasis. Overexpression of tK(UUU), tQ(UUG), and tE(UUC), is known to suppress the phenotypes of yeast strains with hypomodified U_{34}. Importantly, overexpression of these tRNAs also decreased the translational slowdown of their cognate codons and alleviated proteotoxic stress. Using quantitative mass spectrometry we found that the protein content of aggregates from strains lacking either U_{34} modification or cotranslational chaperones largely overlaps. Thus, aggregates are not specifically formed by proteins that are enriched for CAA, AAA and GAA codons.

Taken together, our findings provide the first *in vivo* evidence, that codon-specific translational slowdown, which is induced by wobble uridine hypomodification, negatively affects protein folding. This establishes the critical importance of tRNA anticodon modifications for protein homeostasis. Finally, our findings suggest that the phenotypic expression of tRNA anticodon modification defects mainly stems from toxicity of misfolded proteins.

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356 Multiple functions of a family of 3′-to-5′ polymerases in *Dictyostelium discoideum*

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All canonical DNA and RNA polymerases catalyze nucleotide addition in the direction of 5′-to-3′, but a family of 3′-to-5′ reverse polymerases has been found. These enzymes include the highly conserved $tRNA^{His}$ guanylyltransferase (Thg1), which use the 3′-to-5′ addition activity to play an essential role in maturation of cytosolic $tRNA^{His}$ in eukaryotes. Most eukaryotes contain one unique Thg1 enzyme, but four Thg1 orthologs (DdiThg1 and three Thg1-like proteins (DdiTLP2-4)) have been identified by BLAST in *Dictyostelium discoideum*. To investigate the functions of the four Thg1 orthologs in *D. discoideum* would lead to understanding the versatile roles of this reverse polymerase family in biology. Here we show in vitro and in vivo characterization demonstrating that these four enzymes are all 3′-to-5′ polymerases that carry out independent cellular functions that are important for viability in *D. discoideum*, including G_{-1} addition to cytosolic and mitochondrial $tRNA^{His}$ and 5′-tRNA editing in mitochondria. This study provides the first identification of a bona fide biological function for the Watson-Crick template-dependent 3′-to-5′ polymerase activity that is associated with Thg1-family enzymes. These data also reveal that there is an additional previously unknown, but essential, function for one of the Thg1-related genes in cytosol of *D. discoideum*, an observation that opens the door to identification of further roles for 3′-to-5′ polymerases in biology.

357 Computational identification of RNA editing sites and related mechanisms of regulation

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A-to-I RNA editing carried out by ADAR proteins is a widespread molecular mechanism that plays a crucial role in diversifying gene products. The abundance of RNA editing events and their importance in regulating cellular mechanisms were revealed in recent years by the development of new sequencing technologies. However, the majority of these RNA editing events cannot be associated with regulatory cellular mechanisms.

Here we introduce a new computational method which can be used to identify RNA editing sites with high accuracy. To identify editing events, we perform accurate genome-wide analysis on tissue specific RNA-seq data sets. By applying statistical analysis and using ADAR specific features, we manage to distinguish real editing events from SNPs, and sequencing or mapping artifacts. Moreover, using our highly confident detected set of editing events, we explore the potential effects of RNA editing on the regulation of the transcriptome.

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358 Pilot scale compound screening against RNA editing identifies inhibitors of *Trypanosoma brucei*

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Most mitochondrial mRNAs in trypanosomatid pathogens undergo a unique type of post-transcriptional modification involving insertion and/or deletion of uridylates. This process, termed 'RNA editing,' is catalyzed by a ~1.6 MDa multi-protein complex called the editosome. Knockdown of core editosome proteins compromises the mitochondrial function and, ultimately, cell viability. Therefore, as editosome is unique to trypanosomatids, it serves as an ideal drug target for the many diseases caused by these parasites. The field however, lacks potent editosome inhibitors that could be used for the development of drugs against trypanosomatids and also serve as unique tools to perturb and characterize the various interactions and reaction stages of RNA editing. Here, we screened the library of pharmacologically active compounds ($LOPAC_{1280}$, Sigma) using our previously reported high throughput screening assay to identify RNA editing inhibitors. We report aurintricarboxylic acid, mitoxantrone, PPNDS and NF449 as potent inhibitors of deletion RNA editing, with IC_{50} values ranging from 1 to 5 μM. However, none of these compounds showed inhibition specificity for a catalytic step of RNA editing. We show that mitoxantrone blocks editing by inducing RNA-protein aggregates, whereas the other three compounds interfere with editosome RNA interaction to varying extents. Moreover, our data revealed for the first time that NF449, a suramin analog, was able to effectively kill *T. brucei*, *in vitro*. Thus, the results of this study provide new tools for characterization of editosome and potential discovery of RNA editing inhibitors.

359 Archaeal RNase P

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Correct maturation of tRNAs is crucial for the viability of cells. Cleavage of the pre-tRNA at its 5´-end is performed by RNase P. In the three branches of life, RNase P is build up of one RNA molecule and a different number of proteins, ranging from one in bacteria and nine to ten in higher eukaryotes. The catalytic activity is located on the RNA, characterizing RNase P as a ribozyme. One exception in this concept is found in mitochondria and plant chloroplasts, which harbor a protein only RNase P activity.

The archaeal RNase P is forming a link between the bacterial and eukaryotic RNase P, as it contains one RNA molecule and 5-6 associated proteins. Our aim is to structurally characterize archaeal RNase P.

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360 Directing the timing of ribosomal RNA modification in human cells: the recruitment of late-acting snoRNAs to pre-ribosomal complexes is regulated by the RNA helicase DDX21

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Extensive modification of ribosomal (r)RNAs is key for their biogenesis, structure and function. Most rRNA modifications are 2′-O-methylations and pseudouridylations introduced by box-C/D and box-H/ACA small nucleolar (sno)RNPs, respectively. Their snoRNA component basepairs with the rRNA precursor, guiding the catalytic component of the snoRNP to the target residue. It is generally thought that snoRNPs act co-transcriptionally, while other enzymes function at later stages of ribosome synthesis.

While characterising the functions of the RNA helicase DDX21 we discovered human snoRNAs that are, surprisingly, only associated with late pre-ribosomal complexes, providing the first evidence that snoRNP-mediated rRNA modifications take place at different stages of ribosome maturation in human cells. UV crosslinking experiments identified DDX21 binding sites in the 18S rRNA sequence that cluster in the small ribosomal subunit (SSU) and contain several box-C/D snoRNP modification sites. Interestingly, analysis of the association of these snoRNAs with pre-ribosomes revealed that DDX21 is required for the recruitment of several late-acting snoRNAs to pre-SSU complexes, whilst other snoRNAs that are recruited early, function independently of DDX21. Furthermore, we demonstrated that the helicase activity of DDX21 is required for rRNA modification by these snoRNPs. This suggests that DDX21 functions by remodelling late SSU complexes, enabling snoRNAs to gain access to their rRNA target sites. By analysis of the pre-ribosomal association of multiple snoRNAs we discovered that most snoRNPs are present only in early complexes, others are recruited early intermediates but remain associated longer during pre-ribosome maturation, while several snoRNAs only interact with late pre-ribosomes. Interestingly, both the modifications introduced by these late-acting snoRNAs as well as DDX21 are well conserved in many eukaryotes but are not found in yeast, suggesting that together they constitute a late-acting module required for rRNA modification. Taken together, these observations provide exciting insights into the timing of snoRNP modifications and a new mode of regulation by an RNA helicase in human cells.

361 Diverse roles of the prion-like protein, Mod5, in tRNA-modification and RNA-silencing

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Mod5 is a highly conserved tRNA modifying enzyme that resides primarily in the cytoplasm in eukaryotes where it modifies a small subset of tRNAs, by transferring a isopentenyl group from dimethylallyl pyrophosphate to A37 adjacent to the anticodon. In yeast a small population of Mod5 is bound to nuclear tRNA gene transcription complexes and nascent pre-tRNAs, and is required for silencing RNA polymerase II transcription near tRNA genes (1). Yeast Mod5 can misfold into heritable prion-like aggregates which confer resistance to the fungicide, fluconazole (2) . We have demonstrated that the human homolog of Mod5, TRIT1, complements both the tRNA-modification and tRNA gene-mediated silencing functions in yeast (1). We are currently investigating the folding behavior of Mod5/TRIT1 in yeast and in human cells, addressing the following questions: (1) Does prionization affect the known nuclear or cytoplasmic functions of Mod5? (2) Is the prionization tendency conserved from yeast to humans? (3) Is prionization affected by cellular stress responses? Others have shown that tRNA modification in the cytoplasm is significantly reduced in prion-Mod5 cells, suggesting that prion-Mod5 lacks tRNA modification capabilities presumably due to the insolubility of the Mod5 amyloid aggregate (2). However, our studies demonstrate that Mod5 prionization does not affect tRNA gene-mediated silencing in the nucleus suggesting that either the nuclear pool of Mod5 is aggregation-resistant or that prion-Mod5 in the nucleus remains functional for silencing. It is not currently known whether selection of misfolded Mod5 by fungicide treatment is an evolutionary response to compounds that inhibit the ergosterol pathway or rather that Mod5 evolved prion-like capabilities as part of a more general stress response. Our preliminary data suggests that oxidative stress may also affect Mod5 prionization status, suggesting that prionization of Mod5 may be part of a larger protein misfolding response to cellular stressors.

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362 RET1-DSS1 complex is required for gRNA maturation in *Trypanosoma brucei* mitochondria

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Trypanosomes are parasitic protozoan hemoflagellates that cause serious diseases such as Chagas disease and African sleeping sickness. The trypanosome mitochondrion encloses an unusual DNA structure composed of a few maxicircles and thousands of minicircles. Mitochondrial genes are encoded in maxicircles, but most are encrypted. Therefore, their transcripts require extensive uridine insertion/deletion RNA editing to produce open reading frames. The editing is directed by minicircle-encoded guide RNAs (gRNAs). Mature 50-60 nt gRNAs are generated from ~800 nt precursors by 3′ end nucleolytic processing and subsequent RET1-catalyzed uridylation. We previously demonstrated that TbRET1 repression in *Trypanosoma brucei* leads to a loss of 3′ oligo(U) tails and overexpression of enzymatically inactive TbRET1 triggers accumulation of gRNA precursors (pre-gRNAs). The former observation is consistent with TbRET1's uridyl transferase activity; the latter finding remained puzzling because TbRET1 lacks nucleolytic activity.

Here, we show that TbRET1 forms a stoichiometric complex with a putative 3′-5′ exonuclease TbDSS1 and several proteins without any discernible motifs, which we named TbRDS (TbRET1-TbDSS1) complex. In *S. cerevisiae*, DSS1 interacts with SUV3 helicase to form a mitochondrial degradosome. However, in *T. brucei* we found no evidence of stable DSS1-SUV3 interaction. Repression of TbDSS1 led to a loss of mature gRNAs and accumulation of ~800 nt pre-gRNAs indicating that TbRET1 and TbDSS1 function in the same processing pathway. Furthermore, overexpression of enzymatically inactive TbDSS1 also triggered gRNA processing defects. In addition to minicircle-encoded pre-gRNAs, TbRDS complex also targets maxicircle-encoded messenger and ribosomal RNA precursors leading to generation of mature molecules.

Collectively, our data indicate that nucleolytic processing of pre-gRNAs by TbDSS1 and uridylation of processed pre-gRNAs by TbRET1 are coupled by virtue of both enzymes being assembled into a stable TbRDS complex. Based on our data, we will propose a model for gRNA maturation mechanism at post-transcription.

363 Dynamic modification of tRNA in the yeast *Saccharomyces cerevisiae*

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Interest in the function of numerous post-transcriptional modifications of tRNA bases and sugars that are known to occur in all three domains of life has increased in recent years. While translation-related roles for some modified nucleotides found near the tRNA anticodon are relatively well-established, the biological function of many modifications found in the remaining tRNA body is far less well-understood. Some modifications occurring in the core of the tRNA affect overall stability, and thus, loss of specific modifications may lead to degradation. In addition, cells exposed to oxidative stress or growth arrest may gain additional modifications on certain tRNAs. These data suggest that tRNA modification can be regulated in cells as a way to ensure overall quality and function of the tRNA pool, but the consequences of alternative tRNA modification patterns remain to be fully investigated.

In this work, we show that the yeast m^1G_9 methyltransferase, Trm10, displays the ability to modify additional tRNA substrates, both in vitro and in vivo, beyond the set of tRNA species that are normally modified in wild-type *S. cerevisiae*. We hypothesize that this expanded mode of substrate specificity is advantageous in that it could allow Trm10 to modify non-cognate tRNAs in cells under stress, possibly preserving the structural integrity of the tRNA. Using in vitro activity assays with tRNA chimera, we have identified tRNA elements that affect substrate specificity of yeast Trm10. Moreover, analysis of the modification status of the 5′-end of tRNAs exposed to two different stress conditions using primer extension revealed additional primer extension stops corresponding to positions of known tRNA modifications, including *N-1* methylation at G_9. These data support the hypothesis that the modification status of tRNAs is much more dynamic than previously understood and opens the door to further investigation of the physiological function of alternative tRNA modification patterns in cells.

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364 RNA repair in bacteria and beyond

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Ribotoxins kill cells by cleaving essential RNAs, and RNA repair system exists in nature to counter the damages, exemplified by the RNA repair system from bacteriophage T4 discovered about 30 years ago. Specifically, T4Pnkp process the broken ends of RNA, and T4Rnl1 restores the damaged RNA to its original form. A few years ago and built upon the studies by Shuman and coworkers, our laboratory discovered a bacterial RNA repair system named Pnkp/Hen1 (1). Bacterial Pnkp/Hen1 distinguishes the T4 system by addition of a methyl group at the junction of repair, thus resulting in repaired RNA resisting further damage.

Since the methylated RNA repair product is superior, there is a great incentive for Pnkp/Hen1 to produce it as much as possible. However, the mechanism on how this is achieved was unknown. Our studies revealed that the ligase activity of Pnkp requires activation by the N-terminal domain of Hen1, thus ensuring the opportunity of Hen1 to carry out methylation during RNA repair (2).

The presence of Hen1, however, does not guarantee the efficiency of methylation during repair. We observed that Pnkp/Hen1 forms a heterotetramer in vitro (two copies of each active site), which might be the possible cause. But we were not able to crystallize Pnkp/Hen1 heterotetramer to provide the insight. We have recently discovered a new bacterial RNA repair complex named Pnkp1/Rnl1/Hen1, which forms a heterohexamer in vitro (thus also two copies of each active site). The structure of the Pnkp1/Rnl/Hen1 heterohexamer presented here revealed the molecular basis of efficient methylation during RNA repair as well as overall RNA repair process.

Through bioinformatic analysis, we recently found a gene from Acanthamoeba castellanii encoding a protein containing both Hen1 and Rnl, which may involve RNA repair. Biochemical and structural characterization is currently underway. If our studies show the positive results, it will represent the first example of RNA repair in eukaryota.

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365 Characterising the mechanism by which Inosine-containing dsRNA suppresses interferon induction

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Adenosine deaminases acting on RNA (ADARs) catalyze the deamination of adenosine to inosine (I) within dsRNA. Of the three ADARs (ADAR1–3) described in mammals, only ADAR1 and ADAR2 are catalytically active.

While A-to-I editing can occur selectively within mRNA, hyper-editing of long dsRNA can result in up to 50% of adenosine residues being changed to inosine. Localized changes in RNA structure are likely within hyper-edited inosine-containing dsRNAs (IU-dsRNAs), as IU wobble pairs are weaker than canonical base pairs. Most mammalian editing occurs within non-coding regions of RNA, particularly within repetitive elements such as*Alus*. However, while many RNAs may be extensively edited, the role of IU-dsRNA in cells is poorly understood.

Deletion of ADAR1 in mice gave rise to an embryonic lethal phenotype, where widespread apoptosis and upregulation of interferon-stimulated genes was observed. Whilst several ideas were put forward to explain how ADAR1 might regulate interferon signaling, these speculations were not substantiated. However, our recent findings provide an explanation for how ADAR1 may regulate interferon signaling and apoptosis[1]. We showed that IU-dsRNA in mammalian cells was sufficient *per se* to suppress interferon induction and apoptosis in response to long dsRNA. Moreover, we showed that IU-dsRNA inhibited activation of IRF3, a key factor in the pathway leading to interferon induction. We additionally showed that IU-dsRNA interacted specifically with RIG-I, the cytosolic sensor for dsRNA. We therefore speculated that IU-dsRNA suppressed interferon induction by competing with specific ligands for RIG-I binding.

We have now further investigated the mechanism by which IU-dsRNA suppresses interferon induction in mammalian cells. We have thus shown that both the helicase and C-terminal domain of RIG-I are essential for specific binding to IU-dsRNA. Moreover, competition analyses demonstrated that IU-dsRNA competes with canonical RIG-I ligands for binding. We have also shown that IU-dsRNA suppresses early steps in the pathway prior to activation of IRF3. These data together support the idea that IU-dsRNA downregulates interferon induction by preventing RIG-I activation, and thereby underlines the importance of ADAR1 in mammalian cells.

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366 Modified Amber Force Field Correctly Models the Conformational Preference of Tandem GA pairs in RNA

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Conformational changes are important for RNA function. We used molecular mechanics with all-atom models to understand conformational preference in RNA tandem guanine-adenine (GA) base pairs. These tandem GA base pairs play important roles in determining the stability and structural dynamics of RNA tertiary structures. Previous solution structures showed that these tandem GA base pairs adopt either imino (cis-Watson-Crick/cis-Watson-Crick interaction) or sheared (trans-Hoogsteen/trans-Hoogsteen interaction) pairing depending on the sequence and orientation of the adjacent base pairs. In our simulations we modeled $(GCGGACGC)_2$ (Wu and Turner 1996) and $(GCGGAUGC)_2$ (Tolbert et al., 2007) experimentally preferred as imino and sheared respectively. Besides the experimentally preferred conformation, we constructed models of the non-native conformations by changing cytosine to uracil or uracil to cytosine. We used explicit solvent molecular dynamics and free energy calculation with umbrella sampling to measure the free energy deference of the experimentally preferred conformation and the non-native conformations. A modification to ff10 required, which allowed the guanine bases' amino group to leave the base plane (Yildirim et al., 2009). With this modification, the RMSD of unrestrained simulations and the free energy surfaces are improved, suggesting the importance of electrostatic interactions by G amino groups in stabilizing the native structures.

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367 The DEAH-box ATPase Prp2 catalytically activates the spliceosome by rendering the first step reactants accessible for catalysis without changing the secondary structure of the spliceosomal RNA network

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During catalytic activation of the spliceosome, the B^{act} complex undergoes a major structural rearrangement that is mediated by the DEAH-box ATPase Prp2 and its co-factor Spp2 in an ATP-dependent process, generating the B* complex. While the effect of Prp2′s ATPase activity on the protein dynamics of the catalytic RNP core of the spliceosome has been investigated in some detail (1), its effect on the structure of the RNA-RNA interaction network of the B^{act} complex is poorly understood. Using a purified yeast splicing system (2), we have investigated the spatial organization of the spliceosome's RNA network and its dynamic rearrangement during the Prp2-mediated B^{act} to B* complex transition via chemical RNA structure probing. Our data reveal that the overall secondary structure of the spliceosome's RNA elements does not change during catalytic activation, indicating that Prp2 does not act by disrupting or rearranging RNA base pairs. Several small changes in base accessibility were observed in single-stranded regions of U2 snRNA on both sides of the U2/pre-mRNA branchpoint helix, possibly due to Prp2′s effect on U2 protein remodeling. As the 2′-hydroxyl of the branchpoint is the nucleophile for the first catalytic step of splicing, we assessed susceptibility of the RNA-network to 2′-hydroxyl acylation in B^{act} and B* complexes using SHAPE methodology. Similar to our RNA structure probing results, the global SHAPE modification pattern of the RNA network remained unchanged. However, we observed a dramatically increased reactivity of the 2′-hydroxyl of the branchpoint and of the two nucleotides upstream of the 5′ss. This suggests a substantial increase in the flexibility of these reactants as a direct result of Prp2 action. Taken together, these data suggest that Prp2 repositions the branchpoint helix towards the catalytic center, while at the same time rendering the first step reactants more accessible and thereby preparing for the nucleophilic attack of the branchpoint 2′hydroxl on the 5′ss phosphodiester bond. In summary, our data indicate that Prp2 acts like an RNPase rather than an RNA helicase during catalytic activation. (1) Ohrt et al, RNA, 18:1244 (2012); (2) Warkocki et al, NSMB, 16:1237 (2009).

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368 NMR localization of divalent metal ions in RNA using Mn^{2+}-induced paramagnetic relaxation enhancement and Cd^{2+}-induced chemical-shift perturbation of phosphorothioate RNAs

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Mg^{2+} ions play essential roles in RNA structure and can participate directly in catalysis of certain ribozymes. However, only a few methods are available to precisely localize Mg^{2+} ions in RNA structures. Here, we combined two NMR-based approaches to precisely map divalent metal-ion binding sites in RNA: Mn^{2+}-induced paramagnetic relaxation enhancement (PRE) and Cd^{2+}-induced chemical shift perturbation (CSP) of phosphorothioate RNAs. Mn^{2+}-induced PRE is a common and robust method that relies on the specific line broadening of NMR signals from nuclei that are in proximity (≤ 10 Å) of the paramagnetic metal. It can be used to derive several metal-RNA distance restraints from observable ^{1}H, ^{13}C and ^{15}N signals. However, given the poor dispersion of ^{31}P signals in RNA, it is generally challenging to obtain direct ^{31}P-metal restraints from Mn^{2+}-induced PRE. However, such restraints can be obtained from Cd^{2+}-induced CSP of phosphorothioate RNAs, an approach that relies on the use of chemically synthesized phosphorothioate RNAs containing site-specific sulfur substitution of the nonbonded *pro-*R_p and *pro-*S_p phosphate oxygens. The ^{31}P signal of the phosphorothioate can be readily identified from a simple 1D ^{31}P NMR spectrum. Addition of a thiophilic metal ion, like Cd^{2+}, can induce CSP of the phosphorothioate ^{31}P signal that is informative of its metal coordination mode. This dual NMR approach is demonstrated here for the stem-loop VI domain (SLVI) derived from the *Neurospora* VS ribozyme. Since the NMR structure of this RNA was previously determined by NMR, we added the new set of metal-RNA restraints to existing NOE, dihedral angle and RDC restraints in order to precisely localize divalent metal ion binding sites in SLVI. Interestingly, this study allowed us to identify and characterize several metal-binding sites in RNA, including those associated with a G-U base pair, a GAAA tetraloop, and an S-turn motif. These methods are applicable to a wide variety of RNAs and will increase our insights on the intimate role played by metal ions in RNA structures.

369 RNA 3D Structure in a Nutshell

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The intricate network of interactions observed in RNA three-dimensional structures is often described in terms of a multitude of geometrical properties, including helical parameters, base pairing/stacking, hydrogen bonding and backbone conformation.

We show that a simple one-bead-per-nucleotide representation describing the relative arrangement of nucleobases can account for the fundamental structural properties of RNA. In this framework, canonical Watson-Crick, non-Watson-Crick base-pairing and base-stacking interactions can be unambiguously identified within a well-defined interaction shell.

We validate this representation by performing two independent, complementary tests.

First, we use it to construct a sequence-independent, knowledge-based scoring function for RNA structural prediction, which we show to perform better compared to fully atomistic, state-of-the-art techniques. Second, we define a metric to measure deviation between RNA structures that directly reports on the differences in the base-base interaction network. The effectiveness of this metric is tested with respect to the ability to discriminate between structurally and kinetically distant RNA conformations, comparing favorably to existing techniques.Taken together, our results suggest that this minimalist, nucleobase-centric representation captures the main interactions that are relevant for describing RNA structure and dynamics.

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370 Structural and Thermodynamic Studies of a Remarkably Stable Kissing-Loop Interaction Important for Substrate Recognition by the VS Ribozyme

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Substrate recognition by the *Neurospora* VS ribozyme depends largely on the formation of a magnesium-dependent kissing-loop interaction between stem-loop V (SLV) of the catalytic domain and stem-loop I (SLI) that defines the substrate domain. It has been shown from biochemical studies that the I/V kissing-loop interaction involves three Watson-Crick base pairs and is associated with a structural rearrangement of the SLI substrate from a non-shifted to a shifted conformation. Here, we present a thermodynamic and structural characterization of the VS ribozyme I/V kissing-loop interaction using isolated stem-loop fragments (SLI and SLV). Both isothermal titration calorimetry (ITC) and nuclear magnetic resonance (NMR) spectroscopy studies were conducted with several SLI/SLV complexes using a common SLV, but either shiftable or pre-shifted SLI substrates. From the ITC studies, we show that, under saturating amount of magnesium ions, the affinity of the pre-shifted SLI substrates for SLV is remarkably high, the interaction being more stable than predicted for a comparable duplex. In addition, these ITC studies demonstrate that pre-shifted SLI substrates have higher affinity for SLV than shiftable SLI substrates, and these results allow us to evaluate the energetic cost of the conformational shift in SLI. From the NMR studies, we confirm formation of three Watson-Crick base pairs at the kissing-loop junction and provide direct evidence on the structural rearrangement of shiftable SLI variants in the presence of magnesium and SLV. The NMR structure of a high-affinity SLI/SLV complex demonstrates that both the SLI and SLV loops adopt U-turn structures, which facilitate intermolecular Watson-Crick base pairing. Several other interactions at the I/V interface, including base triples and base stacking help create a continuously stacked structure. These NMR studies provide a structural basis for the high stability of the kissing-loop interaction and lead us to propose a kinetic model for substrate activation by the VS ribozyme.

371 Molecular crowding enhances folding of single ribozyme molecules

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The interior of a cell is crowded with macromolecules, organelles, and cytoskeletal elements that occupy approximately 20-30% of the total cellular volume. This crowding environment results in volume exclusion effects that change the chemical activity of many biological molecules compared to that found in dilute solution. It is therefore crucial to study the influence of macromolecular crowding on the folding of non-coding RNAs as a prerequisite for their efficient cellular function. Taking into consideration Arthur Kornberg's 7th commandment, "Correct for extract dilution with molecular crowding," here we report a single-molecule fluorescence resonance energy transfer (smFRET) study of the two-way junction hairpin ribozyme as a model system in the presence of crowding agent polyethylene glycol (PEG-8000) and/or yeast cell extract to mimic a crowded intracellular environment. We find that PEG stabilizes the more compact, docked conformation of the hairpin ribozyme, consistent with previous ensemble-level studies of other RNAs. We also observe that PEG reduces the concentration of magnesium ions required to stabilize the three- dimensional structure of the hairpin ribozyme. Furthermore, we find the docking kinetics to be heterogeneous in the presence of PEG. To further refine our understanding of the folding dynamics of the hairpin ribozyme in the presence of crowders, we used hierarchical clustering methods that systematically identify individual subpopulations within a larger set of behaviors with finer discrimination than is possible using traditional single molecule kinetic tools. These results provide a wealth of information about the folding dynamics of RNA in a crowded environment as a step towards a deeper understanding of RNA folding inside cells.

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372 Transient RNA structure features are evolutionarily conserved and can be computationally predicted

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State-of-the-art methods in RNA secondary-structure prediction focus on predicting the final, functional structure. However, ample experimental and statistical evidence indicate that structure formation starts immediately during transcription and this **co-transcriptional folding** influences the resultant final RNA structure. Thus, identifying the transient structures that are formed co-transcriptionally may bring insight into understanding how co-transcriptional folding leads to the final conformation *in vivo*. As RNA secondary-structures are currently best predicted by comparative approaches, we therefore investigated whether homologous RNA genes not only assume the same final structure, but also share structural features during the co-transcriptional folding *in vivo*. For this, we compiled a non-redundant data set of 32 transcripts deriving from six different RNA families which constitutes the most comprehensive data set with experimentally confirmed transient and alternative RNA structures so far. We present solid statistical evidence that homologous RNA genes from related organisms fold co-transcriptionally in a similar way (J.Y. Zhu *et al*., Nucleic Acids Res, 2013). In particular, we show that some transient structures are highly conserved with levels similar to those of the final, functional structure. Moreover, we find that the predicted co-transcriptional folding pathways of homologous sequences encounter similar transient structure features, and that these features often coincide with known transient features. We thus also predict candidates for these evolutionarily conserved transient features of co-transcriptional folding pathways *in silico*.

We further expand 4 alignments from the aforementioned dataset using search via covariance model and manual curation in order to share them with the RNA community. These alignments either update the existing Rfam datasets with annotation of transient structures, or introduce new RNA family: (1) Trp operon leader, where alternative structures are coordinated to regulate the operon transcription in response to tryptophan abundance (2) HDV ribozyme, where the self-cleavage activity is modulated via transient structures involving the extended 5′ flanking sequence (3) 5′ UTR of Levivirus maturation protein, where a transient structure temporarily postpones the formation of the final structure that inhibits the translation of maturation protein (4) SAM riboswitch, where the downstream gene expression is regulated by alternative structures upon binding of SAM.

373 Dehydration and cation replacement dramatically improve crystals of large RNAs

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Preparation of well-ordered crystals of large RNAs remains a daunting experimental challenge. This probably reflects the relatively undifferentiated molecular surface of folded RNAs (dominated by a regular array of phosphates), the comparatively low free energy of RNA tertiary structure stabilization, and the resulting tendency of RNAs to be conformationally polydisperse. We have found empirically that dehydration and exchange of counterions can dramatically improve the diffraction properties of some RNA crystals. Two examples from our work are crystals of the glmS ribozyme-riboswitch [1] and of the ternary complex of a T-box riboswitch, its cognate tRNA and an RNA binding protein [2]. Untreated, flash-frozen crystals of the glmS ribozyme diffracted synchrotron X-rays to 3.3 Å resolution. Upon controlled dehydration by soaking into solutions with higher osmolarity, the unit cell contracted by approximately 10%, and diffraction data could be collected that extended to 1.7 Å resolution. Untreated crystals of the T-box ternary complex diffracted X-rays only to 8 Å resolution. A combination of controlled dehydration and exchange of the magnesium an lithium ions needed for crystal growth with strontium (a soft divalent cation), dramatically extended the diffraction limit, allowing the structure to be solved by SAD at 3.2 Å resolution. Because it is a polyanion, RNA is heavily hydrated and surrounded by a diffuse cloud of counterions. It can also site-specifically bind to partially or wholly desolvated metal ions. Hydration and ion binding not only control RNA folding, but also modulate crystallogenesis. Therefore, controlled dehydration and cation exchange are post-crystallization treatments that should be routinely explored for RNA. (This work was supported in part by the Intramural Program of the National Heart, Lung and Blood Institute.) [1] Klein, D.J. & Ferré-D'Amaré, A.R., Science 313:1752 (2006). [2] Zhang, J. & Ferré-D'Amaré, A.R., Nature 500:363 (2013).

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374 Kinetic Dissection and Predictions of an RNA Assembly Process

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To fold to functional structures, RNAs traverse complex free energy landscapes with multiple interconnected pathways. The rates of folding along these pathways determine the time of native state formation, and competition between alternative pathways can lead to different functional or non-functional structures. The ability to predict rate constants for folding pathways is essential for a deep understanding of RNA folding by natural RNAs and the successful design of novel RNAs. We are using a small RNA subdomain within the Tetrahymena group I intron ribozyme, P5abc, and its assembly with the ribozyme core lacking P5abc (EΔP5abc) to test whether the rate of a multi-step folding process can be accurately predicted from knowledge of the component steps. P5abc forms an alternative secondary structure in the absence of Mg2+, and during Mg2+-induced folding the native secondary structure forms in concert with Mg2+ binding and formation of the metal ion core. We generated a series of P5abc mutants that stabilize either the native or the alternative secondary structure to test the simple model that the overall assembly kinetics can be predicted from the equilibrium for P5abc folding in isolation, which is expected to be fast and reversible, and the rate constant for assembly of the natively folded P5abc with EΔP5abc. Thus, we measure the equilibrium for P5abc folding using 15N relaxation dispersion NMR and quantitative footprinting, and we measure assembly of the native P5abc with EΔP5abc by native gel shift analysis using the wild-type P5abc. Together these measured values lead to predicted rate constants for the overall two-step assembly process of the mutants, which are tested by native gel shift. I will present results that provide quantitative tests of this model, establish a regime over which the model holds, and explore the limits of the model and the physical origins of those limits.

375 Probing the dynamics of Ribosome biogenesis in yeast

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Ribosome synthesis in eukaryotes is an incredibly complex process that- besides ribosomal proteins (r-proteins), requires the activity of ~200 ribosome assembly factors. Many of these assembly factors contain enzymatic motifs and are presumed to play crucial roles in remodeling of pre-ribosomes and rRNA folding steps. Although we have a fairly complete picture of the stages at which ribosome assembly factors bind to intermediates, we still lack detailed knowledge of the RNA folding steps that take place and the role of the putative enzymes in this process. Inspired by impressive chemical probing work done by many groups on bacterial ribosomes, we have developed protocols for purification and chemical modification of specific yeast ribosome assembly intermediates. By combining this with high-throughput sequencing we are able to quantitatively measure structural changes and remodeling steps during ribosome synthesis in a single chemical probing reaction. Using this methodology, dubbed ChemModSeq, we discovered that a large number of ribosomal proteins that interact with head domain of the 18S rRNA r- proteins are not in their final conformation. Our results support the notion that many ribosome assembly factors can (also) function as r-protein placeholders. This misplacement of ribosomal proteins correlates with the presence of specific 40S ribosome assembly factors and our results show that the head domain undergoes major remodeling just before the final 18S rRNA cleavage event in the cytoplasm. Our data provide the first nucleotide resolution insights into how assembly factors modulate the assembly of ribosomal proteins and provide a platform for studying the role of NTPases in restructuring/remodeling ribosome assembly.

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376 An Adaptable Pentaloop Defines a Robust Neomycin-B RNA Aptamer with Conditional Ligand-Bound Structures

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Aptamers can be highly specific for their targets, which implies precise molecular recognition between aptamer and target. However, as small polymers, their structures are more subject to environmental conditions than the more constrained longer RNAs such as those that constitute the ribosome. To understand the balance between structural and environmental factors in establishing ligand specificity of aptamers, we examined the RNA aptamer (NEO1A) previously reported as specific for neomycin-B. We show that NEO1A can recognize other aminoglycosides with similar affinities as for neomycin-B and its aminoglycoside specificity is strongly influenced by ionic strength and buffer composition. NMR and 2-aminopurine (2AP) fluorescence studies of the aptamer identified a flexible pentaloop and a stable binding pocket. Consistent with a well-structured binding pocket, docking analysis results correlated with experimental measures of the binding energy for most ligands. Steady state fluorescence studies of 2AP-substituted aptamers confirmed that A16 moves to a more solvent accessible position upon ligand binding while A14 moves to a less solvent accessible position, which is most likely a base stack. Analysis of binding affinities of NEO1A sequence variants showed that the base in position 16 interacts differently with each ligand and the interaction is a function of the buffer constituents. Our results show that the pentaloop provides NEO1A with the ability to adapt to external influences on its structure, with the critical base at position 16 adjusting to incorporate each ligand into a stable pocket by hydrophobic interactions and/or hydrogen bonds depending on the ligand and the ionic environment.

377 The folding of 5′-UTR human G-quadruplexes possessing a long central loop

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G-quadruplexes are widespread four-stranded structures that are adopted by G-rich regions of both DNA or RNA and are involved in essential biological processes such as mRNA translation. They are formed by the stacking of two or more G-quartets that are linked together by three single-stranded loops. Although the maximal loop length is usually fixed to seven nucleotides (nt) in most G-quadruplex predicting software, it has already been demonstrated that artificial DNA G-quadruplexes containing two distal loops that are limited to one nt each and a central loop up to 30 nt long are likely to form *in vitro*. Here, we demonstrates that such structures possessing a long central loop are actually found in the 5′-UTRs of human mRNAs. Firstly, 1453 potential G-quadruplex forming sequences (PG4s) were identified through a bioinformatic survey that searched for sequences respecting the requirement for two single nt long distal loops and a long central loop of 2-90 nt in length. Secondly, *in vitro* in-line probing experiments confirmed and characterized the folding of eight candidates possessing central loops of 10 to 70 nt long. Finally, the biological effect of several G-quadruplexes with a long central loop on mRNA expression was studied *in cellulo* using a luciferase gene reporter assay. Clearly, the actual definition of G-quadruplex forming sequences is too conservative and must be expanded to include the long central loop. This greatly expands the number of expected PG4s in the transcriptome. Consideration of these new candidates might aid in elucidating the potentially important biological implications of the G-quadruplex structure.

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378 Structural analysis of the 5´-UTR of the HIV-1 genome

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The highly structured 5´ UTR of the HIV-1 genome is a ~350-nt RNA that contains motifs critical to viral RNA (vRNA) transcription, reverse transcription, and interactions with viral and cellular factors. The first step of reverse transcription is primer $tRNA^{Lys3}$ annealing to the HIV-1 genome at the primer binding site (PBS), which shares extensive complementarity with $tRNA^{Lys3}$. Subsequently, reverse transcription initiates by NTP addition to the 3´ end of $tRNA^{Lys3}$. Human lysyl-tRNA synthetase, which plays a key role in packaging the tRNA primer, specifically binds to a tRNA anticodon-like element in the HIV-1 genome (Jones et al, (2013) *RNA,* 19:219-29). Using small-angle X-ray scattering (SAXS), the overall shape of the PBS region has been shown to mimic the shape of a tRNA (Jones et al, (2014) *PNAS* 111:3395-400). As SAXS has only provided a low-resolution model for the annealed complex, the details of how the tRNA primer interacts with the PBS region are unknown. The PBS region interacts with $tRNA^{Lys3}$ primarily through an 18-bp duplex formed between the 3´ end of $tRNA^{Lys3}$, but the additional interactions that occur are less clear. These include a 6-bp interaction involving the tRNA variable loop and a 4-bp interaction between the anticodon and an A-rich bulge in the vRNA. To better understand how these additional interactions affect the tRNA/PBS complex, we have examined *in vitro* annealed complexes of HIV-1 PBS RNA (~100 nt) with RNAs of different lengths derived from *in vitro* transcribed $tRNA^{Lys3}$. Using size exclusion chromatography to analyze annealed RNA complexes, we show that RNAs containing all three tRNA/vRNA interactions form complexes more readily than RNAs containing only two of the three interactions. Additionally, we show that extended $tRNA^{Lys3}$ species which mimic the first three nucleotide addition steps of reverse transcription anneal at levels similar to unextended $tRNA^{Lys3}$. Taken together, these results suggest that multiple interactions between $tRNA^{Lys3}$ and the vRNA contribute to the formation of stable annealed complexes. Future work aims to uncover how each interaction between $tRNA^{Lys3}$ and vRNA affect the 3D organization of the initiation complex.

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379 A Novel Interaction Between the 5´ and 3´ ends of the Pea Enation Mosaic Virus (PEMV) Enhances Translation

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We have developed computer tools and protocols that allowed us to model and elucidate the mechanisms of structural control elements within the 3′ UTRs of the Turnip crinkle virus (TCV) and the Pea enation mosaic virus (PEMV). The focus of this presentation is the 3D structure model of the PEMV translation enhancer which links the 5′ end and the 3′ untranslated region (UTR) of its genome via a kissing loop interaction while simultaneously binding ribosomes. Based on the experimental pairing information, we employed our 3D molecular modeling software, RNA2D3D, as well as other 3D structure prediction packages to build models of the PEMV 3′ UTR structural element and of the kissing loop complex it can form with a hairpin partner in the 5′ end of the genome. We employed molecular dynamics (MD) simulations to evaluate the stability of the alternative models and showed that they converge to a T-shaped structure, and this shape is maintained in the kissing-loop complex with the 5′ hairpin. These results strongly suggested that the structure is significant and may play a functional role, and its shape and size similarity to tRNA suggested potential for interactions with ribosomes.

This PEMV structure was named a kissing-loop-T-shaped structure (kl-TSS). Experiments showed that it binds full ribosomes and their subunits, at the same time engaging in a long-distance interaction with the 5′ coding region hairpin. Functionally, the kl-TSS appears to enhance the translation re-initiation. Together with an earlier study on the translation enhancer structure internal to the 3′ UTR of the TCV this work reveals novel, T-shaped structural elements, comparable in size and shape to tRNAs, that bind ribosomes and control the process of translation in plant viruses. Similar mechanisms may exist in other viruses and genomes, as the structural elements similar to the tRNA-like structure in the TCV have also been identified in several viruses.

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380 A two-faced RNA: the crystal structure of a plant virus' tRNA-like sequence reveals the basis for mimicry, structural plasticity, and multifunctionality

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RNA is remarkably functionally diverse. In some cases, a single RNA sequence can perform multiple roles; this multifunctional ability can be conferred by a complex three-dimensional structure. This multifunctionality can also be driven or enhanced by the ability of a given RNA to assume more than one conformational state. This phenomenon is likely biologically important, but a detailed structural understanding of the paradigm of RNA structure-driven multifunctionality is lacking. Model RNAs that can provide insight are the tRNA-like structures (TLS) found at the 3′ end of some single-stranded positive-sense RNA plant viruses. A prototype TLS is the one found in the Turnip Yellow Mosaic Virus (TYMV). This TLS RNA not only acts akin to a tRNA to drive aminoacylation of the viral genomic RNA (gRNA), but also interacts with other structures in the gRNA's 3′ untranslated region, contains the promoter for negative strand synthesis, and influences several infection-critical processes. These features suggest that the structure of the TYMV TLS RNA could provide a glimpse into the structural basis of RNA multifunctionality and plasticity. However, for decades this RNA's high-resolution structure has remained elusive. Here, we present the crystal structure of the complete TYMV TLS to 2.0 Å resolution, similar to the highest resolution structures of authentic tRNA in the unbound state (1.93 Å). Globally, the TLS RNA adopts a shape that mimics tRNA, but it uses a very different set of intramolecular interactions to achieve this shape, to include radical differences in the D-loop and Variable loop. These divergent intramolecular interactions also suggest how the TLS can readily switch between a conformation that favors aminoacylation and a conformation that is necessary for replication. In addition, the TLS structure is 'two-faced': one 'face' closely mimics tRNA and drives aminoacylation, the other 'face' diverges from tRNA and enables additional functionality. The TLS is thus structured to perform several functions and interact with diverse binding partners, and we demonstrate its ability to specifically bind to ribosomes.

381 Are waters around RNA more than just a solvent? - An insight from molecular dynamics simulations

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Hydrating water molecules are believed to be an inherent part of the RNA structure and have a considerable impact on RNA conformation. However, the magnitude and mechanism of the interplay between water molecules and the RNA structure are still poorly understood. In principle, such hydration effects can be studied by molecular dynamics (MD) simulations. In our recent MD studies,(1, 2) we observed that the choice of water model has a visible impact on the predicted structure and structural dynamics of RNA, and in particular, has a larger effect than type, parameterization and concentration of the ions. Furthermore, the water model effect is sequence dependent and modulates the sequence dependence of A-RNA helical parameters. Clearly, the sensitivity of A-RNA structural dynamics to the water model parametrization is a rather spurious effect that complicates MD studies of RNA molecules. These results nevertheless suggest that the sequence dependence of the A-RNA structure, usually attributed to base stacking, might be driven by the structural dynamics of specific hydration. Here, we present a systematic MD study that aimed to (i) clarify the atomistic mechanism of the water model sensitivity, and (ii) discover whether and to what extent specific hydration modulates the A-RNA structural variability. We carried out an extended set of MD simulations of canonical A-RNA duplexes with TIP3P,(3) TIP4P/2005,(4) TIP5P(5)and SPC/E(6) explicit water models and found that different water models provided a different extent of water bridging between 2′-OH groups across the minor groove, which in turn influences their distance and consequently also inclination, roll and slide parameters. Minor groove hydration is also responsible for the sequence dependence of these helical parameters.

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382 High-throughput probing of human lncRNA secondary structure by Mod-seq

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Long noncoding RNAs (lncRNAs) comprise a large proportion of the human transcriptome. Over the past decade, lncRNAs have been increasingly recognized as important regulators of multiple gene expression processes, and a growing number have been associated with human development and diseases. Because they don't encode proteins, lncRNA structures and their interactions with proteins are likely to be crucial for their regulatory functions. However, their massive size (often larger than the ribosome) has made structural study of lncRNA a daunting task. To experimentally determine the secondary structures of lncRNAs, we developed a chemical modification based high throughput probing method, Mod-seq. Compared to traditional RNA secondary structure determination methods, Mod-seq provides substantial improvements in throughput. To evaluate the performance of the Mod-seq method, we compared Mod-seq probing results with *Saccharomyces cerevisiae* rRNAs whose crystal structures and base-pairing information are known. We found that nucleotide positions with higher Mod-seq scores are more likely to be in single-stranded state in both DMS-based and SHAPE-based RNA secondary structure probing experiments, suggesting that Mod-seq is a robust RNA secondary structure probing method. In addition, we used Mod-seq to probe the individual human lncRNAs MALAT1 and snolncRNA-2 *in vitro*. We will present the resulting experimentally driven lncRNA secondary structure predictions.

383 Structure and dynamics of the HIV-1 frameshift element RNA

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The human immunodeficiency virus type 1 (HIV-1) ribosomal frameshift element is a highly structured motif, regulates translation of all virally encoded enzymes, and is a promising therapeutic target. The original model for this motif contains two helices separated by a three-nucleotide bulge. Modifications to this model have been suggested by SHAPE chemical probing of an entire HIV-1 genome. Important additions in the SHAPE-directed model include alternate helical conformations and a larger, more complex domain that includes basepairings outside the conventional domain. These additional pairings support the presence of a secondary frameshift site within the expanded frameshift domain model. In the present work, we use high-affinity locked nucleic acid (LNA) oligonucleotides to examine these two models. We also examined the stability of helices in the frameshift domain by probing in the presence of formamide and inside authentic virions. Our data are consistent with a model in which the frameshift domain is anchored by a very stable helix outside the conventional frameshift domain. Less stable helices within the domain have the propensity to switch from the SHAPE-predicted to the conventional conformation. Translational frameshifting assays with native mutant sequences reveal a functional role for a proposed helical conformation and support the putative secondary frameshift site, both specific to the SHAPE-directed frameshift domain model. These results suggest that the HIV-1 frameshift domain is a complex, dynamic structure and underscore the importance of analyzing folding in the context of full-length RNAs. This work has been supported by National Institutes of Health grant AI068462 (KMW), National Research Service Award F30DA027364 (JTL), Medical Scientist Training Program T32GM008719 (JTL), and Contract No. HHSN261200800001E with Leidos Biomedical Research, Inc. (RG)

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384 Measuring the Evolution of the Small Subunit of the Ribosome

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A large majority of RNA never gets translated to protein yet the standard ways to measure natural selection are aimed at those RNA that do code for a protein. The standard method of measuring natural selection entails taking the ratio between the rates of non-synonymous and synonymous substitutions. That method is not appropriate for non-coding RNA in which every substitution, insertion or deletion can disrupt the ensemble of structures and their associated function. To address this issue, we present a method for measuring the evolution of a non-coding RNA in relation to a more basal sequence. This method is able to identify when a set of sequence changes are the result of random mutation or selection. When applied to 72 ribosomal small subunit sequences from *Escherichia coli*, and in relation to the accepted consensus sequence, a general pattern of evolution emerges; a single or a few mutations increases the diversity of structures which is then followed by selection, which subsequently reduces this diversity.

385 Biochemical investigation of RNA thermosensors

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Bacterial systems use a variety of mechanisms in order to respond to temperature change. One such mechanism is translational regulation through restriction of ribosomal access to particular mRNAs. This effect can be mediated by RNA thermosensors, RNA elements that form temperature-sensitive structures regulating the expression of the mRNA of which they are a part. These elements are most commonly found in the 5′-untranslated region (UTR) of bacterial genes involved in the heat shock response, cold shock response, and pathogenic virulence. We are studying RNA thermosensors involved in the heat shock response. Under standard conditions, these thermosensors adopt a structured conformation that prevents ribosomal access to the Shine-Dalgarno sequence and/or start codon of the mRNA. Melting of the secondary structure under elevated temperature allows ribosomal binding and elicits protein translation. Our initial efforts have focused on a short thermosensor found in the 5′UTR of the *Salmonella enterica htrA* gene. We have explored the use of SHAPE (Selective 2′-Hydroxyl Acylation analyzed by Primer Extension), followed by capillary electrophoresis, to investigate this thermosensor. Our ultimate goal is to further characterize the molecular determinants that control the temperature-dependent behavior of additional natural thermosensors involved in the heat shock response.

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386 Alternate base pairing and conformational changes observed in loop A of the hairpin ribozyme

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The catalytic cycle of the hairpin ribozyme begins with the rate-limiting ‘docking’ between loops. The kinetic rates of docking previously reported by ourselves and others indicate that conformational sampling may be a factor that limits the docking transition rates. To assess conformational sampling in the hairpin ribozyme, we have run several explicit solvent Molecular Dynamics (MD) simulations of hairpin ribozyme loop A domain totaling 2.4 μs. We observed two dominant conformers and multiple minor states differing in hydrogen bonding and base stacking in the loop region. Targeted Molecular Dynamics (TMD) was used to model the pathway between the major and minor conformations using CHARMM36/NAMD. The Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) approximation was applied to predict conformational energy differences between the two observed conformations. MMPBSA correctly predicted the major conformations to be lower in free energy than the minor conformations. The barrier height between the major and minor conformations was reasonable based on the free energy difference between the starting and target conformations consistent with the explicit simulation results. These observations underscore the rugged energy landscape with multiple accessible shallow energy minima for loop A of the hairpin ribozyme, consistent with a possible role for conformational sampling in the observed slow rates of docking.

387 The ydaO riboswitch forms two symmetry-related pockets for targeting c-di-AMP second messenger

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The *ydaO* riboswitch, involved in sporulation, osmotic stress responses and cell wall metabolism, has been shown to target the second messenger c-di-AMP with subnanomolar affinity. We have solved the 2.73 Å structure of c-di-AMP bound to the *Thermoanaerobacter tengcongenesis ydaO* riboswitch, thereby identifying a five-helical scaffold containing a partially zippered-up bubble, a pseudoknot and long-range tertiary base pairs. Highlights include the unanticipated identification of two c-di-AMP binding pockets per riboswitch, related by pseudo two-fold symmetry, both within and between pockets. The bound adenine rings of both bound c-di-AMP molecules are wedged between bases of the riboswitch, and stabilized by stacking, base-sugar and sugar-sugar intermolecular hydrogen bonding interactions, thereby explaining the inability of the *ydaO* riboswitch to bind c-di-GMP and c-GAMP. The structural studies are complemented by ITC-based binding studies as a function of mutants mediating key tertiary intermolecular contacts. The *T. tengcongenesis ydaO* riboswitch, like its *C. elegans* counterpart, appears to function through a transcription termination mechanism, with the c-di-AMP bound state representing an 'off' switch.

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388 Interaction between an octameric RNA structure and different divalent and trivalent metal ions as revealed by x-ray crystallography

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The principle of charge neutralization and electrostatic condensation require cations to overwhelm the repulsive forces of the negatively charged backbone of RNA to adopt its three-dimensional structure [1, 2]. A precise structural knowledge of RNA-metal ion interaction is crucial to understand the role of metal ions in the catalytic or regulatory activity of RNA [1, 3]. In our study we use an octameric RNA duplex as a model system to investigate the coordination of various metal ions to specific binding sites and to understand the interactions between metal ions and RNA.

New technical approaches of the synchrotron radiation facility at the Swiss Light Source (SLS) were applied to obtain high-resolution data that is required for a detailed structural analysis. We were able to solve the crystal structure of the octameric RNA duplex in presence of six different di- and trivalent metal ions such as calcium, cobalt, copper, manganese, strontium and terbium. Hence, the study extends the knowledge of the influence of metal ions for conformational changes in RNA structure

The results reveal the strong influence of cations for a more compact RNA structure, although the kind of metal ions employed has structurally no particular influence. We considered different parameters to carefully assign the positions of the metal ions and suggest two prevalent positions in the investigated octameric RNA structures. One is located at the phosphate backbone; the second cation is in the centre of the RNA, interacting by a particular innersphere binding to O4 of uracil in presence of calcium, cobalt and copper. Further we could describe for the first time a RNA structure associated with copper.

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389 Structure prediction benchmarking's not dead

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We can assume that sequence alone does not contain enough information to find the correct RNA structure every time. Comparative analysis and the analysis of suboptimal predictions is the way to go if we want to find a new RNA structure in practice.

That's why we refined benchmarking for RNA structure prediction. Since we cannot assume to always find the correct structure, we don't ask a yes-or-no question, but rather look at the number of correctly folded structures for each program.

Since many tools explore a large part of the folding space of the molecule, we need strategies to measure the performance of the tools on all these alternative results, focusing on strategies to circumvent the "sequence only" input approach. These strategies use base pairing masks to guide the structure prediction algorithm.

We propose the following:

1) sequence only, that means we fold and check the Matthews Correlation Coefficient, as it is current practice for benchmarking RNA structure prediction.

2) extract statistics from the folding results, such as a number of suboptimals, components under application of an energy barrier, selecting reocurring basepairs - and refeed them into the folding process as a mask for the structure prediction program to "zoom in" on a specific feature.

3) supply fractions of known crystallography or NMR data as a folding mask, to benchmark prediction with additional information (e.g. the canonical basepairs from a crystal, 50% of it, etc).

4) if information (RMDetect) suggests a motif which has structural features that are not scored in the prediction model (e.g. base triples), we can transform this knowledge into a mask to boost the motif.

In this way, we predict structural data tailored to the external information we have about the molecule. The structure prediction process becomes user centric, since each strategy has a different application domain and external information.

With the right strategy it is possible to predict structures with noncanonical basepairs, which gives a more complete image of the structure, and makes sure that less folding paths are missed when we view the predicted structures as a dynamic process.

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390 RNA structure analysis by SHAPE-MaP

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RNA structure plays a functional role in many biological processes. However, RNA structure modeling remains challenging, particularly for large RNAs. Incorporation of chemical probing data from selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) significantly improves RNA secondary structure modeling accuracies. In this work, we develop a concise approach for gathering SHAPE chemical probing data using massively parallel sequencing. We analyze both small dynamic RNAs, including the thiamine pyrophosphate riboswitch, and large RNAs including bacterial ribosomal RNAs and an entire HIV-1 RNA genome. This approach, termed SHAPE mutational profiling (SHAPE-MaP), takes advantage of the observation that reverse transcriptase will incorporate a mutation or deletion in the sequence of the nascent cDNA when encountering a SHAPE adduct. Coupled with fully automated data analysis and RNA folding algorithms, we were able to produce structure models that reproduced ≥ 90% of accepted base pairs for RNAs with known secondary structures. SHAPE-MaP analysis produced a structure model that fully recapitulated previously identified structures in the HIV-1 genome and identified previously unknown structures, including three pseudoknots. All three pseudoknots impacted viral fitness or RNA structure upon mutation, with a particularly strong effect observed upon disruption of a pseudoknot in the 3′ untranslated region of HIV-1, immediately adjacent to the polyadenylation signal. These applications demonstrate that SHAPE-MaP can interrogate local and large-scale RNA structures both in small RNA transcripts and in large complex RNAs at nucleotide resolution. SHAPE-MaP is independent of sequencing platform and library construction scheme, yields accurate and high-resolution secondary structure models, detects scarce and low abundance RNAs, allows the structural consequences of sequence polymorphisms to be disentangled in single experiments, and will ultimately democratize RNA structure analysis.

391 Calculation of loop probabilities using an RNA partition function

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RNA secondary structure prediction is widely used to analyze RNA sequences. In an RNA partition function calculation, free energy nearest neighbor parameters are used in a dynamic programming algorithm to estimate statistical properties of the secondary structure ensemble. Previously, partition functions have largely been used to estimate the probability that a given pair of nucleotides form a base pair, the stacking probability of two pairs, or the accessibility to binding of continuous nucleotides. Here, we show that an RNA partition function can also be used to analytically calculate the probability of hairpin loops, internal loops, bulge, and multibranch loops at a given position. Benchmarking on a large number of RNA sequences with known secondary structures indicated that loops that were calculated to be more probable were more likely to be present in the known structure. This calculation is important for drug discovery because small molecules are known to bind secondary structure motifs that could be found in RNAs with this method. [1]

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392 The role of a non-coding SNP in COPD predisposition

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Disease-associated SNPs (single nucleotide polymorphisms) that map to non-coding regions could affect post-transcriptional regulatory mechanisms by changing RNA structure. A recent genome-wide association study identified a SNP in the 5′ UTR of SERPINA1 as being associated with an increased risk of developing Chronic Obstructive Pulmonary Disease (COPD) among smokers. The SERPINA1 gene is particularly interesting in that the full diversity of mature transcripts originate almost entirely from the 5′UTR region through a combination of 2 transcription start sites and alternative splicing. Though the 5′ UTR is a potential site of regulation, its RNA structure is completely uncharacterized. Computational predictions using SNPfold suggest that the disease-associated SNP is likely to significantly impact the structural ensemble of this 5′ UTR. Here, we use SHAPE to investigate how alternative splicing and SNPs alter the conformation of the 5′ UTR of SERPINA1. Additionally, we use luciferase assays to determine how the different versions of the UTR impact translation. Surprisingly, neither the SNP nor the different splicing events significantly change the RNA structure. The RNA structure is extremely robust even very close to alternatively spliced junctions. Together these experiments will help us to understand how non-coding SNPs can contribute to disease mechanisms.

393 Structural Studies of the 5′-Untranslated Region of the HIV-1 Genomic RNA

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During virus assembly, all retroviruses, including HIV, specifically encapsidate two copies of full-length viral genomic RNA in the form of a non-covalently linked RNA dimer. A number of studies suggest that dimerization is mediated through a highly conserved 35-nucleotide RNA stem-loop, the dimerization initiation site (DIS), in the 5′-untranslated region (UTR) of the genomic RNA via an intermolecular kissing interaction between two DIS loops. The structures formed by the 5′-UTR RNA and the *in vivo* mechanism by which genome dimerization occurs are still not well understood. We will present results from measurements using fluorescence, nuclear magnetic resonance (NMR), and small-angle X-ray and neutron scattering (SAXS and SANS), which are being used to analyze the structure of the 5′-UTR of the HIV-1 genomic RNA and guide the building of structural models. Small-angle scattering data are being evaluated using SASSIE, a suite of computational methods for biological small-angle scattering currently under development at the NIST Center for Neutron Research (NCNR). SASSIE employs Monte Carlo-based configuration sampling over the six main-chain torsion angles to calculate an ensemble of flexible RNA structures that can then be filtered against experimentally-derived constraints. We will present data for the 5′-UTR as both a monomer and a dimer as well as for sub-domains of the 5′-UTR, and also in the absence and presence of the HIV-1 nucleocapsid protein, NCp7, which has been implicated as an RNA chaperone in the genome dimerization process. Knowledge of these structures could potentially guide the development of novel therapeutics that target HIV-1 genomic RNA before packaging and/or upon introduction to the cell after infection, and may lead to new treatments for other retroviruses as well.

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394 *RNA structural elements and protein interactionsthat regulate HIV genome splicing*

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Alternative splicing is a key event of the HIV replication cycle; however, little is known about the RNA structures and protein interactions that regulate splice site selection. Acceptor site A7 is one of the better-characterized sites, where its activity along with donor site D4 is required to remove the Rev Responsive Element and produce multiply spliced transcripts encoding *tat, rev,* and *nef*. The activity of A7 is suppressed by an intronic splicing silencer (ISS), a bipartite exonic splicing silencer (ESS3a/b), and activated by an exonic splicing enhancer (ESE3). The hnRNP A1 protein binds the silencer elements to effectively block ASF-ESE3 interaction, thereby inhibiting A7 usage. Available splicing models assert hnRNP A1 disrupts RNA secondary structure through 3′-5′ cooperative assembly; however, footprinting studies show discrete protection patterns of the RNA structure within the hnRNP A1-ssA7 complex. To gain insight into the molecular mechanisms that regulate ssA7, we have investigated the structural, biophysical, and biochemical basis of hnRNP A1 recognition of the ISS and ESS3 elements. Here, we present solution NMR structures of the isolated ISS stem loop (55-nt) and ESS3 stem loop (27-nt). We've also used calorimetric and NMR titrations to determine binding surfaces and epitopes. Lastly, we present a structural model of the UP1-ESS3 complex that is derived from NMR-STD, mutagenesis, and crystallographic studies of UP1 bound to a short RNA oligo. The structural model offers insight into how hnRNP A1 binds RNA stem loop structures, and suggest a unique binding mechanism for this biologically important complex.

395 Validating the fragment-based drug discovery strategy for targeting biological RNAs: Lead fragments specifically bind and remodel the TPP riboswitch

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Microbial resistance to the current clinical arsenal of antibiotics is emerging at an alarming rate, and multidrug-resistant infections are a major world health concern. Novel classes of antibiotics that target cellular processes distinct from the current repertoire of antibiotics offer a promising route for drug development. One attractive group of cellular targets for the development of novel antibiotics are riboswitches, which are structured regions of mRNA that modulate gene expression in response to the intracellular concentration of a variety of small molecules. Thiamine pyrophosphate (TPP) riboswitches regulate essential genes in pathogenic bacteria by changing conformation upon binding intracellular TPP. Fragment-based approaches, in which weakly-binding small molecule "fragments" (Mr < 300 Da) are identified in modestly-sized screens and then elaborated into more potent ligands, have emerged as a promising method for drug discovery[1]. Although extensively used against protein targets, only recently has this approach been applied to RNA, leading to the discovery of fragments that bind a TPP riboswitch[2]. Crystallographic studies now show that, despite having micromolar K_ds, four different fragments bind the TPP riboswitch site-specifically, occupying the pocket that recognizes the aminopyrimidine of TPP[3]. Unexpectedly, as visualized in the fragment co-crystals, the unoccupied site that would recognize the pyrophosphate of TPP rearranges into a structure distinct from that of the cognate complex. This idiosyncratic fragment-induced conformation, also characterized by small-angle X-ray scattering (SAXS) and chemical probing (SHAPE), represents a possible mechanism for adventitious ligand discrimination by the riboswitch, and suggests that off-pathway conformations of RNAs can be targeted for drug development. Our structures, together with previous screening studies, demonstrate the feasibility of fragment-based drug discovery against RNA targets.

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396 Characterization of Synthetic Regulatory RNA Structures and Interactions in *E. coli* with SHAPE-Seq

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As the field of Synthetic Biology grows, researchers are increasingly turning to synthetic RNAs for controlling gene expression across a variety of applications. However, creating these synthetic regulators is challenging, and is often done through a laborious process of testing incremental changes to the RNA's sequence based on empirical or computational assumptions about how the RNA fold in the cell. In many cases these mutants are not functional for reasons that are hypothesized to be due to mis-folding in the cellular environment. To remedy this, we demonstrate how Selective 2′-Hydroxyl Acylation analyzed by Primer extension Sequencing (SHAPE-Seq) can be used to characterize RNA secondary structures of synthetic RNA regulators in *E. coli* cells. We also show how SHAPE-Seq's leverage of next-generation sequencing allows for simultaneous measurement of multiple RNA structures within the same cell, which we show can unveil nucleotide-resolution models of RNA-RNA interactions within the cellular environment for both transcriptional and translational regulators. The extra level of information obtained with SHAPE-Seq can then be used to guide RNA circuit design and provide a more complete picture of the mechanism of action of RNA regulators.

397 The role of RNA structure in the alternative splicing regulation of the apoptotic factor Bcl-X

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Apoptosis (or Programmed Cell Death, PCD) is a cellular mechanism that enables the systematic execution of harmful or superfluous cells during development. The Bcl-2 family of proteins majorly regulates the intrinsic pathway, mediated through the mitochondria. Of interest is one of the pro-survival proteins, Bcl-X_L.

Bcl-X_L is an alternatively spliced isoform of the BCL-X (or BCL2L1) gene. *Bcl-X* pre-mRNA is relatively simple, consisting of three exons and two introns. Exon 2 harbours two alternative 5′ splice sites, which produce the two main transcripts: *Bcl-*X_S is the short, pro-apoptotic isoform and *Bcl-*X_L is the long, pro-survival isoform. Bcl-X_S is expressed at high levels *in vivo* in cells that experience a high rate of turnover, such as developing lymphocytes, whilst Bcl-X_L is expressed in higher levels in post-mitotic cells and cancer cells.

The *Bcl-X* pre-mRNA is affected by many splicing factors. Certain regions of *Bcl-X* bind various proteins known to regulate splicing, such as hnRNP A1, H/F and SR proteins. hnRNP F is of particular interest as this protein binds directly to G tracts in the region between the two alternative 5′ splice sites. It has been suggested that the role of hnRNP F in splicing is to retain these G tracts in a single-stranded conformation, preventing secondary structures from forming, including G-quadruplexes.

To determine the overall native structure of *Bcl-X*, RNA footprinting was performed to obtain a 2D secondary structure of the pre-mRNA. *In vitro* splicing assays reveals that GQC-05, a known G-quadruplex stabilizer of DNA, is able to simultaneously decrease the X_L isoform while also increasing the X_S isoform, increasing the X_S/X_L ratio significantly. When doing RNA Footprinting with a titration of this molecule, structural changes could be observed, suggesting that its effect is from directly binding to the RNA. In addition, when comparing deaza-GTP transcript to a normal rGTP transcript, further structural changes could be observed. Such changes can be localized to the 5′ splice sites. All these observations give us further insight into the role of structure on 5′ splice site selection in *Bcl-X*.

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398 Automated Design of RNA 3D Structure

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RNA engineering holds promise for the development of customized, in vivo logic circuits, sensors, and machines, but advances to date have depended on expert intuition, time-consuming selection methods, or trial-and-error refinement. Here we present an automated toolkit for RNA 3D design by integrating recent algorithms for high-resolution RNA structure prediction, a wealth of knowledge from the RNA crystallography and structural bioinformatics communities, and insights drawn from recent protein engineering advances. Our strategy is to assemble RNA 3D motifs, which are well-defined geometric arrangements of interacting nucleotides, to build custom *de novo* RNAs. These motifs are modular and retain their 3D structure independently of their original RNA scaffold. To create motif libraries for the RNA Element DESIGN (REDESIGN) toolkit, we extensively characterized all high-resolution crystallographic structures to isolate bulges, hairpins, junctions and tertiary contact motifs. Each motif is characterized by the displacement and rotation between its base pair edges. Motifs can then be assembled in a near infinite number of combinations by aligning the base pair edges of two or more motifs together. These assembled *de novo* RNA segments can then be inserted into RNA structures of interest to rigidify specific areas, add flexibility, connect two different RNAs together, or to add new tertiary contacts as well as a suite of other functions. To demonstrate the wide applicability of our REDESIGN toolkit (https://github.com/jyesselm/REDESIGN), we focus on two paradigmatic RNA design challenges: thermostabilizing RNA 3D structure and designing an RNA aptamer to bind a target. Using REDESIGN, we report novel rationally-designed 3D solutions for both challenges with structural validation from SHAPE chemical mapping experiments. The REDESIGN toolkit will help to elucidate principles for RNA design, solidifying the critical foundation required for rational engineering of RNA.

399 Abstract Withdrawn

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401 Unexpected common developmental programs in *D. melanogaster* and *C. elegans* revealed by RNA-Seq of developmental timecourses

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Drosophila melanogaster and *Caenorhabditis elegans* are two well-studied model organisms in developmental biology. Their morphological development differ greatly, yet we postulated that there may nonetheless be underlying shared developmental programs employing orthologous genes. The availability of modENCODE RNA-Seq data for developmental stages of the two organisms enabled a transcriptome-wide comparison study to address this question. We undertook a comparison of their developmental time courses, seeking to identify commonalities in orthologous gene expression. For every stage in each organism, we select "stage-associated genes" which are defined as relatively highly expressed at that stage compared with others. To test the the commonality of *D. melanogaster* and *C. elegans* stages in terms of orthologous gene expression, we used an overlap statistic - the number of orthologous gene pairs associated with both stages - and computed a statistical significance.

We first carried out the statistical test on pairs of intra-specific stages within *D. melanogaster* and within *C. elegans*, and we found that temporally adjacent stages in both species exhibit high overlap in gene expression, supporting the validity of this approach. Additionally, fly female adults and worm adults are mapped with fly and worm embryos, respectively, due to maternal gene expression.

We then compared orthologous gene expression between the species. We found an unexpected strong collinearity in the timecourse from early embryos to late larvae in both worm and fly - showing that these two very different species express significant numbers of orthologous genes in parallel developmental programs. Moreover, a second parallel pattern was found between fly prepupae through adults and worm late embryos through adults, consistent with the second large wave of cell proliferation and differentiation in the fly life cycle. The results also indicate that that the developmental program in worm has been partially duplicated in fly, in which one set of genes is expressed in embryo through larvae, and a partial duplicate of this set of genes expressed in later development. Our results constitute the first comprehensive comparison between *D. melanogaster* and *C. elegans* developmental time courses, and provide new insights into similarities in the development of these two species.

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402 Dissecting the expression landscape of RNA-binding proteins in human cancers

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RNA-binding proteins (RBPs) play important roles in cellular homeostasis by controlling gene expression at the post-transcriptional level forming ribonucleoprotein (RNP) complexes. In the present study, we explore the expression of more than 800 RBPs in sixteen healthy human tissues and their patterns of dysregulation in cancer genomes from The Cancer Genome Atlas project .We show that genes encoding RBPs are consistently and significantly highly expressed compared with other classes of genes, including those encoding regulatory components such as transcription factors, miRNAs and long non-coding RNAs. We also demonstrate that a set of RBPs, numbering approximately 30, are strongly upregulated (SUR) across at least two-thirds of the nine cancers profiled in this study. Analysis of the protein-protein interaction network properties for the SUR and non-SUR groups of RBPs suggests that path length distributions between SUR RBPs is significantly lower than those observed for non-SUR RBPs. We further find that the mean path lengths between SUR RBPs increases in proportion to their contribution to prognostic impact. We also note that RBPs exhibiting higher variability in the extent of dysregulation across breast cancer patients have a higher number of protein-protein interactions. We propose that fluctuating RBP levels might result in an increase in non-specific protein interactions, potentially leading to changes in the functional consequences of RBP binding. Finally, we show that the expression variation of a gene within a patient group is inversely correlated with prognostic impact. Overall, our results provide a roadmap for understanding the impact of RBPs on cancer pathogenesis.

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403 Identification of structural requirements for VapBC toxin-antitoxin interactions

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Type II Toxin-antitoxin (TA) systems are ubiquitous in prokaryotes and have several biological functions including the control of dormancy, formation of persisters and programmed cell death. TA systems typically form a two-gene operon. This operon encodes a protein toxin that suppresses metabolic pathways within the bacteria, and another protein antitoxin that counteracts the function of the toxin. The antitoxin inhibits the activity of its cognate toxin by directly interacting with it to form a protein complex. Type II toxin-antitoxin systems are classified into nine families, the largest being the VapBC family, which is defined by the presence of a putative PIN endoribonuclease domain found in prokaryotes and eukaryotes. The antitoxin proteins appear to consist of two distinct motifs; a DNA-binding motif in the N-terminal region that functions as a transcriptional repressor for the autoregulation of the TA operon, and an antitoxin motif in the C-terminal region that binds to and inactivates the toxin. A major question is how the specificity of TA interaction is determined such that there is no cross talk between VapBC pairs in organisms with multiple copies of related TA operons. The antitoxin VapB4 from *M. tuberculosis* is a small protein that is 85 amino acid residues in length. Our results show that 31 amino acid residues in the C-terminal region of VapB4 are necessary and sufficient to neutralize the toxicity of its cognate toxin VapC4. We used a genetic screen to isolate random mutations in this 31 amino acid region that disrupt its ability to neutralize VapC4. The mutations, at 14 different positions, cause loss of the antitoxin activity in vivo. Surprisingly, our findings indicate that the same mutations in context of full-length VapB4 do not disrupt its antitoxin activity in vivo, suggesting that the amino acid residues in N-terminal two-thirds of full length VapB4 may contribute to the stability of VapBC toxin-antitoxin interactions.

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404 An RNA-based, Generalizable Synthetic Genetic System for Dynamic Regulation

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Biological systems adjust endogenous metabolite levels against environmental perturbations through their embedded dynamic regulatory mechanisms. These native control strategies typically involve sensing intracellular metabolites, subsequent genetic computations, and feedback loops to regulate the level of metabolites. Such dynamic control schemes have also been widely used by synthetic biologists in building synthetic genetic systems to control metabolite production over external uncertainties. One major challenge in implementing such genetic systems is the lack of a generalizable system architecture to facilitate the design process through matching the activity levels between different genetic components. To address this challenge, we designed an RNA-based synthetic regulator, exploiting the high flexibility in ligand sensing and tunability in regulating gene expression of RNA. We selected theophylline as the metabolite of interest to implement a proof-of-concept regulator in engineered yeast hosts, and established several design considerations for implementing such RNA-based regulators. We first prototyped an open-loop regulator, which consists of a theophylline-responsive RNA switch (sensor), a synthetic transcriptional device (amplifier), and a GFP reporter (actuator). The synthetic transcription device is designed to amplifier RNA signal generated by the sensor and then activate the downstream reporter. We characterized the open-loop regulator across a range of theophylline concentrations and achieved a maximum dynamic range of 70-fold under optimized conditions. We subsequently implemented the closed-loop form of the regulator by replacing the GFP reporter with an engineered demethylase enzyme that converts caffeine precursor to theophylline product. We tested the closed-loop regulator under different fed precursor levels and measured the product accumulation over time. The positive feedback activity was established from the synthetic regulator within the first 20 hours of growth, and approximately 200 mM of theophylline was produced over the course of the experiment. In contrast, the production level was approximately 100-fold lower in the absence of the synthetic regulator. Our work demonstrates a generalizable strategy for implementing RNA-based genetic regulators that are capable of sensing metabolites, computing proper genetic response and establishing a 100-fold change in production. This RNA-based architecture provides a plug-and-play feature, which minimizes re-design efforts when applying this strategy to other metabolite-enzyme pairs and other regulatory schemes.

405 Intron retention as a prevalent gene regulatory mechanism in T cells

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T cell activation is a well-established model for cellular response to exogenous stimulation. Using stand-specific RNA-seq to profile the transcriptome of resting and activated T cells, we observed an unexpected high level of introns retained in polyadenylated transcripts. Intron retention is prevalent in resting T cells and is significantly reduced upon cell T activation. Further Integration of Pol II and H3K36Me3 ChIP-seq data demonstrated that intron-retained transcripts are less stable than properly spliced transcripts. Strikingly, the decreases in intron retention level correlate with the increases in steady-state mRNA level, suggesting that intron retention may be broadly involved in gene regulation. Supporting this notion, majority of the genes upregulated in activated T cells are accompanied with a significant reduction in intron retention. Of them, 185 genes are likely to be predominantly regulated at the posttranscriptional level, and are highly enriched in the proteasome pathway that is essential for proper T cell proliferation and cytokine release. Taken together, our study uncovered a novel posttranscriptional mechanism mediated by global intron retention. We speculate that it may bypass the requirement for de novo transcript synthesis and thus shorten the responding time to extracellular stimuli such as acute infection.

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406 Defining the role and the mechanism of RNA localization to the mitotic apparatus

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The subcellular localization of RNA molecules is an important mechanism for modulating the spatio-temporal dynamics of gene expression, which plays key roles in many fundamental cell biological and developmental processes. Although this process was first thought to be limited to few specialized transcripts, recent genome wide studies suggest that it is actually a highly prevalent regulatory mechanism in organisms ranging from bacteria to humans. Among the 70% of mRNAs estimated as localized in *Drosophila* embryos, a set of ~30 transcripts are targeted to structures of the mitotic apparatus (mitotic spindles, astral microtubules, centrosomes). Many of these transcripts encode key regulators of mitosis and emerging studies suggest that their targeting to the mitotic apparatus is functionally important for the coordination of cell division. Like other post-transcriptional steps, mRNA trafficking depends on interactions between *Cis*-regulatory elements residing within the RNA molecule and *Trans*-acting RNA binding proteins (RBPs). However, the mechanism responsible for targeting mitotic mRNAs and the precise functional roles of this localization during mitosis remains poorly understood. Thus, we aim to identify *Cis* and *Trans* regulators that specifically drive mRNA localization to centrosomes, a key organelle in the organization of microtubules, and mitotic spindle in eukaryotic cells. For this, we are first combining biochemical and cell biological approaches, such as structure-function studies coupled to FISH and IF, and computational analysis to characterize the *Cis*-regulatory motifs responsible for localizing candidate mitotic mRNAs. We then initiated an *in vivo* RNA-interference screen in *Drosophila* embryos, directed against ~20 RBPs, selected on the basis of their biochemical enrichment within the mitotic apparatus and their functional importance in cell division, which could be involved in the trafficking and localized translation of mitotic mRNAs. Loss of function of several of these RBPs leads to specific cell division defects and genome instability in developing embryos, implicating these factors in the regulation of mitotic mRNAs. This project will help define new mechanisms of cell division control mediated by localized RNAs.

407 The Hepatitis B Virus Post-Transcriptional Regulatory Element Promotes Nuclear Export of Unspliced mRNAs by Recruiting TREX via ZC3H18

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Viral RNA elements that facilitate mRNA export are useful tools for identifying cellular RNA export factors. Our study revealed that hepatitis B virus post-transcriptional element (PRE) is one such element, and we identified several new cellular mRNA export factors using PRE. We found that PRE drastically enhances the cytoplasmic accumulation of cDNA transcripts independent of any viral protein. Systematic deletion analysis revealed the existence of a 116-nt functional Sub-Element in PRE (SEP1). The RNP that forms on the SEP1 RNA was affinity purified, and TREX components as well as several other proteins were identified. TREX components and the SEP1-associating protein ZC3H18 are required for SEP1-mediated mRNA export. Significantly, ZC3H18 directly binds the SEP1 RNA and interacts with TREX, suggesting a role of ZC3H18 in recruiting TREX to SEP1-containing mRNAs. Requirements for SEP1-mediated mRNA export are similar to those for splicing-dependent mRNA export. In agreement with these similarities, the SEP1-interacting proteins ZC3H18, ARS2, Acinus and Brr2 are required for efficient nuclear export of polyA RNAs. Together, our data indicate that SEP1 enhances mRNA export by binding ZC3H18, which subsequently recruits TREX. The new mRNA export factors that we identified might be involved in cap- and splicing-dependent TREX recruitment to cellular mRNAs.

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408 Abstract Withdrawn

409 Roles of Los1, Msn5, and Mtr10 in tRNA nuclear-cytoplasmic dynamics in *Saccharomyces cerevisiae*

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In eukaryotes, mature tRNAs move bidirectionally between the nucleus and the cytoplasm. Three members of yeast importin-b family, Los1, Msn5, and Mtr10 have been implicated in tRNA subcellular movement. Here we developed formaldehyde crosslinking-based *in vivo* pull-down assays to study the *in vivo* interactions of b-importins with tRNAs. It has been proposed that Los1 participates in the primary and re-export of tRNAs to the cytoplasm, but Msn5 participates only in the re-export of tRNAs encoded by intron-containing genes. Consistent with this proposal, Los1 binds to both intron-containing and mature tRNAs, whereas Msn5 preferentially binds to mature tRNAs. Mtr10 affects tRNA nuclear import, but our data provide no evidence for Mtr10 binding to tRNA, implicating that Mtr10 indirectly functions in tRNA subcellular dynamics. *In vitro* studies report that Msn5 binds short dsRNAs and uncharged tRNA, in conflict with our *in vivo* genetic and biochemical data. To address this, we assessed whether other proteins aid *in vivo* specificity of Msn5 for spliced tRNA. Tef1 (vertebrate eEF1A), implicated in tRNA nuclear export, co-purifies with Msn5 in a RanGTP-dependent manner. Our studies and the studies in other organisms support the model that Msn5 forms a cooperative quaternary complex with Tef1, RanGTP, and aa-tRNA to export mature, probably charged tRNAs to the cytoplasm. Thus, Msn5 appears to regulate the tRNA re-export step via interaction with Tef1 to derive its tRNA binding specificity.

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410 *Drosophila* Imp is required for localization of germ plasm mRNAs

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mRNA localization and local translation play important roles in regulating the distributions of proteins that regulate cellular and developmental asymmetry. Two mRNAs, *oskar* (*osk*) and *nanos* (*nos*) have been established as useful models for studying the roles and mechanisms underlying such post-transcriptional regulation. During mid-stages of *Drosophila* oogenesis, *osk* mRNA is transported by Kinesin to the posterior of the oocyte where it is then translated. At the posterior, Osk nucleates formation of the germ plasm, the specialized cytoplasm that specifies germ cell formation in the embryo. At late stages of oogenesis, numerous mRNAs, including *nos* and *osk*, accumulate in the germ plasm via a diffusion/entrapment process. Among germ plasm RNAs, *nos* is unique in that it not only functions in germline development, but its translation at the posterior of the embryo to produce a protein gradient of Nos protein is essential for abdominal segmentation.

While localization of both *osk* and *nos* is essential for their function, an inefficient localization mechanism leaves the bulk of these mRNAs distributed throughout the oocyte and embryo cytoplasm where they must be translationally silenced. This translational repression is critical, as Osk and Nos must be excluded from the embryo anterior where their expression leads to ectopic germ cells and inhibition of anterior structures, respectively. Although several regulatory elements that mediate regulation of *nos* have been identified, relatively few factors have been identified that act through these elements. We have used an RNA affinity approach to identify additional factors regulating *nos*; from this analysis we identified Igf-II mRNA binding protein (Imp) as a putative *nos* binding protein. Previously, Imp had been identified as an *osk* mRNA binding protein; however, mutation of *imp* did not appear to perturb *osk* regulation. We have now demonstrated a role for Imp in the localization of both *osk* and *nos* mRNAs in the embryo, and our data suggest that Imp may be involved specifically in the accumulation of germ plasm mRNAs at late stages of oogenesis. Additionally, we present data characterizing the interaction of Imp with *nos* regulatory factors, supporting a role for Imp in *nos* regulation.

411 A role for platelet microparticles in the intercellular transfer of mRNA regulatory microRNAs

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Introduction: Platelets are anucleate elements of the blood that contain an abundant and diverse array of microRNAs, which are known as master regulators of mRNA translation. Since platelets (i) are at the interface between leukocytes and the endothelium, and (ii) may release microparticles (MP) upon activation, we hypothesized that microRNA-containing, platelet-derived microparticles (PMP) can be internalized by macrophages and modulate gene expression and function.

Material & Methods: Human platelets were stimulated with thrombin, and the PMP were isolated and characterized by flow cytometry prior to co-incubation with cultured human primary macrophages. Internalization of PMP was analyzed by confocal microscopy, whereas the mRNA and microRNA profiling of macrophages was performed by micro-array and quantitative PCR (qPCR).

Results: Containing functional Argonaute 2 (Ago2)•microRNA complexes, PMP internalized by macrophages mainly associated with the endoplasmic reticulum, where components of the microRNA pathway are also located. Micro-array and reporter gene activity assays confirmed the enrichment and functionality of PMP-derived microRNAs in macrophages. PMP induced changes in the mRNA transcriptome of primary macrophages, and the accumulation of PMP-derived microRNAs correlated with downregulation of their mRNA targets. Phenotypically, exposure of macrophages to PMP did not induce apoptosis, as reported for endothelial cells, but enhanced their capacity for phagocytosis.

Conclusion: Our results demonstrate that PMP can mediate delivery of functional platelet-derived Ago2•microRNA regulatory complexes to recipient cells, such as primary macrophages, and modulate their gene expression programming and function. PMP may thus act as a natural vehicle for microRNAs, contribute to intercellular signaling and condition the circulatory system under specific health conditions associated with platelet activation.

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412 Identification of consensus element from human naturally intronless mRNAs that promotes the export of cDNA

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Mechanism on the export of human naturally intronless mRNAs has long been elusive. We have previously reported the presence of Cytoplasmic Accumulation Region (CAR) in the coding sequences of three such mRNAs (Proc Natl Acad Sci, 2011). Further work revealed a ten-nucleotide consensus CAR element that could recruit TREX and other export related factors and promote the export of b-globin cDNA (Nucleic Acids Res, 2013). However, it is currently unknown whether all of the human intronless mRNAs contain similar elements. To investigate this possibility, we first carry out bioinformatic analysis on the coding sequences of 679 naturally intronless mRNAs from the Intronless Gene Database using MEME algorithm. The analysis reveals six 10-nt consensus elements present in more than 90% of all sequences analyzed. To test whether these elements function in mRNA export, we insert tandem repeats of each element upstream b-globin cDNA and the localization of transcripts after transfection is determined by RNA-FISH. This leads to the identification of one motif that significantly promotes the export of b-globin cDNA. No cryptic splicing is observed using RT-PCR, indicating that the effect on export is due to the element inserted. Our finding raises the possibility that all of the human naturally mRNAs contain cis element facilitating their export.

413 Importin β1 mRNA Localization for Cell Growth Control

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Size homeostasis is one of the most fundamental aspects of biology and given cell types exhibit characteristic size limitations. How can large cells such as neurons assess their own lengths and adjust their metabolic output accordingly? We recently proposed that neurons might sense the lengths of their axonal microtubule cytoskeleton by a bidirectional motor-based scanning mechanism, and that the fluctuating retrograde signal generated by this mechanism can provide input for the coordinated regulation of neuronal biosynthesis and axonal growth (Rishal et al., *Cell Reports 1*, 608-616 [2012]). Here we show that depletion of importin β1 from axons by a 3′UTR variant knockout, or by targeting of an RNA-binding protein involved in its axonal localization, enhances neuronal outgrowth. Moreover, similar perturbations affect the morphology and size of fibroblast and HeLa cells in culture. Thus, subcellular localization of importin β1 mRNA affects cell size and growth control mechanisms.

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414 CTIF is involved in mRNA export in human cells

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In eukaryotic cells translation of messenger RNA (mRNA) can be initiated either on transcripts associated with the cap-binding complex (CBC; consisting of CBP80 and CBP20) or on transcripts with the eukaryotic translation initiation factor (eIF) 4E bound to the cap. Together with eIF4G and eIF4A, eIF4E forms the eIF4F-complex, which mediates translation initiation during the bulk of cellular protein synthesis. Functionally substituting for eIF4G, the CBP80/20-dependent translation initiation factor (CTIF) has been reported to be part of the CBC-dependent translation initiation complex [1,2]. CTIF consists of a N-terminal CBP80-binding domain and a conserved C-terminal MIF4G domain [1]. This MIF4G domain has been shown to mediate the interaction between CTIF and different factors such as eIF3g and the stem-loop binding protein (SLBP) [2,3]. Here we provide evidence that CTIF, besides its function in translation initiation, is also involved in mRNA translocation from the nucleus to the cytoplasm, possibly through a direct interaction with the nuclear export factor NFX1/TAP. Taken together our results suggest that CTIF can function as a platform that interacts with proteins involved in different steps of the mRNA metabolism.

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[2] Choe J. et al. (2012) The Journal of biological chemistry 287:18500-18509

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415 hnRNP K Regulates Translation of Cytoskeletal-associated mRNAs for Vertebrate Axon Outgrowth through JNK Signaling

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Heterogeneous nuclear ribonucleoprotein K (hnRNP K) has long been recognized as a multifunctional protein involved in multiple aspects of RNA trafficking and gene expression, but its physiological roles in the intact organism are only beginning to be elucidated. Using the South African claw-toed frog, *Xenopus laevis*, as a model system, we have discovered that hnRNP K regulates both embryonic and regenerative axon outgrowth by coordinately controlling nucleocytoplasmic trafficking of the mRNAs of multiple cytoskeletal proteins used to organize neuronal cytoskeletal polymers into the arrays that form the axon. To understand how these actions of hnRNP K might be directed by cell signaling pathways orchestrating axonogenesis, we focused on the role of its phosphorylation in embryonic *Xenopus* neurons by c-Jun N-terminal kinase (JNK), which both phosphorylates hnRNP K and is similarly required for axonal outgrowth. Pharmacological inhibition of JNK, as well as knockdown of hnRNP K expression, inhibited both axon outgrowth and translation of hnRNP K-regulated, cytoskeletal-related RNAs in these neurons. These defects were alleviated by expressing phosphomimetic, but not phosphodeficient, forms of hnRNP K. Immunohistochemical and biochemical analyses further indicated that phosphorylation of the JNK-target site on hnRNP K occurred mainly in perinuclear, cytoplasmic regions of neuronal perikarya, on the side facing the axon. Whereas this phosphorylation was unnecessary for intracellular localization of and RNA binding by hnRNP K, it was required for loading hnRNP K-targeted, cytoskeletal-related RNAs onto ribosomes. These data provide the first evidence of a role for JNK phosphorylation of hnRNP K in the post-transcriptional control of an important biological process - axon outgrowth - and indicate a mechanism whereby the synthesis of structural proteins used to build the axon can be coordinated with the demand for these materials as axon outgrowth proceeds. *Supported by NSF - IOS (BGS) and an AAUW dissertation fellowship (EJH)*.

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416 Localization analysis of predicted pseudo cleavage furrow-localized mRNA in *Drosophila* early embryos

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The regulation of protein localization in cell is very important for cellular differentiation and development. One of the mechanisms of the spatial regulation of protein localization is the preceding mRNA localization. Previous global analysis for mRNA localization in early *Drosophila* embryos revealed that a number of mRNAs are subcellulary localized. However the molecular mechanisms of the mRNAs localization are mostly unrevealed.

In early *Drosophila* embryogenesis, consecutive thirteen times nuclear divisions occur without cytokinesis and result in multinucleate cell called a syncytium. After fertilization, nuclei divide seven times synchronously and then most of the nuclei move to the surface of the embryo during the following three times nuclear divisions. Between 10th and 13th nuclear division, transient invagination of plasma membrane, known as a pseudo cleavage furrow (PCF), arise between nuclei. We found that localization patterns of some mRNAs reported previously were very similar to the spatial pattern of PCF formation. Therefore, as a first step to investigate whether these mRNAs localize at the PCF, we carried out RNA *in situ* hybridization combined with immunostaining for a PCF maker protein in *Drosophila* embryos. The obtained data revealed that *canoe* (*cno)* mRNA, one of the predicted PCF-localized mRNAs, was localized at the tip of the PCF, but not detected at the surface of syncytial blastoderm embryos at nuclear division cycle 10 to 13. In contrast, the *cno* mRNA was not detected at the cellularization furrow formed during 14th nuclear division. These results suggest that the differences between the components of the tip of the PCF and the cellularization furrow are important for the localization of *cno* mRNA. Because the localization patterns of other predicted PCF-localized mRNAs such as *scraps, polychaetoid, diaphanous, CG43427* are similar to that of *cno* mRNA, we predict these mRNAs are also localized at the PCF. In addition, because it is reported that the proteins encoded by the predicted PCF-localized mRNAs are localized at the PCF, the localization of the mRNAs at the PCF might be responsible for an efficient supply of those proteins to the PCF in order to support the rapid nuclear divisions during the syncytial blastoderm stage.

417 Identification of non-canonical mRNAs targeting to the ER and analysis on subcellular localization of mRNAs by RNA imaging system

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Secretory and membrane proteins have a signal peptide, which is recognized by the signal recognition particle (SRP) as their proteins emerge from the ribosome. This signal peptide recognition via the SRP directs mRNA/ribosome/nascent polypeptide complexes to the ER membrane. Although recent analyses have indicated mRNAs encoding nuclear and cytoplasmic protein are also directed to the ER in SRP-independent manner, the molecular mechanism and biological significance is still unknown. As the first step to reveal the molecular mechanism, we identifid the ER-directed mRNAs not bearing a signal sequence from *Drosophila* S2-DRSC cells with cell fractionation and RNA-seq analysis. We identified 9,707 transcripts from the ribosome-stripped rough microsome, ER enriched fractions. In the top 100 of enriched mRNAs in the fractions, we couldn't identify any obvious signal sequence in 97 each mRNA. These data suggest that a wide variety of mRNA are targeted to the ER SRP-independently and this targeting mechanism is operating regularly in the cells. In parallel with the identification of the mRNAs, we have established RNA visualization system mediated by GFP in the S2-DRSC cells to analyze the ER targeting mechanism of the mRNA. By using this system, we visualized *Vm34Ca* and *smt3* mRNA encoding secreted protein as a positive control and cytoplasmic protein as a negative control, respectively. While *Vm34Ca* mRNA formed foci adjacent to the ER, *smt3* mRNA dispersed throughout the cytosol. The foci associated with *Vm34Ca* mRNA disappeared in the *srp54k*, a core component of SRP, knockdown cells or in the signal sequence-deleted *Vm34Ca* mRNA expressing cells. These data suggest that this imaging system is suitable to visualize mRNA targeting to the ER in the S2-DRSC cells. Using this system, we examined the subcellular localization of *yorkie* (*yki)* mRNA, one of the mRNAs not bearing signal sequence in ribosome-stripped rough microsome fractions. Yki is a transcription co-activator, known as an oncogene. The *yki* mRNAs also formed foci adjacent to the ER and the foci were unaffected by knockdown of *srp54k*. These results indicate that *yki* mRNA is targeted to the ER SRP-independently. Currently, we are trying to identify cis-element of *yki* mRNA required for ER targeting.

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418 Luzp4 Defines a New mRNA Export Pathway In Cancer Cells

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Cancer/Testis Antigens (CTAs) are encoded by genes that are normally expressed in human germ cells and trophoblast but are also aberrantly expressed in various cancers. Their restricted expression pattern in normal tissues and their ability to elicit immune response in cancer patients make them promising targets for customized immunotherapies. However, 70 % of the CTAs known to date have no clearly defined function.

Here, we report that the cancer/testis antigen gene LUZP4/CT-8/HOM-TES-85 encodes a canonical mRNA export adaptor.

Using a wide range of experimental approaches, we show that Luzp4 is an RNA-binding protein that integrates into the TREX complex and interacts directly with the RNA helicase Uap56 and the mRNA export receptor Nxf1. In support of these results, we demonstrate that Luzp4 functionally complements an RNAi-mediated depletion of the canonical mRNA export adaptor Alyref by rescuing the global mRNA export pathway and restoring cellular growth.

Interestingly, we found that the presence of the Luzp4 protein in cancer cells is associated with low levels of the Alyref protein and vice versa. We observed this inverted correlation of expression in 80 % of the cancer cell lines tested. Furthermore, analysis of Luzp4 function in cancer cells showed that it supports the nucleocytoplasmic transport of multiple transcripts required for various aspects of cellular growth and invasion of tissues by cancer cells. This finding was confirmed by a dramatic reduction of cancer cells proliferation upon knockdown of Luzp4 by RNAi.

Overall, this study assigns a function to a member of the poorly characterized Cancer/Testis Antigens family. It defines the cancer/testis antigen Luzp4 as an mRNA export adaptor used by some cancer cells to sustain cell proliferation.

419 Hrp1p and Vts1p contribute to targeted degradation of nitrogen specific mRNAs in dynamic environments

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In both unicellular and multicellular organisms, a healthy cell is able to respond correctly to specific environmental cues. In steady-state conditions, stable transcript levels of active genes are ensured through ongoing RNA degradation in the cytoplasm. Furthermore, intrinsic properties of each mRNA, as well as the RNA binding proteins (RBPs) associated with it, determine the transcript's translation efficiency. In dynamic environments, transcriptional regulation has been thought to be the driving force behind the changes in transcriptome composition. It is becoming increasingly apparent however, that differential binding of RBPs can also have an effect on the stability of specific transcripts, facilitating the rapid remodeling of the gene expression program.

In this study, we re-visit the Nitrogen Catabolite Repression (NCR) regulon in *S. cerevisiae* in order to investigate non-siRNA mediated transcript elimination under dynamic conditions. NCR gene products are involved in the utilization of secondary nitrogen sources and are therefore highly expressed when the environment is poor in nitrogen and repressed under optimum growth conditions. Using experimental transitions from poor to rich nitrogen sources and time course experiments, we find evidence for accelerated degradation of these transcripts. We use bioinformatics and biochemical techniques to identify for the first time Hrp1p—an hnRNP-like protein—and Vts1p—a member of the Smaug protein family—as RBPs that bind NCR transcripts. We study the specificity of these RBPs using RNA immunoprecipitation (RIP) experiments in dynamic environments. We further use mutant strains to dissect the specific effect of these proteins in transcript destabilization and explore their coordination with the known RNA decay pathways. Finally, we combine our data to generate an integrated functional network underlying the post-transcriptional regulation of this environmentally regulated regulon.

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420 Mechanistic insights of SIDER2 retroposon-mediated mRNA decay in *Leishmania*

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Regulation of gene expression in *Leishmania,* an evolutionary early-branched unicellular parasitic protozoan, occurs almost exclusively post-transcriptionally. We have reported previously that the *Leishmania* genome harbors truncated versions of formerly active retroposons (500-nt average size), SIDERs (**S**hort **I**nterspersed **DE**generate **R**etroposons), that are located predominantly within 3′-untranslated regions (3′UTRs) and participate in distinct RNA networks. Members of the SIDER2 subfamily promote rapid mRNA destabilization by a novel mechanism involving endonucleolytic cleavage without prior deadenylation. Primer extension analysis and RNase protection assays mapped the putative endonucleolytic cleavage site within the second 79-nt SIDER2 signature sequence, which represents the most conserved sequence amongst SIDER2 retroposons. To better delineate the sequence and structural requirements for SIDER2-mediated mRNA decay, we carried out deletion and mutagenesis studies within the putative cleavage region of signature II. Our results indicate that a predicted stem-loop secondary structure at the 3′ end of signature II plays a crucial role in the decay process as point mutations abolishing this structure resulted in high accumulation of the SIDER2-bearing reporter transcript. Interestingly, this structure is predicted in several other functional SIDER2 sequences. We also showed that signature II without the rest of SIDER2 sequence was sufficient in promoting rapid degradation. In order to identity protein factor(s) contributing to the SIDER2-mediated mRNA decay, we developed an optimized MS2 tethering approach using reporter transcripts with two MS2 hairpins upstream of 3′UTRs harboring or not a SIDER2 sequence and a tandem MS2 coat protein construct. Immunoprecipitation and mass-spectrometry studies with parasites co-expressing the SIDER2-MS2 reporter transcripts and the MS2 protein tagged with an HA epitope revealed few SIDER2-interacting RNA-binding protein candidates. These include members of the CCR4-NOT deadenylation complex, a member of the PUF family, a 5′ to 3′ exoribonuclease XRN homolog, and other RNA-binding proteins of unknown function. Experiments are in progress to investigate the individual contribution of these candidate proteins in mRNA degradation and their specific binding to the SIDER2 regulatory regions.

421 Splicing-factor SRSF1's dual function in nonsense-mediated mRNA decay: NMD fast-forward and UPF1 dephosphorylation

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We previously showed that splicing factor SRSF1 promotes nonsense-mediated mRNA decay (NMD), a splicing- and translation-dependent quality-control mechanism that degrades mRNAs with premature termination codons (PTCs). Here, we show that SRSF1 has dual functions in NMD. First, transcript-bound SRSF1 increases the binding of UPF1 to mRNAs via direct interaction starting in the nucleus, and bypassing UPF2 recruitment in the cytoplasm. Moreover, SRSF1-tethered *HBB* (β-globin) transcripts bound by UPF1 show an increase in bound exon-junction-complex (EJC) components in the nucleus, and a decrease in the cytoplasm, compared to transcript bound only by UPF1. This observation suggests that in the presence of SRSF1, transcript-bound EJCs are displaced more rapidly by ribosomes, due to fast-forwarding of NMD. Second, SRSF1 through its RRM2 facilitates UPF1 dephosphorylation, enabling its recycling into the active form, by promoting/stabilizing the interaction between phospho-UPF1 and SMG6, an endonuclease involved in RNA cleavage. Moreover, the strengthening of this interaction via SRSF1's RRM2 ensures the stabilization of SMG6 on PTC-containing transcripts, presumably facilitating SMG6's endonucleolytic function.

SRSF1's mode of action in NMD resembles that of EJC and NMD factors, as it promotes NMD when positioned downstream of a PTC. Moreover, the effect of SRSF1 on NMD of an intronless PTC-containing *HBB* transcript is significantly reduced, compared to the intron-containing *HBB* transcript, suggesting that splicing and/or EJC deposition increases the effect of SRSF1 on NMD. Lastly, SRSF1 enhances NMD of PTC-containing endogenous transcripts that result from various events. This function is abrogated by deletion of RRM2, consistent with this domain's involvement in UPF1 dephosphorylation.

Our findings reveal an alternative mechanism for UPF1 recruitment, and shed light into the dephosphorylation steps of UPF1, uncovering a novel connection between splicing and NMD. SRSF1's role in the mRNA's journey from splicing to decay has broad implications for gene-expression regulation and human genetic diseases.

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422 Identification and characterization of novel factors that act in the NMD pathway in nematodes, flies and mammals

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The nonsense-mediated mRNA decay (NMD) pathway is a quality control mechanism that selectively degrades mRNAs that contain premature termination codons (PTCs) preventing the accumulation of truncated proteins. Importantly, this quality control mechanism not only degrades mRNAs harboring nonsense mutations but also regulates an important fraction of the transcriptome. Previously, several evolutionarily conserved core NMD factors have been identified and characterized and a mechanistic insight into how this surveillance mechanism operates in eukaryotes has been partially uncovered.

Here, we have conducted a genome-wide RNAi screen that has resulted in the identification of several novel NMD genes in the nematode C. elegans. These novel genes include the GTPase, ngp-1, the nuclear pore protein complex, npp20, as well as aex-6, which encodes a Rab protein involved in vesicular trafficking. Interestingly, ngp-1 and npp-20 are required for proper larval development in nematodes, which is in contrast to most smg genes that are not essential in C. elegans. These genes are conserved throughout evolution and their human homologs GNL2 (ngp-1), SEC13 (npp20) and RAB27B (aex-6) are also required for NMD in human cells. We also show that noah-2, which is present in Drosophila melanogaster, but absent from humans, is an NMD factor in insects. Interestingly, we show that transcripts encoding different NMD factors were sensitive to depletion of GNL2 and SEC13 suggesting that these novel NMD factors participate in a conserved NMD negative feedback regulatory loop, as has been demonstrated for core NMD factors. Altogether, these results demonstrate the complexity of the NMD pathway and suggest that yet uncovered novel factors may act to regulate this process.

423 mRNA decay is altered in Myotonic Dystrophy patient cells

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Type 1 Myotonic dystrophy (DM1) is a chronic and progressive autosomal dominant neuromuscular disease resulting from a trinucleotide repeat expansion in the 3′UTR of the dystrophia myotonia protein kinase (DMPK) gene. DM1 symptoms are characterized by myotonia, endocrine defects, muscle wasting, and cardiac conduction defects. Pathogenesis is linked to the accumulation of the toxic repeat-containing RNA in nuclear foci and sequestration of RNA-binding proteins, such as Muscleblind (MBNL1). MBNL1 and CELF1, another RNA binding protein whose function is affected in DM1, have both been implicated as regulators of mRNA decay. This leads to the hypothesis that the stability of cellular mRNAs is altered in DM1 due to the presence of mutant DMPK mRNA and this altered stability may contribute to pathogenesis of the disease.

Evaluation of the patient myoblast cells demonstrated that they have phenotypic features associated with DM1, including; the expression of nuclear foci, an elevated abundance of CELF1 protein, and documented splicing defects. Preliminary data indicate that mRNAs from three transcripts that were previously shown to be bound by CELF1: SOX9, TUT1, and ZNF37A, are stabilized in DM1 patient myoblasts compared to control cells. Moreover, knockdown of mutant DMPK mRNA in DM1 patient cells results in restoration of decay to rates similar to those in myoblasts from normal individuals. These data support the hypothesis that mRNA stability is affected in myotonic dystrophy and that the altered stability results from the expression of mutant DMPK mRNA.

Sequencing of mRNA (RNA-seq) will be used to perform a global analysis in these samples to evaluate changes in mRNA decay, splicing, and polyadenylation that result from expression of the toxic repeat-containing DMPK RNA. The results of these experiments will be used to identify changes in mRNA metabolism which may contribute to the pathogenesis of DM1 and may also uncover new avenues for therapeutics.

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424 Insight into the hydrolytic activity of Decapping Scavenger enzymes: effect of His tag sequence location and product inhibition

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Decapping scavengers (DcpS) are involved in the last stage of eukaryotic mRNA degradation process which determines the lifetime of different transcripts in living cells and therefore plays an important role in the post-transcriptional control of gene expression. DcpS enzymes catalyze the hydrolysis of residual cap structure m^7GpppN or short capped oligonucleotide resulting from exosom-mediated 3′ to 5′ digestion of deadenylated transcript. They utilize an evolutionary conserved HIT motif (His-X-His-X-His) to bind substrates and cleave 5′,5′-triphosphate bridge within the cap, releasing m^7GMP and ppN or diphosphate terminated oligo mRNA containing less than 10 nucleotides. DcpS enzymes are homodimeric proteins with two independent active sites (both sufficient for substrate binding and hydrolysis) and two structural domains in each subunit: highly conserved C-terminal domain containing HIT motif and more variable N-terminal domain. Most recombinant DcpS enzymes used in a variety of biochemical and biophysical studies were obtained as N-terminal His-tagged proteins.

Here we compare hydrolytic activity of N-terminally and C-terminally His-tagged DcpS enzymes towards dinucleotide cap analogs modified within 7-methylguanosine moiety (m^7GpppG, et^7GpppG, bn^7GpppG, $m_2^{2,7O'}$GpppG, $m_2^{3,7O'}$GpppG and $m_3^{2,2,7}$GpppG). Enzymatic digestion of these dinucleotides was tested by means of fluorescence and HPLC at two temperatures, 20°C and 37°C. To calculate the hydrolysis rate of cap analogs, the time-dependent increase of fluorescence intensity, caused by the removal of intramolecular stacking upon the cleavage of triphosphate bridge, was recorded. Our data demonstrate differences in the reaction velocities when two forms of DcpS were used in enzymatic assays (N-terminally His-tagged DcpS versus C-terminally His-tagged DcpS).

Kinetic analysis of DcpS-mediated hydrolysis of m^7GpppG, et^7GpppG and bn^7GpppG indicate the influence of reaction products (m^7GMP, et^7GMP, bn^7GMP) on the overall hydrolysis rate. This effect has not been investigated so far. Our preliminary results suggest that m^7GMP, et^7GMP and bn7GMP decrease the observed initial velocities of dinucleotide hydrolysis. To further adress the phenomena of inhibition *via* reaction products, we estimated the IC50 values for m^7GMP, et^7GMP and bn^7GMP. Among the three monophosphates examined, the most efficient product inhibition is observed for m^7GMP.

425 Assessing the "geometry" of the TRAMP and exosome complexes

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The exosome complex plays major roles in RNA 3′->5′ processing and surveillance activities. The TRAMP complexes, including 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. Structural analyses of Mtr4 have 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 intact Mtr4 and an Mtr4-Arch construct, lacking the Arch domain. Mtr4-Arch showed greatly reduced association with pre-rRNA species, indicating an important role in surveillance of ribosome synthesis. CRAC was also performed on the exonuclease Rrp44, in strains expressing Mtr4-Arch or with a mutation that occludes the central channel (Rrp41-channel) within the exosome. This reveals how Mtr4 affects the interaction of the core exosome with different substrate classes and the dependence of different substrates on threading through the exosome. The results indicated a role for the Mtr4 Arch domain in pre-mRNA and pre-tRNA surveillance. We conclude that the Arch domain recruits Mtr4 to the 3′ ends of pre-mRNA introns and tRNAs, and targets them to Rrp44 via the exosome channel.

In CRAC analyses, RNAs are footprinted by the exosome. The cDNA length distribution of the library should therefore distinguish between RNA species that thread through the central channel of the exosome (~33nt fragments) or pass directly to the Rrp44 active site (~10-12 nts). RNA sequence length distributions for Rrp44 were compared in the WT strain and mutants for the exonuclease, endonuclease, or central channel. This indicated that different RNA classes follow distinct pathways to access Rrp44. The distribution of hits among RNA classes in size-fractionated part of the library reveal striking differences, confirming that different classes of RNA go through specific pathway to access Rrp44. Moreover, in Rrp41-channel strain, intermediate length fragments accumulated, suggesting that RNAs can use an additional, as yet unidentified, pathway through the exosome.

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426 A genome-wide comparative study identifies novel *cis*-regulatory elements involved in nuclear mRNA decay

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Post-transcriptional regulatory mechanisms are important to our understanding of development and evolution, metabolism and the pathogenesis of diseases, including many cancers. Such regulatory mechanisms are mediated by *cis*-regulatory sequence elements, typically located within the untranslated regions (UTRs) of mRNAs. These elements control mRNA maturation and processing, export from the nucleus, sub-cellular localization, stability and translation by recruiting *trans*-acting factors. Several comparative genomics studies have identified many unknown regulatory sequences within mammalian UTRs, however, their functions and corresponding mechanisms remain to be discovered.

We performed genome-wide comparative studies to identify novel candidate *cis*-regulatory elements within 3′UTRs - these studies indicate the likely existence of hundreds of novel elements within mammalian 3′UTRs. Tissue-culture reporter assays revealed a function in post-transcriptional regulation for a set of novel elements predicted only by their conservation. In this study we focused on the functional investigation of two *cis*-regulatory elements that share significant sequence identity to one another. These conserved elements can be found in hundreds of different 3′UTRs of mRNAs, which encode mainly transcription factors and proteins that are involved in signaling pathways. Systematic experimental investigation excluded a function in translation, nuclear export, mRNA processing and transcription, but clearly indicated a function in nuclear RNA decay. Transcripts harboring these elements undergo rapid decay prior to the completion of normal transcript processing. We are currently focusing on identifying *trans*-acting factors required for this nuclear decay pathway.

427 GU-rich element-containing transcripts are differentially regulated through alternative ploydenylation and CELF1 phosphorylation following T cell activation

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The RNA-binding protein, CELF1, regulates the destabilization of a network of mRNA transcripts though binding to GU-rich elements (GREs) in their 3′untranslated regions (3′UTRs). In primary human T cells, GREs and CELF1 control a posttranscriptional network of transcripts that encode regulators of cell growth and apoptosis. To characterize the components of this network, we immunoprecipitated CELF1 from resting T cell cytoplasmic extracts, and CELF1-associated mRNAs were analyzed by microarray hybridization. Approximately 1200 CELF1 target transcripts were identified, and they exhibited significant enrichment of GREs in their 3′UTRs.

CELF1 target transcript subsets exhibited distinct expression patterns following T cell activation. Some transcripts were turned off rapidly in the first hours following activation while others were turned on at later time points. We hypothesized that GRE-mediated mRNA decay played an important role in the rapid coordinated down-regulation of transcripts that control cell growth and apoptosis in the first hours following T cell activation, but more complex mechanisms were involved at later time points. For example, we found that transient stimulus-dependent phosphorylation of CELF1 6 hours following T cell activation blocked its ability to bind to GRE-containing mRNAs, in a manner that correlated with transiently increased expression of these transcripts. Subsequent dephosphorylation of CELF1 at later time points restored CELF1's ability to bind to target transcripts and mediate their decay.

In addition to regulation through CELF1 phosphorylation, we showed using next-generation RNA sequencing technology that a large subset of GRE-containing transcripts were shortened in activated T cells through alternative polyadenylation (APA), resulting in loss of GREs from these transcripts. Removal of GREs from these shortened transcripts rendered them inaccessible to CELF1-mediated regulation following T cell activation.

These results suggest that GRE-containing CELF1 target transcripts are differentially regulated through CELF1 phosphorylation and APA following T cell activation. A subset of CELF1 target transcripts are regulated over time through transient phosphorylation of CELF1 following T cell activation, whereas another subset of CELF1 target transcripts are exempt from CELF1-mediated regulation by loss of their GREs through transcript shortening due to alternative polyadenylation.

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428 Glucocorticoid receptor triggers rapid mRNA degradation by recruiting PNRC2 and Upf1

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Glucocorticoid receptor (GR) functions as nuclear receptor for controlling various physiological processes including inflammation. GR plays an additional role in rapid mRNA degradation by acting as an RNA-binding protein. However, the behind molecular details remains unknown. Here, we show that GR, loaded onto 5′UTR of target mRNA independently of a ligand-binding, recruits Upf1 and Dcp1a to the 5′UTR via its interaction with PNRC2 in a ligand-dependent manner, so as to elicit rapid mRNA degradation, which we term GR-mediated mRNA decay (GMD). We also identify de novo cellular GMD substrates using microarray analysis. Among them is CCL2 mRNA. Intriguingly, GMD is involved in chemotaxis of THP-1 cells by regulating the level of CCL2 mRNA. Our data thus reveal a novel mRNA decay pathway, GMD, which is mechanistically distinct from nonsense-mediated mRNA decay.

429 Mechanism of nonsense-mediated decay inhibition by a retroviral RNA stability element

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The nonsense-mediated mRNA decay (NMD) pathway recognizes and degrades transcripts containing long 3′ untranslated regions (3′UTRs). To maintain proper gene expression, retroviruses have evolved RNA elements capable of protecting transcripts from 3′UTR length-dependent decay. The best-characterized retroviral NMD-antagonizing element, the RNA stability element (RSE) of Rous sarcoma virus, stabilizes both retroviral and synthetic reporter mRNAs containing long 3′UTRs. This large (~ 400 nt) RNA segment sits immediately downstream of the viral *gag-pro* termination codon, preventing it from being recognized as premature. However, despite the RSE's robust anti-NMD activity, its mechanism of action has remained unclear.

By purifying ribonucleoprotein complexes associated with mRNAs containing RSE or control sequences, we have identified a set of proteins specifically associated with the RSE. We will present evidence that one of these binding partners, the polypyrimidine tract binding protein 1 (PTBP1) is the primary host factor responsible for RSE activity. Mutation of putative PTBP1 binding sites in the RSE abolishes its activity, and depletion of PTBP1 via RNAi reduces RSE-containing transcript stability. Further, artificial recruitment of PTBP1 immediately downstream of an NMD-inducing stop codon fully stabilizes mRNAs, while recruitment to sites in the 3′UTR distant from the stop codon fails to protect from NMD. This position-dependence mirrors that of the wild-type RSE sequence, supporting a model in which the RSE primarily functions by recruiting PTBP1 to viral RNAs. We propose that the binding of PTBP1 downstream of stop codons restricts the access of the core NMD factor UPF1 to mRNAs, disrupting its ability to detect potential NMD substrates.

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430 DRBD13 as a regulator of AU-rich element (ARE) containing mRNAs

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Trypanosoma brucei, a unicellular protozoan parasite responsible for African sleeping sickness, alternates its life cycle between a mammalian host and an insect vector. Regulation of gene expression in trypanosomes is mainly post-transcriptional (mRNA stability and translational control), mostly through interactions between *trans*-acting RNA-binding proteins and *cis*-acting regulatory elements in 3′ untranslated region (3′-UTR) of mRNAs. Using a novel computational framework, we recently discovered a highly conserved AU-rich element (ARE), AUUUAUU, in 3′-UTRs of many trypanosomatid genes that potentially control mRNA abundance across different life stages. Follow up computational and experimental analysis led to the identification of DRBD13 as one of the *trans*-acting factors involved in the ARE-mediated regulation in trypanosomes. Here, we studied the mechanism of DRBD13 gene regulation through identification of its RNA targets and associated proteins. DRBD13 immunoprecipitation followed by RNA sequencing together with available transcriptome data in *T. brucei*, indicate that DRBD13 is preferentially associated with mRNAs encoding proteins for cell membrane organization. Consistent with this observation, DRBD13 over-expression led to striking changes in *T. brucei* morphology including expression of mammalian form specific variant surface glycoprotein (VSG) mRNAs in insect form of trypanosomes. This suggests that DRBD13 may act as a regulator of gene expression for events leading to changes in membrane composition of the parasite. Furthermore, tandem affinity purification of tagged DRBD13, showed its association with translational complex constituents (eIF4E and eIF4G) as well as components of the deadenylation machinery (Not1, Not5 and Caf40). The later observation is consistent with destabilizing effect of DRBD13 on its target mRNAs supported by artificially tethering the protein to a reporter mRNA and the absence of DRBD13 from the actively translating polysomal fraction. Thus, our data suggest that DRBD13 may be a crucial determinant of the stability of mRNAs encoding proteins that are involved in the assembly of cell membranes.

431 High resolution analysis of *cis*-regulatory sequences within the HMGA2 3′UTR

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The 3′ untranslated regions (3′UTRs) of mRNAs regulate transcripts by serving as binding sites for regulatory factors, which include small non-coding RNAs (such as microRNAs (miRNAs)) and RNA binding proteins (RBPs). Binding of such *trans*-acting factors can control the rates of mRNA translation and decay, as well as other aspects of mRNA biology. To better understand the role of 3′UTRs in gene regulation, we have performed a detailed analysis of a single mammalian 3′UTR with the primary goal of identifying the complete set of regulatory elements within a single 3′UTR. We chose HMGA2, an oncogene whose overexpression in cancers often stems from mutations that remove 3′UTR regulatory sequences. The highly conserved and almost 3000 nucleotide long HMGA2 3′UTR contains multiple target sites for the tumor-suppressor miRNA let-7, and recent studies indicate it may serve a dual role as a let-7 sponge. We used reporter assays in human tissue culture cells to generate a high resolution map of *cis*-regulatory information across the HMGA2 3′UTR at 50 nucleotide resolution. Many sites across the length of the UTR showed regulatory potential, a number of which were up-regulating and most had not been described previously. Importantly, the overall location and impact of regulatory sites was similar when assayed in different cell lines (both mouse and human derived) and between HMGA2 3′UTRs from 3 different species (mouse, human and chicken). We were also interested in determining the extent to which 3′UTR regulatory elements interact with and affect one another. By systematically comparing the regulatory impact of HGMA2 segments of different sizes we were able to determine that most regulatory sequences function independently of one another, though a small number of segments showed evidence of interactions between elements. We are currently working on identifying the specific sequences and *trans*-factors responsible for the regulatory effects we observed. By fully characterizing one 3′UTR, we hope to better understand some of the principles involved in 3′UTR mediated gene regulation. Also, a clearer understanding of HMGA2 regulation may benefit our understanding of its oncogenic roles in cancer.

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432 Nucleases involved in rRNA degradation in yeast during apoptosis and autophagy

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Several stress response pathways, including oxidative stress, apoptosis and autophagy, involve extensive degradation of ribosomal RNA, accompanied by accumulation of specific rRNA intermediates. To identify enzymes and factors responsible for this process we have analyzed contribution of various endo- and exonucleases to rRNA fragmentation. These included mitochondrial nuclease Nuc1 a homolog of human apoptotic protein EndoG, that induces apoptotic death; endonucleases RNAse P and MRP, involved tRNA or rRNA processing, respectively; cytoplasmic 5′-3′ exonuclease Xrn1 active in mRNA decay; components of the exosome complex that functions in processing and degradation of several RNAs; and finaly vacuolar ribonuclease Rny1, that cleaves tRNA molecules under various stress conditions. We show that oxidative stress and autophagic rRNA decay mechanisms are generally separate, with Nuc1 and RNase MRP being mainly responsible for the former and Rny1 for the latter pathway. In both cases however, rRNA fragments generated endonucleolytically are further trimmed by exonucleases, mainly the exosome and Xrn1.

In contrast, components of both 18S and 25S “non-functional rRNA decay” (NRD), that eliminates malfunctionning ribosomes, are not required for stress-related rRNA destruction.

These data point to activation of diverse metabolic pathways by different stress stimuli.

433 Investigating an RNA element that inhibits mRNA nuclear export

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Quality control (QC) of mRNA represents an important regulatory mechanism for gene expression in eukaryotes. Transcripts that are misprocessed or arise from non-conserved intergenic regions, often encode for toxic proteins and therefore need to be eliminated. However, despite the significant amount of spurious transcripts generated in the nucleus, the vast majority of cytoplasmic polyadenylated RNA codes for protein. This is because the eukaryotic gene expression machinery effectively evaluates whether or not a transcript has all the features of a bona fide translationally competent mRNA. However, if a transcript lacks these features, it is retained in the nucleus and degraded by several QC pathways. Here we identify an RNA element that inhibits mRNA nuclear export when present in the 3′ untranslated region (UTR). This element consists of the consensus 5′ splice site motif. Data from the literature suggests that U1 snRNP binds to such RNAs and results in 3′ end misprocessing. We have shown that a fraction of RNAs with this aberrant 3′ end configuration is rapidly degraded. The fraction that evades decay is retained in the nucleus, likely in nuclear speckles, dynamic structures that act as a depository for splicing and mRNA nuclear export factors. Genome wide analysis reveals that this 5′ splice site motif is depleted in the 3′ UTR, but is not depleted in intergenic regions. This bias suggests that the presence of a strong 5′ splice site motif adjacent to the cleavage site in the 3′end is deleterious for gene expression and is consistent with the inhibitory role exerted by the aberrant 3′ end configuration in our model transcript.

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434 Functional analysis of IMP3, a RNA-binding protein

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RNA-binding proteins (RBP) regulate multiple steps of post-transcriptional gene expression, and it is important to know the target RNAs of RBPs for the understanding the biological function of RBPs. It was reported that IMP3, an RBP, stabilizes CD44 mRNA. It has been proposed that IMP3 is a useful indicator of the prognoses of several cancers, such as colorectal, ovarian, gastric carcinoma. IMP3 has a potential not only to be simply a marker of prognosis in cancer cells, but also to regulate cancer progression. However, little is known about the function of IMP3 in cancer development because RNA targets of IMP3 are unclear. In this study, we tried to identify RNA targets of IMP3 through genome-wide analysis.

Initially, we determined the expression levels of whole transcripts in IMP3-depleted cells by deep sequencing analysis. The expression levels of 419 transcripts were increased in IMP3-depleted cells. We also identified 2,218 transcripts as candidates of IMP3 binding RNAs by RNA immunoprecipitaion followed by deep sequencing analysis (RIP-seq). Among them, 110 transcripts were up-regulated in IMP3-depleted cells, and we judged these 110 transcripts are the potential IMP3 targets. We measured the stability of these RNA targets of IMP3 in IMP3-depleted cells using BRIC-method, an inhibitor-free method for directly measuring RNA stability. We showed that EIF4EBP2 and MEIS3 were stabilized by depletion of IMP3. Co-immunoprecipitaion analysis revealed that IMP3 interacts with ribonucleases such as RRP4 and XRN2. Moreover, the retarded proliferation of IMP3-depleted cells was partially rescued by EIF4EBP2 depletion. There was an inverse correlation between the expression level of IMP3 and EIF4EBP2 in human lung adenocarcinoma tissue. Our results suggest that IMP3 promotes the degradation of certain RNA targets such as EIF4EBP2, leading to regulate cell proliferation. This is a first demonstration that IMP3 is a RNA destabilizing factor for a set of transcripts, and we showed, in part, the function of IMP3 in cancer cells.

435 Control of Trypanosome Gene Expression by RNA Binding Protein 10 (RBP10)

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Trypanosomes, unlike other eukaryotes, control their gene expression mainly post-transcriptionally. RNA binding proteins (RBPs) are crucial to this process. RBP10 is an essential cytoplasmic protein only expressed in the mammalian infective bloodstream forms; it is undetectable in the vector infective-procyclic forms. Alteration of RBP10 expression in these two life stages causes a widespread effect on the transcriptome [1]. A subset of mRNAs greatly affected encodes proteins involved in the energy metabolism of the bloodstream forms. Interestingly, RBP10 over-expression in the procyclic forms promotes expression of these mRNAs which results in impaired growth. The mechanism of action of RBP10 is still unknown and attempts to identify a common motif in the 3′UTRs of RBP10 mRNA targets have not been successful.

Tethering of RBP10 to a reporter mRNA reduces its abundance and represses its translation. We have shown the C-terminal part of RBP10 is necessary and sufficient to induce this inhibitory effect. In addition, the reporter mRNA distribution on the polysomes is shifted from heavy to upper polysomal fractions of the sucrose gradient. This suggests a possible role of RBP10 in translation repression. We have evidence by yeast two hybrid and co-immunoprecipitation that RBP10 interacts with eIF4E interacting protein (eIF4E-IP). Interestingly, in Leishmania, eIF4E-IP binds to one of the four eIF4E isoforms (eIF4E-1); this interaction directs its role during translation in a life-stage specific manner [2]. In a genome-wide tethering screen, we identified both eIF4E-IP and eIF4E-1 act as down-regulating proteins. Therefore, we speculate eIF4E-IP might be recruiting RBP10. Currently, using polysome analysis combined with deep sequencing we are investigating the effects of RBP10 on loading of trypanosome mRNAs onto the polyribosomes.

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436 SMG6 mediated degradation of nonsense mRNA requires phosphorylation-independent interaction with the helicase domain of UPF1

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Eukaryotic mRNAs with premature translation-termination codons (PTCs) are recognized and eliminated by nonsense-mediated mRNA decay (NMD). NMD targeted mRNAs can be degraded by different routes that all involve phosphorylated UPF1 (P-UPF1) as a starting point. The endonuclease SMG6, which cleaves mRNA near the PTC, is one of three known NMD factors thought to be recruited to nonsense mRNAs by interaction with P-UPF1, leading to eventual mRNA degradation. By MS2-mediated tethering of SMG6 and mutants thereof to a reporter RNA combined with knockdowns of various NMD factors, we demonstrate that besides its endonucleolytic activity, SMG6 also requires UPF1 and SMG1 for inducing RNA decay. Our experiments revealed a phosphorylation-independent interaction between SMG6 and UPF1 that is important for SMG6-mediated mRNA decay and using yeast two hybrid assays, we mapped this interaction to the unique stalk region of the UPF1 helicase domain. This region of UPF1 is essential for SMG6-mediated reporter RNA decay and also for NMD. Our results postulate that besides recruiting SMG6 to its RNA substrates, UPF1 is also required to activate its endonuclease activity.

437 Poly(A)-specific ribonuclease (PARN): Poly(A)-specificity is coupled to events of translocation and hydrolysis

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Poly(A)-specific ribonuclease (PARN) is a divalent metal-ion dependent poly(A)-specific, processive and cap-interacting 3′-5′ exoribonuclease that efficiently degrades poly(A) tails of eukaryotic RNAs. PARN is unique among the poly(A) degrading nucleases, being the only one that has the capacity to directly interact during poly(A) hydrolysis with both the m^7G-cap structure and the poly(A) tail of the mRNA. On the basis of biochemical and structural evidence we have developed a working model for PARN action (Virtanen et al., *Crit. Rev. Biochem. Mol. Biol.*, **48**, 192-209). The model defines two alternating reaction steps, a translocation event that pushes the scissile bond of the poly(A) substrate into the catalytic center of the active site followed by a hydrolytic event that cleaves the scissile bond. The two reaction steps are repeated and one AMP residue is released in each cycle. We have performed kinetic analyses and used divalent metal ions as probes to decipher mechanisms of the translocation and hydrolytic events. To interpret our results we developed a kinetic scheme for the degradation of trinucleotide substrates. Using this scheme we found that the translocation step is the rate determining step and is tightly coupled to adenosine recognition/specificity.

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438 The effects of disruptions in ribosomal active sites and in intersubunit contacts on ribosomal stability in growing *Escherichia coli*

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Although ribosomes are very stable under most conditions, ribosomal degradation does occur in diverse groups of organisms as a response to specific stresses or environmental conditions. While the non-functional ribosome decay (NRD) in yeast has recently been well characterized, very little is known of the mechanisms that initiate ribosomal degradation in bacteria. My studies of *Escherichia coli* rRNA mutations allow to test specific hypotheses about the mechanism of ribosome degradation in growing cells. We discovered that mutations in the 16S rRNA decoding centre (G530U and A1492C) and 23S rRNA active site (A2451G), which induce NRD in yeast, fail to do so in *E. coli*. In contrast, NRD-inducing mutation, U2585C, in 23S rRNA causes degradation of both the large and small ribosomal subunits in *E. coli*. We further tested the role of ribosome-inactivating mutations (A1912G, A1919G, A1960G and ΔH69) in ribosomal intersubunit contacts. Deletion of Helix 69 of 23S rRNA and the point mutation A1912G in the same helix did not destabilize ribosomes, while expression of mutations A1919G in H69 and A1960G in H71 led to degradation of both mutant and wild-type ribosomes in growing cells. Surprisingly, the A1919G mutant ribosomes are stable in stationary cells, as are wild-type ribosomes during IF3 overexpression, which dissociates ribosomal subunits and consequently stops cell growth. Our results suggest that ribosomal degradation in growing *E. coli* is not simply a result of exposing inactive or dissociated ribosomes to the degradation machinery. It is rather a controlled process, which is dependent on *de novo* protein synthesis and degrades both the mutant and wild-type ribosomes.

439 Structural basis for binding of Pan3 to Pan2 and its function in mRNA recruitment and deadenylation

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The conserved eukaryotic Pan2–Pan3 deadenylation complex shortens cytoplasmic mRNA 3′ polyA tails to regulate both mRNA stability and translation. The exonuclease activity resides in Pan2, and efficient deadenylation requires Pan3 although its mechanistic role is unclear. We have used a combination of structural and biochemical studies to understand the function of Pan3 within the complex. We show that Pan3 binds to the region of Pan2 that links its N-terminal WD40 domain to the C-terminal part that contains the exonuclease, with a 2:1 stoichiometry. The crystal structure of the Pan2 linker region bound to a Pan3 homodimer shows how the unusual structural asymmetry of the Pan3 dimer is used to form an extensive high-affinity interaction. This binding allows Pan3 to supply Pan2 with substrate polyA RNA, facilitating efficient mRNA deadenylation by the intact Pan2–Pan3 complex to regulate gene expression.

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440 Regulation of mRNAs involved in copper homeostasis by the Nonsense-mediated mRNA decay pathway

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The nonsense-mediated mRNA decay pathway (NMD) is an mRNA degradation pathway that degrades specific mRNAs. More specifically, the NMD pathway is responsible for degrading mRNAs with premature termination codons, as well as many natural mRNAs. In *Saccharomyces cerevisiae*, five features have been shown to target natural mRNAs for NMD-mediated degradation. One of these features is the presence of an atypically long 3′-UTR. The *S. cerevisiae CTR2* mRNAs play a role in copper homeostasis, and are regulated by the NMD pathway. We found that the regulation of *CTR2* mRNAs by NMD is due to the presence of an atypically long 3′-UTR; and a second NMD contributing feature, the length of the *CTR2* open reading frame. Here, we examined seven other *S. cerevisiae* mRNAs that are involved in copper homeostasis and were predicted to be sensitive to the NMD pathway. Out of these seven mRNAs, five were predicted to have a long 3′-UTR. We determined which of the mRNAs are direct or indirect NMD targets, and the extent to which the long 3′-UTRs are the NMD targeting mechanisms. Additionally, we examined multiple transcripts under low copper conditions. We found that low copper growth conditions affect NMD sensitivity of *MAC1* mRNA. *MAC1* is a copper sensitive transcription factor that regulates genes involved with high affinity copper transport. This study provides insight into how NMD regulates mRNAs from the same functional group.

441 Pumilio proteins bind and regulate mRNAs associated with progression of Parkinson's disease in humans

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Parkinson's disease (PD) is the most common motor system disorder and is caused by the loss of dopamine-producing neurons. Coding mutations have been identified in several PD-associated genes, yet it is often the over-expression of these genes that is implicated in the disease. Post-transcriptional regulation of mRNA stability and translation are key steps in gene expression control in disease progression. Typically, cis elements located in the 3′ untranslated region (UTR) of mRNAs recruit microRNA (miRNA) complexes or regulatory proteins that influence mRNA decay and/or translation rates. One class of 3′ UTR regulatory proteins is the Puf family. In humans, the Puf proteins Pum1 and Pum2 are expressed widely, including in stem cells and brain tissue. At the molecular level, Puf proteins directly elicit translation initiation repression through protein interactions that inhibit cap-binding events, or stimulate deadenylation and decapping steps of decay through interactions with mRNA decay machinery. We have identified several novel targets of Pumilio regulation that are involved in PD progression. Utilizing SH-SY5Y neuronal cells, we knocked down Pum1 and Pum2 expression and evaluated endogenous mRNA and protein levels of SNCA, LRRK2 and SAT1. Upon knockdown, mRNA and protein levels of target genes increased, indicating Pumilio acts to down-regulate these genes. To validate the direct nature of this regulation, we utilized a luciferase-based reporter assay to evaluate 3′UTRs of target genes for their ability to confer Pumilio regulation. Preliminary data suggests that the 3′UTR of target genes is sufficient for regulation, and upon overexpression of Pum1 the levels of luciferase decrease, suggesting that Pumilio acts through the 3′UTRs. In a reciprocal experiment, knockdown of Pum1 and Pum2 lead to an increase in luciferase levels. Mutation of Pumilio response elements (PREs) located within 3′UTRs caused an increase in luciferase, validating the direct nature of Pumilio regulation. We are currently investigating if Pumilio regulation of target genes is cooperative with the miRNA regulatory system to provide a fine-tuned mechanism of controlling protein levels, which when aberrantly expressed can lead to a disease state.

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442 Probing interactions of NMD factors in a distance-dependent manner by BioID

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The understanding of molecular mechanisms requires the elucidation of protein-protein interaction *in vivo*. For large multi-factor complexes like those assembling on mRNA, co-immunoprecipitation assays often identify many peripheral interactors that complicate the interpretation of such results and that might conceal other insightful mechanistic connections. Here we address the protein-protein interaction network for key factors in the nonsense-mediated mRNA decay (NMD) pathway in a distant-dependent manner using BioID[1,2]. In this novel approach, the mutant *E. coli* biotin-protein ligase $BirA_{R118G}$ is fused to the bait protein and biotinylates proximal proteins promiscuously. Hence, interactors positioned close to the bait in vivo are enriched by streptavidin purification and identified by mass spectrometry or western blotting. We present a validation of the BioID assay and preliminary results for close interactors of UPF1 and other key players in NMD.

1 Roux, K. J., Kim, D. I., Raida, M. & Burke, B. A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. *J Cell Biol* **196**, 801-810, doi:10.1083/jcb.201112098 (2012).

2 Roux, K. J., Kim, D. I. & Burke, B. BioID: a screen for protein-protein interactions. *Curr Protoc Protein Sci* **74**, Unit 19 23, doi:10.1002/0471140864.ps1923s74 (2013).

443 The human DIS3 nuclease clears the cell of pervasive transcription products

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The human genome is pervasively transcribed but most transcripts originating from non-coding parts of the genome are expressed at very low levels. DIS3 is a catalytic subunit of the nuclear exosome complex and contains exonucleolytic (RNB) and endonucleolytic (PIN) active domains. It was found that the RNB domain of DIS3 is mutated in about 10% of multiple myeloma (MM) patients.

In order to identify human DIS3 targets genome-wide we have conducted comprehensive RNA-seq experiments of samples isolated from HEK293 cells in which wild-type DIS3 has been replaced by various mutants bearing single and double substitutions, including the MM associated ones, and RNB and PIN catalytic mutations. These analyses were supplemented with UV Cross-Linking and Immunoprecipitation (CLIP) experiments.

The most prominent effect of DIS3 mutations was observed on pervasive transcription products. Promoter Upstream Transcripts (PROMPTs) accumulate robustly, especially in double mutants. Nearly all (98.8%) known PROMPTs were upregulated, overall increasing from 0.14% to 0.77% of mapped reads in RNA-seq experiments, with a fraction accumulating nearly 50-fold. PROMPTs represented 7% of reads in CLIP experiments. Interestingly, there is no correlation between PROMPT accumulation and expression of neighboring genes. Moreover, RNAs originating from unannotated genomic regions rose from 10% of mapped reads in cells expressing wild-type DIS3 to 25% in cells expressing double mutants. The reads are generally quite dispersed but in some cases we were able to assemble novel transcripts. The second most prominent increase was that of snoRNA precursors. For long intergenic non-coding RNAs we generally observe downregulation rather than accumulation, with the single exception of a PIN domain mutation, in which several lincRNA are accumulated. The overall trend among mRNAs is a slight decrease but we do see accumulation of some species. Moreover, in PIN domain-only mutants we see very strong downregulation of a few interesting mRNAs. Finally, there is no general accumulation of reads corresponding to tRNAs, introns, transposones or repeats, suggesting that these transcripts are degraded by other nucleolytic enzymes.

Overall, our data indicate that DIS3 is responsible for the main nucleoplasmic exosome activity, which is degradation of pervasive transcription products and snoRNA processing.

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444 An exon junction complex-independent role of Y14 in mRNA stability control

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Eukaryotic mRNA biogenesis involves a series of interconnected steps including nuclear pre-mRNA processing, mRNA export and surveillance. The exon-junction complex (EJC) is deposited on newly spliced mRNAs and functions to coordinate several post-splicing mRNA biogenesis steps. The EJC core protein, Y14, is important for nonsense-mediated mRNA decay and promotes translation. We have previously reported that Y14 binds the cap structure of mRNAs and inhibits the activity of the decapping enzyme Dcp2. Using reporter assays, we found that Y14 can prevent mRNA degradation. To understand more about the structural basis for the Y14-cap interaction, we performed the protein homology modeling and determined the residues of Y14 involved in cap binding. Mutagenesis study revealed that W73 was critical for cap binding. Mutations of this residue disrupted the cap binding activity of Y14 but did not prevent its interaction with Dcp2. Therefore, the cap binding and decapping inhibition activities of Y14 may be biochemically uncoupled. Moreover, we assessed the biological function of Y14 in cap binding and mRNA stability control. We found that Y14 associated with a set of mRNAs including previously reported Dcp2 targets, and that depletion of Y14 destabilized the newly synthesized but not steady state population of these mRNAs. Experiments are still underway to decipher the biological significance of EJC-independent functions of Y14.

445 The Mtr4 Ratchet Helix and Arch Domain Act in Concert to Promote RNA Unwinding

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Mtr4 is a conserved superfamily-2 RNA helicase that activates exosome mediated 3′-5′ turnover in nuclear RNA surveillance and processing pathways. As a member of the Trf4-Air2-Mtr4 polyadenylation (TRAMP) complex, Mtr4 unwinds polyadenylated RNA substrates and presents them to the exosome for degradation. Prominent features of the Mtr4 structure include a four domain ring-like helicase core and a large arch domain that spans the core. The "ratchet helix" within the helicase core is positioned to interact with the bases on the 3′ end of poly-adenylated RNA substrates. However, the contribution of these interactions to Mtr4 activity is poorly understood. Here we show that strict conservation along the ratchet helix is particularly extensive for Mtr4 as compared to related helicases. Mutation of residues along the ratchet helix alters RNA unwinding rates in both Mtr4 and TRAMP, affects Mtr4 RNA affinity, and results in slow growth phenotypes *in vivo*. We also identify a mutation on the ratchet helix that influences Mtr4 affinity for polyadenylated substrates. Previous work indicates that deletion of the arch domain has minimal effect on Mtr4 unwinding activity. Surprisingly, we now show that the combination of archless and ratchet helix mutants completely abolishes helicase activity and produces a lethal *in vivo* phenotype. These studies demonstrate that the ratchet helix modulates helicase activity and suggest that the arch domain plays a previously unrecognized role in unwinding substrates.

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446 Role of Pat1 in the Lsm1-7-Pat1 complex function

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A major mRNA decay pathway conserved in all eukaryotes is the 5′ to 3′ pathway. The first step in this pathway is deadenylation or poly(A) shortening. Following deadenylation, the oligoadenylated mRNA is decapped by the Dcp1-Dcp2 decapping enzyme and the capless message is subsequently degraded by the 5′ to 3′ exonuclease Xrn1. Decapping is a rate limiting crucial step in this pathway and the Lsm1-7-Pat1 complex is essential for normal rates of decapping *in vivo*. This complex is made up of the seven Sm-like proteins Lsm1 through Lsm7 and the Pat1 protein all of which are conserved in all eukaryotes. It selectively binds to the 3′-ends of oligoadenylated mRNAs *in vivo* and the purified complex exhibits strong intrinsic binding preference for oligoadenylated RNAs over polyadenylated RNAs *in vitro*. After binding the mRNAs, this complex facilitates their decapping via additional post-binding steps that are poorly understood. The RNA binding properties of this complex are important for mRNA decay *in vivo* and the Sm domain of the Lsm1 subunit is an important determinant of such properties. Pat1 is another key subunit of this complex whose functions are not well understood. Pat1 is implicated in translation repression and other functions like p-body assembly that do not seem to be related to its association with the Lsm1-7 complex. In order to gain insight into the role of Pat1 as part of the Lsm1-7-Pat1 complex, we have studied the Lsm1-7 complex purified from *pat1*Δ cells and recombinant Pat1 fragments purified from *E. coli*. The results of these studies will be presented.

447 Genome wide identification of the RNA 3′ uridylation in humans

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RNA polyadenylation and uridylation are known to be essential for the RNA quality control and gene expression regulation. These processes are mediated by enzymes from the non-canonical polyA polymerase family. The events of the untemplated RNA extension are closely connected with the 3′ to 5′ end degradation process. Exonuclease DIS3L2 that is involved in Perlman syndrome development was recently reported to be the missing component of the uridylation mediated degradation of the let-7 miRNA precursors during the cell differentiation. Here we report on the RNA binding studies that were performed by crosslinking in vivo and immunoprecipitation (CLIP) method of human DIS3L2 nuclease uncovering a wide range of the DIS3L2 substrates *in vivo*.

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448 The mechanism of RNA decay by an Rrp6-associated RNA exosome

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The eukaryotic RNA exosome is a multi-subunit ribonuclease (RNase) that functions in pathways that control RNA decay, maturation, and quality control of nuclear and cytoplasmic RNAs. The nine-subunit exosome core (Exo9) includes a prominent central channel formed by two asymmetric rings composed of six RNase PH-like proteins and three S1/KH domain proteins. While the yeast Exo9 core is devoid of RNase activity it associates with two RNases, Rrp44 in the cytoplasm and nucleus and Rrp6 in the nucleus. It has been demonstrated through structural and functional assays that Rrp44 uses the entirety of the Exo9 central pore to channel RNA substrates into its active site. Recent biochemical results suggested that Rrp6 also utilizes the central channel to degrade RNA. However, the position of Rrp6 on Exo9, and how it uses the core to process RNAs has remained unclear. We have determined a 3.3Å crystal structure of *Saccharomyces cerevisiae* Rrp6 bound to Exo9, engaged with single-stranded polyA RNA. The structure positions Rrp6 directly above the S1/KH ring, with its catalytic domain hovered above the Exo9 central channel. The 3′ end of RNA is directed from the Rrp6 active site into the Exo9 S1/KH ring via interactions conserved in eukaryotes, in a path partially overlapping, but opposite that observed in the crystal structure of an Rrp44-associated exosome. Our structure also reveals a potential gating mechanism for how Rrp6 activates Rrp44 in the context of the nuclear exosome by inducing Exo9 channel widening. Finally, we present biochemical data suggesting that the RNA path to Rrp6 is exclusively dependent on the S1/KH ring, unlike that observed for Rrp44 RNA substrates. These experiments provide further evidence for exosome component interdependence – how Rrp6 and Rrp44 influence each other's activities in a core-dependent manner, and how presentation of a particular RNA to the Exo9 core may dictate its fate for distributive versus processive decay.

449 Asp/Glu residues interacting with RNA hydrogen bond acceptors - What sorcery is that?

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Interactions with nucleic acids involving cationic species (mainly Na^+, K^+ and Mg^{2+}) are relatively well characterized. Conversely, less is known about anionic species interacting with nucleic acids. In the last decade some investigations aimed at clarifying anion-RNA interactions (1) and in particular the ones involving Asp/Glu side chains (2). Favored nucleobase binding sites emerged; more specifically, guanine Watson-Crick sites were observed to dominate over other possibilities without excluding less obvious interaction patterns.

We report here, along with a classification of regular Asp/Glu binding sites, several surprising instances of close contacts between Asp/Glu side chains and hydrogen bond acceptors in nucleic acid systems. A remarkable example involves a contact with a backbone phosphate group that interacts simultaneously with a protonated form of a sulfate anion (PDB code: 2DRB; pH: 7.5). These counterintuitive contacts involving Asp/Glu residues generally considered as negatively charged and nucleic acid electronegative atoms can only be understood by considering the neutral forms of these amino acids. To confirm that these interactions are not anecdotal, a survey of the PDB was performed. This survey collected a large array of short hydrogen bond contacts between Asp and Glu residues with themselves and with other electronegative atoms, therefore stressing that their neutral form is more frequent than previously thought.

The purpose of this study is to highlight the implications of such unexpected hydrogen bond network contributions to the understanding of protein/nucleic acid interactions. As a main outcome of this study, it is proposed that the neutral Asp and Glu amino acids have to be included into an expanded bestiary of interacting residues leading to novel recognition patterns. These motifs will certainly have to be considered in forthcoming nucleic acid/protein prediction studies.

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2. Kondo, J., Westhof, E. (2011) Classification of pseudo pairs between nucleotide bases and amino acids by analysis of nucleotide-protein complexes. *Nucleic Acids Res* **39,** 8628-8637.

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450 Effects of G-Quadruplex Formation on Biophysical Properties of QRRM1-RNA complex of hnRNP H1

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The hnRNP H1 protein is a member of the ubiquitously expressed heterogeneous nuclear ribunucleoproteins (hnRNPs) subfamily. hnRNP H1 has three Quasi-RRM domains and Glycine rich repeats. Studies have shown that hnRNP H1 binds poly G segments with high affinity, but the binding preferences of each sub Q-RRM are unknown. All members of this family act as a trans regulator of alternative splicing; however, the mechanism of how each protein of this family regulates alternative splicing is poorly understood. As a step toward gaining a better understanding of hnRNP H1 nucleic acid interactions, we studied NMR solution structure of Q-RRM1 domain that was solved by our collaborator Dr. Ramelot. Furthermore we used NMR for determining the amino acid residues interacting with poly G oligos to elucidate the site of interaction on Q-RMM1. Poly G regions of nucleic acid tends to form a structured region called G-quadruplex. G-quadruplex structures are four stranded structure formed by at least three consecutive guanine in nucleic acid strands. NMR experiments demonstrate the intermolecular G-quadruplex structures formed by some of oligos we studied. G-quadruplexes structure in DNA and RNA strands are quite different in their stability. RNA G-quadruplexes has been poorly studied; however, these studies suggest a regulatory function by switching from a single strand to a G-quadruplex and vice versa. Our studies affirm that Q-RRM1 only binds to a single strand oligos and the thermodynamic stability of G-quadruplexes will determine the binding properties of Q-RRM1 and nucleaic acid complex. Additionally isothermal titration calorimetry was utilized for studying biophysical properties of the Q-RRM1 interaction with each poly G oligos. As a result hnRNP H1 can play a role in regulation by switching from a single strand to G-quadruplex structure in the site of splicing with poly G motifs.

451 Abstract Withdrawn

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452 MECP2 is post-transcriptionally regulated during hiPS cell neurodevelopment into human neurons

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The methyl CpG-binding protein 2 gene (MECP2) is a transcriptional modulator whose control of protein abundance is crucial for neurological physiology. Mutations in MECP2 cause the neurodevelopmental disorder Rett syndrome. MECP2 protein level is progressive increased during neurodevelopment, and the lack of direct correlation between changes in MECP2 mRNA and protein levels suggests a post-transcriptional component in MECP2 gene regulation. MECP2 transcripts are alternatively polyadenylated and the full 8.5kb MECP2 3′UTR contains various sequence elements known to be fundamental for post-transcriptional regulation. We hypothesize that an interplay of alternative polyadenylation to selectively include or exclude sequence elements, and their interaction with cell-type specific RNA-binding proteins (RBPs) is responsible for post-transcriptionally regulating MECP2. To test this, we use human induced pluripotent stem cells (hiPSC) and their ability to differentiate into neurons to model neurodevelopment. Our data shows that MECP2 is indeed post-transcriptionally regulated during neurodevelopment, where a discrete 2 fold increase in the transcript levels is largely exceeded by the increase in protein abundance. That increase in MECP2 protein level is in part attributed to an increase in the transcript half-life. We demonstrated that MECP2 transcripts are degraded by P-body components in hiPSCs and polysome profiling showed that these transcripts are highly enriched in the monosome fractions in iPSCs, suggesting that a regulated release from P-bodies and a switch to the polysome fraction must occur to increase the protein levels in neurons. To determine the post-transcriptional elements present in the MECP2 3′UTR, reporter plasmids containing fragments of the 3′UTR were constructed. Two regions were shown to regulate translation and transcript stability during neurodevelopment. Gain-and-loss of function assays using RBP genes are being performed to investigate their role in MECP2 post-transcriptional regulation. In conclusion, our study proposes a model to explain how MECP2 protein abundance is controlled during neurodevelopment. Since deregulation of MECP2 expression causes different neurodevelopmental disorders, understanding the molecular mechanisms underlying MECP2 regulation will contribute to the development of treatments for such diseases.

453 G-quadruplex thermal stability delays DHX36 RNA helicase activity

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Several DExD/H helicases such as DHX36 (RHAU) are known to unwind both DNA and RNA G-quadruplexes (G4) in an ATP-dependent manner. Such G4 helicases are generally thought to unwind G4 to maintain genomic integrity and to prevent abortive transcription/translation at G4-prone sites. However, the effect of substrate G4 stability on helicase unwinding and ATP consumption remains uncharacterized. Additionally, the manner in which the helicase recognizes and unwinds G4 is lacking due to the absence of structures of G4-helicase complexes. The lack of attention on G4 substrate stability is due to the prevailing view that G4-forming motifs uniformly impart the same biological effects (e.g., varying G4 structures act identically as a "roadblock" for RNA polymerases to impede translation). Though seemingly intuitive, nucleic acid substrate stability is not necessarily proportional to helicase unwinding rates. While the DEAD protein eIF4A was shown to have an unwinding rate inversely proportional to dsRNA stability, other helicases have been shown to display unwinding rates independent of substrate stability.

Here, we present a systematic in vitro study on the impact of G4 thermodynamic stability on DHX36 activity. We modulated G4 stability by (1) varying G-tract length and (2) using small molecule G4 ligands. We show that increased G4 stability delays rather than halts DHX36-mediated G4 unwinding. Surprisingly, ATP consumption was not coupled to unwinding, unlike what has been found for other G4 helicases such as WRN and BLM. Taken together, these results suggest that the unwinding mechanism of DExD/H helicases on dsRNA substrates is similar to that of DHX36 on the radically different G4 substrate. Our findings show that G4 stability controls the rate with which a helicase unwinds the G4. G4 stability may thus be (1) a variable in determining the severity of biological events such as abortive transcription or translation and play (2) a role in controlling gene expression through the helicase-mediated processes of transcription and translation.

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454 Defining the Role of Staufen1 in Developing and Mature Skeletal Muscle *In Vivo*

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We have shown that Staufen1 expression is markedly and specifically increased in skeletal muscles from Myotonic Dystrophy Type 1 (DM1) mice and patients (Ravel-Chapuis et al., J. Cell. Biol., 2012). Furthermore, overexpression of Staufen1 in DM1 rescues: 1) the nuclear export and translation of mutant DMPK mRNAs and 2) the aberrant alternative pre-mRNA splicing of the Insulin Receptor and Chloride Channel 1. These advances represent new opportunities to better understand the complex DM1 pathology and the development of novel therapies. Our most recent work also shows that increased expression of Staufen1 negatively impacts myogenic differentiation of C2C12 cells via c-myc dependent and SMD-independent mechanisms (manuscript submitted). Together, these findings highlight the importance for investigating the role of Staufen1 *in vivo* during embryonic and post-natal muscle development as well as in adult muscle. To this end, we generated a muscle-specific Staufen1 transgenic mouse model under the control of the Muscle Creatine Kinase (MCK) promoter/enhancer regulatory cassette. Preliminary observations show that mice overexpressing Staufen1 in striated muscle are viable. Protein analyses confirm muscle-specific expression of the MCK-Staufen1-HA3 transgene. Histological analyses with Hematoxylin and Eosin staining of Tibialis Anterior (TA) muscle cross-sections revealed that 4.5-week-old muscles have an increase in total fiber number as well as smaller fiber cross-sectional area as compared to wildtype littermates. Further analyses of the MCK-Staufen1-HA3 mice will prove invaluable to decipher the role of Staufen1: 1) to examine the impact of Staufen1 overexpression on embryonic and post-natal muscle development, 2) in alternative splicing; and 3) to ascertain the therapeutic potential of Staufen1 for DM1 muscle. Collectively, results from these studies will increase our understanding of the role of Staufen1 in basic muscle biology while also providing novel insights regarding the potential of DM1 therapeutic strategies based upon Staufen1 overexpression.

455 Climbing the vertebrate branch of U1A/U2B″ protein evolution

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In the vertebrate lineage of the U1A/U2B″/SNF protein family, the U1A and U2B″ proteins bind to RNA stemloops in the U1 or U2 snRNPs, respectively. However, their specialization is fairly recent, as they evolved from a single ancestral protein. The progress of their specialization (subfunctionalization) can be monitored by the amino acid sequence changes that give rise to their modern RNA binding specificity. Using ancestral sequence reconstruction to predict the intermediates on the evolutionary branch, a probable path of sequential changes is defined for U1A and U2B″. The RNA binding affinity for U1A/U2B″ protein ancestors was measured using modern U1 and U2 snRNA stemloops and RNA stemloop variants, to understand how the proteins' RNA specificities evolved.

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456 Interaction of OAS1 with the 5′ and 3′ terminal region RNAs of West Nile virus

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West Nile virus (WNV) has a positive sense RNA genome with conserved structural elements in the 5′ and 3′ -untranslated regions. Antiviral immunity to WNV is partially mediated through the production of a cluster of proteins known as the interferon stimulated genes (ISGs). The 2′ 5′-oligoadenylate synthetases (OAS) are key ISGs that help to amplify the innate immune response. Upon interaction with viral double stranded RNA, OAS enzymes become activated and enable the host cell to restrict viral propagation. Studies have linked mutations in the *OAS1* gene to increased susceptibility to WNV infection, highlighting the importance of OAS1 enzyme. Here we report that the region at the 5′-terminal region (5′-TR RNA) of the WNV genome comprising both the 5′-UTR and initial coding region is capable of OAS1 activation *in vitro*. This region contains three RNA stem loops (SLI, SLII, and SLIII), whose relative contribution to OAS1 binding affinity and activation were investigated using electrophoretic mobility shift assays and enzyme kinetics experiments. The combination of SLI/II and SLII/III retained the binding and activation of OAS1 respectively comparable to the 5′-TR RNA. Similar binding and activation studies were performed for the 3′-terminal region (3′-TR RNA). As the 3′-5′-TR RNA panhandle formation is necessary for viral genome RNA replication, we were interested to study its interaction with OAS1. Binding and kinetic studies were performed to obtain information on OAS1 interaction with the 3′-TR RNA and 3′-5′-TR RNA. Experiments are being carried out to study structural aspects of the RNAs and its complex with OAS1.

457 Structural and Dynamic Investigation on ETR-3 RRM3 and its Interaction with AU-rich RNAs

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The ELAV type RNA-binding protein 3 (ETR-3) is a RNA binding protein involved in many aspects of RNA metabolism. ETR-3 interacts with UG repeats in the context of splicing [1]. Sequence specificity for UG repeats and notably the UGUU motif has been confirmed by systematic evolution of ligands by exponential enrichment (SELEX) [2]. However, ETR-3 has also been shown to regulate the cyclooxygenase-2 (COX-2) mRNA stability and translation by binding to AU-rich sequences in the 3′UTR region [3].

ETR-3 belongs to the CUG-BP and ELAV like factor (CELF) family. The members have two RNA recognition motifs (RRM) separated from a third one by a divergent domain (approx. 200 amino acids). The RRMs are extremely conserved among the family; over 90% identity between ETR-3 and CUG-BP1, its closest homolog. The solution structure of CUG-BP1 RRM3 in complex with $(UG)_3$ has been solved by NMR spectroscopy[4]and X-ray crystallography structures of CUG-BP1 RRM1 and RRM2 bound to RNAs containing the UGUU motif have been published [5]. Although these structures provide very detailed insight in the binding mode to UG rich RNAs, it remains unclear how ETR-3 recognizes AU rich RNAs and how it regulates mRNA stability and translation.

In our effort to understand how ETR-3 recognizes AU rich RNA, we determined the structure of RRM3 with 5′-UUUAA-3′ and highlighted the differences with the mode of recognition of UG rich RNAs. We subsequently designed mutants of residues at the binding interface and studied their interaction with RNA by NMR. Unexpectedly, mutation of aromatic residues that are not found in canonical RNPs resulted in an increase in affinity compared to the wild type. This unveiled conformational changes that are correlated to the RNA binding.

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458 Double stranded RNA sensing by 2′-5′ oligoadenylate synthetases

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The 2′-5′ oligoadenylate synthetases (OASs; OAS1, 2, and 3) are a family of interferon (IFN) inducible, regulated RNA polymerases that utilize ATP substrate to produce 2′-5′ linked oligoadenylates (2-5A). 2-5A activate a kinase family receptor, RNase L, to induce regulated RNA decay as a protective mechanism in the IFN response. Our work aims to decipher how double stranded RNA (dsRNA) regulates OAS activity. To understand the mechanism by which dsRNA activates OAS1, we solved the crystal structure of human OAS1 in complex with dsRNA and 2′-dATP. This structure revealed sequence independent dsRNA recognition as well as large conformational changes that occur upon dsRNA binding. Our subsequent work showed OAS3 to be a high affinity dsRNA binding protein with a more complex activation mechanism that requires long dsRNA. Together these data expand our knowledge of OAS regulation and begin to explain why vertebrate genomes encode multiple OAS isozymes.

459 Dead End, a protein counteracting miRNA-mediated repression of tumour suppressor genes, contains non-canonical RNA binding domains

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The protein Dead End (Dnd1) is necessary for proper development of germ cells in zebrafish and mammals [1]. Truncations in the Dnd1 gene have been shown to promote the formation of germ-cell tumors [2]. Recently the molecular function of Dnd1 has been exposed as a negative modulator of miRNA activity, when it was shown that it can counteract repression of several tumour suppressor genes (p27, LATS2) by the miR-221 family by blocking the accessibility of the mRNAs targeted by these miRNAs [3,4,5]. It was suggested that Dnd1 blocks access of miRNAs to their targets by binding to conserved U-rich regions (URRs) in close proximity to the miRNA seed sequences in the mRNA target 3′UTRs. Dnd1 contains two RNA recognition motifs (RRMs) a dsRBD (double-stranded RNA binding domain) the function of which has not been described in the literature.

To understand how Dnd1 recognizes its targets and inhibits miRNA-based gene silencing at the molecular level we aim to solve the three-dimensional structure of the Dnd1 RNA binding domains in complex with RNA derived from one of Dnd1's 3′UTR-targets. We have identified short RNA oligomers derived from the p27 tumour suppressor mRNA 3′UTR that bind to the Dnd1 RRM1 and double RRM12 as shown by NMR chemical shift perturbation mapping and ITC. In addition, ITC measurements have shown that both RRMs are necessary for tight binding, suggesting that the two RRMs are working cooperatively in recognizing their mRNA-targets.

The solution structure of RRM12 shows that Dnd1's both RRMs contain non-canonical helical and beta-hairpin extensions to the classical RRM fold. The RNA binding site includes these non-canonical elements in addition to the RRM1 canonical beta-sheet RNA interaction surface. NMR based structure determination of a RRM12-p27-3′UTR complex is underway.

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460 Solution Conformation of Adenovirus Virus Associated RNA-I and its Interaction with PKR

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The host innate immune system is the first line of defense against viral infection. The presence of highly structured RNAs of many viral genomes is a key extracellular and intracellular indicator of viral infection. Double-stranded RNA-dependent Ser/Thr protein kinase (PKR) is a key antiviral protein found in human cells that is produced in response to viral infection. After initial binding to viral double-stranded (ds) RNA through double-stranded RNA binding motifs (dsRBMs), PKR self-associates followed by its autophosphorylation. In turn PKR phosphorylates its substrate eukaryotic initiation factor 2α, which slows the translation of viral proteins, thus helping the host cell response. Interestingly, adenovirus can, in part, evade this line of defense by transcribing a non-coding RNA molecule (VA_I) that inhibits PKR from performing its enzymatic function. VA_I consists of three base-paired regions; the apical stem responsible for the interaction with the dsRBMs of PKR, the central stem required for inhibition, and the terminal stem. We present solution conformations of VA_I, VA_I lacking the terminal stem ($VA_I\Delta TS$), and apical stem of VA_I (VA_IAS) determined using small-angle x-ray scattering that indicate extended conformations that are in good agreement with their known secondary structures. Solution conformation of VA_IAS in complex with the dsRBMs of PKR indicated that the apical stem interacts with both dsRNA-binding motifs. $VA_I\Delta TS$ in complex with the dsRBMs of PKR demonstrates that the apical stem interacts with both dsRNA-binding motifs whereas the central stem is not involved in interaction highlighting its importance in the inhibition of PKR. Finally, mutation of the inhibitory central stem of VA_I while leaving the apical stem intact has enabled determination of key structural features required for the inhibition of a key innate immune protein, PKR.

461 EWS-FLI1 Reduces RNA Helicase A Activity

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RNA Helicase A (RHA) has critical roles in modulating transcription, splicing and translation as a scaffolding protein. One of RHA's protein partners, Ewing Sarcoma specific fusion protein EWS-FLI1 requires RHA to enable oncogenic transformation. We developed a small molecule called YK-4-279 which blocks the binding of EWS-FLI1 to RHA complex formation and leads to apoptosis in Ewing sarcoma cells. We hypothesized that EWS-FLI1 interferes with RHA helicase function. We purified recombinant full-length RHA protein and evaluated its helicase activity with or without EWS-FLI1 in biochemical and biophysical levels. The recombinant RHA functioned as active helicase in addition to facilitate annealing of RNA. We determined that EWS-FLI1 interfered with the helicase activity of RHA in a dose dependent manner as shown in strand separation assay. However, EWS-FLI1 did not interfere with the ATPase or the annealing activity of RHA. The Michaelis-Menten kinetics of helicase assays with varying concentration of EWS-FLI1 did not reveal the inhibition mechanism. We discovered that EWS-FLI1 binds to dsRNA besides RHA in gel based assays. Th small molecule YK-4-279 negated the inhibitory effect of EWS-FLI1 on helicase activity of RHA in an enantiomer specific manner. We also observed that EWS-FLI1 caused RHA to form higher molecular volume complexes with RNA and EWS-FLI1 in atomic force microscopy. Moreover, the addition of YK-4-279 to RHA-EWS-FLI1 and RNA binding mixture led to decreased the number of these composite complexes among EWS-FLI1-RHA and RNA, and increased the number of the monomeric complexes of RHA-RNA and EWS-FLI1-RNA as observed in AFM. RNA-IP sequencing also demonstrated the both EWS-FLI1 and RHA bound to same RNAs in the Ewing Sarcoma cells. We concluded that EWS-FLI1 modifies the function of RHA by both binding to dsRNA and changing the RNA binding behavior of RHA in addition to being an aberrant transcription factor in Ewing Sarcoma oncogenesis. The small molecule YK-4-279 blocking the binding of EWS-FLI1 to RHA interaction leads to negation of the modulation on helicase activity and thus causes apoptosis.

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462 Biochemical characterization of peripheral domain effects on the activity of a DEAD-Box protein required for ribosome biogenesis in *S. cerevisiae*

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Ribosome processing and assembly is a highly ordered and dynamic process that involves many accessory proteins. To date, over eighteen DExD/H-box proteins (putative unwindases) are implicated in modulating RNA-RNA, RNA-protein, or protein-protein interactions as well as facilitating nucleases during rRNA processing [1]. DExD/H-box proteins achieve *in vivo* RNA specificity by targeting protein cofactors or RNA substrates through their N-terminal and/or C-terminal peripheral domains. For example, Rok1p is a *S. cerevisiae* DEAD-box protein essential for early 40S processing. Several studies suggest or illustrate that Rrp5, an RNA-binding protein, facilitates the association of Rok1p and pre-rRNA [2-4]. The binding of Rrp5 is believed to support Rok1p mediated formation of the pre-A_2 duplex[4]. To investigate the role that peripheral domains play in the DExD/H-box protein catalytic cycle, the degree of ATP hydrolysis and duplex displacement with Rok1p domain-deletion-variants were examined. We have previous demonstrated that Rok1p is a RNA-dependent ATPase with a k_{cat} of 19 ± 1 min^{-1} and $K_{m,ATP}$ of 0.31 ± 0.07 mM. In the absences of either the N-terminal and/or C-terminal peripheral domain, the rate of hydrolysis as well as the apparent binding affinity for ATP slightly increases. The $K_{m, RNA}$, however, weakens by up to 7-fold. Besides ATP hydrolysis, unwinding was observed with a variety of simple RNA duplexes in an ATP-dependent fashion. For two of the three domain-deletion-variants, the unwinding rate was affected to varying extents by the length of the RNA loading strand. This comparative examination suggests that peripheral domains can regulate the intrinsic activity of DEAD-box proteins as well as confer substrate specificity.

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463 Hepatitis delta antigen requires a flexible, quasi-double-stranded RNA secondary structure for binding and condensing HDV RNA

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Hepatitis delta virus (HDV) is an RNA virus that causes the most severe liver disease of the hepatitis viruses. An RNA-protein complex (RNP) formed by the interaction of the hepatitis delta antigen (HDAg) and HDV RNA is required for both virion formation and for viral replication, which occurs via redirection of host RNA polymerase II to RNA-dependent RNA transcription. The unique 1680 nt single-stranded RNA genome of HDV is circular and folds into an extended quasi-double-stranded hairpin with intermittent internal loops and bulges. HDAg specifically binds this structure with high affinity. Outside of an unusual length requirement (~300 nt), the RNA structural determinants for RNP formation are not well understood. We have investigated the RNA sequence and structure features required for HDAg binding with in vitro binding analysis of mutations to a 311 nt HDV RNA segment. Our results indicate that the characteristic RNA structure of HDV RNA, specifically the presence of internal loops and bulges, is the major determinant of HDAg RNA binding specificity. Surprisingly, primary RNA sequence is not recognized by the protein. In addition, base substitutions with inosine or diaminopurine indicated that binding affinity is correlated with increased flexibility of the RNA. Thus, we suggest that the internal loops and bulges contribute flexibility to the quasi-double-stranded RNA structure that allows HDAg binding. Consistent with this idea, selective 2′OH acylation analyzed by primer extension (SHAPE) applied to free and HDAg-bound HDV RNAs indicated that the distinctive secondary structure of the RNA is preserved when bound to HDAg. However, the analysis also indicated that many predicted unpaired positions in the RNA became more dynamic in the RNP. Atomic force microscopy analysis of RNPs formed in vitro revealed complexes reminiscent of nucleosomes in which the HDV RNA is significantly condensed by bending or wrapping. Our results support a model in which the internal loops and bulges in HDV RNA contribute flexibility to the quasi-double-stranded structure that allows RNA bending and condensing by HDAg. We continue to study the role of RNA structure and shape in directing the assembly of HDAg into RNPs with the full length 1680 nt RNA genome.

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464 Role for arginine methylation of RNA binding protein HnRNPUL1 in DNA damage signalling

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The DNA damage response (DDR) is an important cellular process to maintain genomic stability. Failure to repair DNA lesions often leads to the accumulation of genetic alterations causing genomic instability, an emerging hallmark of cancer. Several enzymatic activities that post-translationally modify proteins have been involved in the DDR. Recent research has implicated proteins involved in RNA metabolism to play a role in DDR, including RNA helicases and RNA binding proteins (RBPs) such as RBMX and DDX17, which have been identified to participate in the DDR pathway (Adamson et al., 2012). HnRNPUL1 was shown to bind NBS1 and CtIP and participate in the DDR (Polo et al.,2012).

RNA binding proteins are known targets of protein arginine methyltransferases (PRMTs) (Côté et al.,2003). Since arginine methylation of MRE11 regulated their functions in DDR, arginine methylation could potentially regulate the functions of RBPs involved in DDR (Boisvert et al.,2005). Herein we investigated if arginine methylation could regulate the function of HnRNPUL1 in the DDR.

We show that hnRNPUL1 is indeed arginine methylated, as confirmed by the arginine methylation assay with PRMT1. Three prominent RGG/RG motifs of hnRNPUL1 are methylated. Furthermore, we show that hnRNPUL1 is arginine methylated in vivo using mass spectrometry. Moreover, PRMT1 co-immunoprecipitates with hnRNPUL1 and the knockdown of PRMT1 via siRNAs confers a hypo-methylation status of hnRNPUL1. Mutants harboring R to K mutations of the identified methyl-arginines were generated. Flag-hnRNPUL1 wild type is arginine methylated in vivo, however, the Flag-hnRNPUL1R-K mutant was not. Moreover, PRMT1 does not co-immunoprecipitate with the flag-hnRNPUL1R-K mutant protein. Foci formation studies of GFP-hnRNPUL1 and GFP- hnRNPUL1R-K mutant post laser scissor damage demonstrate unique recruitment and exclusion patterns, respectively (Coulombe Y. & Masson J.Y). Furthermore, we show that NBS-1 does not co-immunoprecipitate with the Flag-HnRNPUL1R-K mutant, suggesting that methylation of hnRNPUL1 is essential for its interaction with NBS1.

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465 iCLIP analysis 2.0: Precise binding site assignment in the presence of RBP-dependent readthrough cDNAs

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Gene function depends on the regulated and dynamic formation of RNA-protein complexes. Following the development of crosslinking and immunoprecipitation (CLIP) methods combined with high-throughput sequencing such RNA-protein interactions can be characterized in vivo and on a global scale.

Individual-nucleotide resolution CLIP (iCLIP) currently represents the most precise technology to determine the localization of binding sites of RNA-binding proteins (RBP). iCLIP is based on the principle that cDNA synthesis terminates precisely at the site where the immunoprecipitated polypeptide is crosslinked to the RNA.

Here, we have analyzed the iCLIP libraries of different RBPs including U2AF65, eIF4A3, SRSF3 and hnRNP L in exons compared to introns. We find that the exonic start sites of the fragments of eIF4A3, SRSF3 and hnRNP L, but not U2AF65, depend on the length of the fragment. With increased fragment length, the start sites are shifted upstream relative to their known region of RNA binding. Whereas this effect is very pronounced in reads that map to exons, it is less noticeable in intronic reads.

Therefore, we suggest that the exonic binding sites can be assigned from fragments that were sequenced completely from the 5′ to the 3′ solexa primers by using the center of these fragments. For eIF4A3, SRSF3 and hnRNP L, this approach could correct for the difference in the positioning of the exonic read start sites, and better align it to the known RNA binding motifs of these proteins.

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466 Post-transcriptional gene regulation by Roquin binding to the mRNAs of ICOS and Ox40

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Acitvation of T helper cells leads to induced expression of costimulatory receptors including the inducible costimulator Icos or the TNF receptor Ox40. Such activation-induced costimulators are essential for T cell differentiation and effector function. Posttranscriptional gene regulation alters translation and stability of mRNAs. This level of regulation mediates fast changes of protein expression and controls immune cell decisions.

Previous work has shown that the *Icos* mRNA is regulated by the RNA-binding protein Roquin-1. A single amino acid exchange (M199R) in this protein results in severe autoimmunity in mice driven by T cell activation and accumulation of follicular helper T cells. All T cells of these mice have highly increased Icos levels on their cell surface. In contrast, a conditional Roquin-1 knock-out showed moderately elevated Icos expression on T cells but no autoimmunity. These findings suggested that Roquin-1 function is compensated upon gene deletion. It also suggested the existence of additional targets of Roquin in the control of autoimmunity. Analyzing a conditional mouse model of combined Roquin-1 and -2 deletion in T cells we show that Roquin-2, the paralog of Roquin-1, can functionally compensate for loss of Roquin-1 in T cells. The loss of both proteins resulted in severe T cell activation and increased differentiation of Tfh cells as well as highest expression levels of Icos. To identify additional mRNA targets of Roquin-1/2 we performed microarray analyses of Th1 cells upon acute deletion of Roquin-1/2. In this approach we identified Ox40 as a potential Roquin target. Comparable to *Icos*, the *Ox40* mRNA was bound by Roquin-1/2, which resulted in the downregulation of Ox40 on the cell surface. Recently, a Roquin-recognized *cis*-element, the so-called constitutive decay element (CDE), has been described in the *Tnf* 3′UTR. This regulatory RNA element forms a stem-loop structure, which was sufficient for Roquin-induced degradation of a reporter mRNA. An RNA element with the CDE-consensus is found at the 3′ end of the *Icos* 3′UTR, however it is absent in the *Ox40* 3′UTR. Our current research therefore focuses on the characterization of the Roquin-1 binding to the mRNAs with the aim to decipher the requirements of this interaction.

467 HuR and AU-rich elements regulate the induction of AChR β-subunit mRNAs after skeletal muscle denervation

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AU-rich elements (AREs), located in the 3′UTR of labile transcripts are known to be key in mediating mRNA decay. By binding to AREs, RNA-binding proteins, such as HuR, post-transcriptionally regulate expression of genes involved in myogenesis. In this context, HuR stabilizes and increases MyoD and myogenin mRNA levels. In differentiating muscle cells, HuR binds to acetylcholinesterase (AChE) transcripts via direct interaction with AREs. This is of particular interest since AChE is localized at the neuromuscular junction (NMJ) in mature muscle. Defective NMJ and sciatic nerve transection (causing muscle denervation) result in impaired neuromuscular transmission leading to muscle weakness and atrophy. Previous work has shown that muscle denervation transcriptionally increases expression of acetylcholine receptor (AChR)β-subunit mRNAs. Nevertheless, it remains also possible that post-transcriptional mechanisms are involved in the overexpression of AChRβ-subunit induced by denervation.

We therefore examined whether post-transcriptional events can indeed regulate expression of AChRβ-subunit mRNAs in response to denervation. First, analysis of mouse denervated muscle showed an increase of up to 4-fold in mRNA levels for numerous elements involved in the formation and maintenance of NMJ such as AChRβ-subunit, Dok7, LRP4, MuSK, myogenin and rapsyn, together with a decrease in AChE mRNA levels. In order to determine whether post-transcriptional mechanisms are involved in the induction of AChRβ-subunit mRNAs in response to denervation, we injected mouse tibialis anterior muscle with luciferase constructs containing either AChRβ-subunit 3′UTR or AChRβ-subunit 3′UTR containing a mutated ARE, and examined luciferase activity 3 and 7 days post-denervation. Under these conditions, we observed a 85% increase in luciferase activity 3 days post-denervation with AChRβ-subunit 3′UTR construct, suggesting that post-transcriptional events control expression of endogenous AChRβ-subunit mRNAs in denervated muscle. ARE mutation prevented this increase, thereby highlighting the critical role of this element in mediating changes in AChRβ-subunit mRNA stability. We also show that HuR mRNA and protein levels are increased after denervation. Finally, we demonstrate that HuR binds to the AChRβ-subunit transcripts in cultured myotubes.

Taken together, these results demonstrate the contribution of post-transcriptional events in regulating expression of AChRβ-subunit mRNAs in denervated muscle and point toward a central role for HuR in mediating synaptic plasticity.

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468 Binding Determinants of Pokeweed Antiviral Protein to REV HIV-1 RNA

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The pokeweed antiviral protein (PAP), isolated from the plant *Phytolacca americana*, is an N-glycosidase that removes purines from RNA. PAP exhibits antiviral activity by depurinating nucleotides within the open reading frames of several different viral RNAs. Damaged viral RNAs are not efficiently translated by ribosomes, resulting in a marked reduction in viral protein synthesis. The heterologous expression of PAP in virus infected cells inhibits virus production without affecting translation of cellular mRNAs. The apparent specificity of PAP for viral RNA is not well understood and it is not known if PAP binds to a primary sequence or secondary structure of viral RNA. This research was conducted to distinguish the importance of either a conserved primary RNA sequence or a secondary structure in the preferential binding of PAP to RNA. The HIV-1 REV was chosen for this study as our recent findings show the RNA is a target for depurination by PAP. The interaction of PAP with HIV-1 REV RNA was compared to other *in vitro* transcribed RNA substrates using northwestern blotting, electrophoretic mobility shift, and filter binding assays. PAP bound selectively to REV RNA and a known native target, the 28S rRNA sarcin/ricin loop, compared with GAPDH RNA and Arg-tRNA. Footprinting analysis of PAP to REV RNA showed no sequence homology to the known binding site on 28S rRNA. We are currently investigating whether the secondary structure of the PAP binding site in REV resembles PAP's native binding in rRNA by conducting SHAPE analysis of the REV RNA *in vitro*. Compensatory mutations and subsequent binding assays will confirm the important structural features required for PAP binding to an RNA template. This research provides new knowledge on how PAP interacts with RNA, namely that PAP binds to particular structural motifs instead of a specific sequence.

469 IMP3 RNP Safe House prevents miRNA-Directed *HMGA2* mRNA Decay in Cancer and Development

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The IMP3 RNA-binding protein is associated with metastasis and poor outcome in human cancer. Using solid cancer transcriptome data, we found that *IMP3* correlates with *HMGA2* mRNA expression. Cytoplasmic IMP3 granules contain *HMGA2* mRNA, and IMP3 dose-dependently increases *HMGA2* expression. *HMGA2* mRNA is regulated by *let-7*, and *let-7* antagomiRs make *HMGA2* mRNA refractory to IMP3. Removal of *let-7* target sites eliminates IMP3-dependent stabilization, and IMP3-containing RNPs are depleted of Ago1-4 and miRNAs. The relationship between *Hmga2* mRNA and IMPs also exists in the developing limb bud where IMP1 deficient embryos show dose-dependent *Hmga2* mRNA down-regulation. Finally, IMP3 RNPs contain other *let-7* targets, including *LIN28B* mRNA, and a global GSEA analysis demonstrates that miRNA-regulated transcripts in general are up-regulated following IMP3 induction. We conclude that IMP3 RNPs may function as cytoplasmic safe houses by preventing miRNA-directed mRNA decay of oncogenes during tumor progression.

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470 A novel method to examine RNA-protein interactions reveals insights into HIV-1 RNA genome packaging

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Conventional methods used to map the footprints of proteins onto RNA examine reactivity/accessibility of parts of the RNA molecule. Where the RNA is no longer accessible/reactive to the reagents, it is assumed that a protein is bound. However, this can provide an incomplete or misleading picture: for example, a protein binding to one strand of a helix and causing the opposite strand to bind elsewhere can have no apparent footprint, leaving protein binding site and corresponding structural change undetected. We have modified a method of cross-linking RNA-protein complexes (Urlaub et al, J Biol Chem (2000), 275, 41458-41468), and coupled it with SHAPE (selective 2′OH acylation analysed by primer extension) to generate a novel, rapid way of examining RNA-protein interactions. This high-throughput cross-linking/SHAPE identifies sites of interaction between RNA and protein and the corresponding structural changes within the RNA. Briefly, RNA-protein complexes are UV cross-linked at 254nm, denatured and protease-treated to leave a residual peptide at the cross-link site. These are mapped using reverse transcription and a fluorophore labelling and analysis system similar to that of high-throughput SHAPE. In parallel, the RNA only and RNA-protein complex are subjected to high-throughput SHAPE to model the structural changes occurring to the RNA upon protein binding. We have developed the technique using the well characterised interaction of HIV-1 TAR RNA with the Tat protein, and validated it using the MS2 phage RNA stem-loops and coat-binding protein. We then applied it to study HIV-1 packaging.

The HIV-1 genome is selected for packaging by the viral structural protein, Gag. This highly specific interaction allows Gag to recognise the genomic viral RNA amongst the wealth of other RNA species in the cytoplasm. The details of this interaction, however, remain unclear. One high-affinity binding site has been observed (SL3) but packaging appears to be a multi-step process and is likely to involve structural changes in both genomic RNA and Gag protein. Our technique identifies the regions in the genomic RNA to which Gag protein binds and the RNA structural changes that ensue, enabling us to build a model for how the HIV-1 packaging process proceeds.

471 Induced RNA structural changes upon substrate recognition by the Thiostrepton-Resistance methyltransferase (Tsr) are necessary for catalysis

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Ribosomal RNA posttranscriptional modifications are essential for ribosome maturation and fidelity and can also confer a high level of bacterial resistance to ribosome-targeting antibiotics. This resistance mechanism is commonly employed by antibiotic-producing bacteria to protect themselves against their own products, but is becoming increasingly prevalent in human and animal pathogens. In the thiostrepton producer Streptomyces azureus, the Thiostrepton-Resistance methyltransferase (Tsr) methylates the antibiotic binding site on the 23S ribosomal RNA thereby inhibiting its binding and action against the bacterial ribosome. To deepen our understanding of how antibiotic-resistance conferring, rRNA modification enzymes recognize their RNA substrates, we have biochemically dissected Tsr's substrate recognition mechanism using RNA structure probing and assess binding of Tsr to RNA and enzymatic activity. We find that Tsr induces significant changes in the RNA structure upon binding that are essential for proper substrate recognition and catalysis. Tsr is composed of an amino-terminal (NTD), and carboxyl-terminal domain (CTD) that have been assigned a primary role in substrate recognition and catalysis respectively. We show that in fact both domains are important for RNA binding. Our data suggest a model in which the NTD unfolds the RNA tertiary structure and recognizes a RNA secondary structure element distant from the site of methylation in a step necessary for catalysis. These findings provide new insights into the intricacies of substrate recognition for antibiotic-resistance conferring and other RNA modifying enzymes that recognize the bacterial ribosome.

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472 Stepwise assembly of the pluripotency factor Lin28 on the terminal loop of let-7 miRNA precursors

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Lin28 inhibits the biogenesis of let-7 miRNAs through direct interactions with let-7 precursors. Previous biochemical and structural studies have described seemingly inconsistent data for Lin28 binding to the terminal loop of pre-let-7 RNAs (TL-let-7), since most studies report a 1:1 binding stoichiometry, but three different Lin28-binding sites have been identified on TL-let-7 RNAs. Here, we reconcile these data by examining the binding mechanism of Lin28 to the terminal loop of pre-let-7g (TL-let-7g) using biochemical and biophysical methods. We first characterize the binding of Lin28 to TL-let-7g variants and short RNA fragments derived from TL-let-7g using electrophoretic mobility shift assay (EMSA). In these studies, we identify three independent binding sites for Lin28 on TL-let-7g, in agreement with previous studies. Using a stoichiometric binding assay, we then determine that Lin28 assembles in a stepwise manner on TL-let-7g to form a stable 1:3 complex. Both RNA-binding domains of Lin28 contribute to this stepwise assembly on TL-let-7g; the cold-shock domain (CSD) is responsible for remodelling the terminal loop of TL-let-7g, whereas the NCp7-like domain facilitates the initial binding to TL-let-7g. This stable binding of multiple Lin28 molecules to the terminal loop of pre-let-7g is also observed with other precursors of the let-7 family, but not with other pre-miRNAs tested. Fluorescence studies with several TL-let-7g RNAs containing single 2-aminopurine modifications indicate that formation of the 1:1 complex significantly remodels the internal loop by affecting residues within the three Lin28 binding sites, whereas formation of the 1:2 and 1:3 complexes has more localized effects on individual Lin28-binding sites. Based on our data, we propose a model for stepwise assembly of the 1:1, 1:2 and 1:3 pre-let-7g/Lin28 complexes. Finally, we show that stepwise multimerization of Lin28 on pre-let-7 is required for maximum inhibition of Dicer cleavage of at least one member of the let-7 family. Similarly, multimerization of Lin28 on pre-let-7 may be important for orchestrating the activity of the other factors that regulate let-7 biogenesis.

473 Molecular details of nucleic-acid binding by the C. elegans splicing protein SUP-12

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The selective inclusion or exclusion of exons by alternative splicing generates transcript variety from a single gene. This process is regulated in response to external stimuli, as well as in a time- and location-dependent manner such as during tissue development. Disruption of the alternative splicing leads to potential abnormalities and disease via the generation of miss-timed or aberrant protein isoforms. We are currently investigating the molecular basis by which alternative splicing is regulated in multicellular organisms, in particular the development of tissue in *C. elegans*. As a model system, the fibroblast growth factor receptor *egl-15* gene forms a muscle-specific isoform driven by the coordinated binding of the splicing factor SUP-12 together with a member of the Fox-1 family of splicing proteins such as ASD-1. The expression of these proteins is restricted to muscle cells where they bind to the *egl-15* pre-mRNA and due to the use of a mutually exclusive exon produce an EGL-15 isoform with altered fibroblast growth factor specificity. We have used a combination of NMR spectroscopy and isothermal titration calorimetry (ITC) to determine molecular details of nucleic-acid binding by the RNA recognition motif (RRM) domain from SUP-12. Surprisingly, we found that SUP-12 displays equally strong binding to RNA or DNA centred on the sequence G-U/T-G-U/T-G. The ability to bind to these two classes of nucleic acid was instrumental in determining the atomic details of nucleotide recognition. A series of protein mutations coupled with an extensive range of nucleotide sequence variants was used to confirm this association mechanism. In addition, the structural details were used to design strategic mutations to quantitatively perturb the splicing pattern in live nematodes and measured by using a fluorescent mini-gene reporter and large object flow cytometer (COPAS) instrument. The *in vivo* splicing perturbation correlates with the in vitro findings, and highlight a narrow tolerance for changes in the affinity between SUP-12 and the pre-mRNA binding site. We have also extended our study to look at the interplay between SUP-12 and the second protein factor, ASD-1.

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474 Identification of novel G-quadruplexes recognized by the helicase RHAU, a human quadruplex resolvase

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RHAU (RNA helicase associated with AU-rich element, also known as DHX36) is an ATP-dependent RNA helicase (DExH/D family) that preferentially binds and unwinds G-quadruplexes [1]. G-quadruplexes spontaneously form from sequences containing consecutive runs of guanines (G-tracts), and assemble into stable stacks of planar G-quartets that rely on non Watson-Crick base pairing and monovalent cations for their stability[2]. G-quadruplexes play a critical regulatory role in a number of contexts, particularly in the regulation of RNA stability and gene transcription. We have identified 304 novel RHAU-interacting RNAs through immunoprecipitation of RHAU followed by subsequent cloning and sequencing of the co-purified RNA. Of these RNAs, 20 were further validated by co-immunoprecipitation/RT-PCR experiments. This remarkable number of interactions suggests that RHAU has broad implications in controlling RNA metabolism/regulation. An exciting outcome from this screen was the identification of PITX1 (paired-like homeodomain transcription factor) messenger RNA. PITX1 is known to act as a tumour suppressor via transcriptional activation of p53 and RASAL1, and transcriptional suppression of hTERT [3; 4; 5]. We present data demonstrating that RNA quadruplexes in the 3′-untranslated region of PITX1 mRNA are RHAU binding sites, and we detail our investigations into the functional mechanisms whereby regulation of PITX1 expression is achieved by RHAU. We conclude by presenting our preliminary structural characterization of G-quadruplex recognition by RHAU.

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475 RRP43 temperature sensitive mutants affect exosome assembly and the interaction between the complex and other cellular proteins

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The yeast exosome is a conserved multiprotein complex essential for processing and degradation of a wide variety of RNAs. The complex is formed by a nine-subunit core that associates with two hydrolytic 3′-5′ exoribonucleases. Although catalytically inert, the assembly of this nine-subunit core seems to be essential for the exosome activity, as mutations in regions that do not directly bind RNA or are not in the active sites of the exonucleases impair the function of the complex in the cellular context. Previously isolated mutations in the exosome core subunit Rrp43p have been shown to affect the function of the complex. With the aim of investigating the effect of these mutations on the complex stability and activity, Rrp43p and its mutant forms were purified by means of the TAP method. Mass spectrometry analyses showed that lower amounts of the exosome subunits are co-purified with the mutant Rrp43p proteins. Additionally, by decreasing the stability of the exosome, other non-specific protein interactions are favored, allowing for the co-purification of different proteins with functions unrelated to the RNA exosome. Samples purified from mutant cells exhibited increased exonuclease activity, suggesting higher dissociation constants for these mutant complexes. Therefore, data reported here indicate that complexes containing a mutant Rrp43p exhibit decreased stability and provide an explanation for the RNA degradation defect observed in cells expressing such mutant complexes. In addition, we are currently interested in identifying other proteins that might associate with the exosome and influence its assembly, stability and activity.

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476 Structural characterization of a novel eukaryotic family of proteinaceous RNase P

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The RNase P activity is ubiquitous and consists of the 5′ maturation of pre-tRNAs. For a long time it has been thought that all RNase P were ribozymes. However, the characterization of the human mitochondrial RNase P revealed a novel kind of RNase P composed of proteins only, called PRORP for "Proteinaceous RNase P" [1]. Whereas in human mitochondria PRORP is formed by a complex of three subunits, RNase P activity in *Arabidopsis thaliana* is held by a single protein. There are three homologs in *A. thaliana*: PRORP1 is located to mitochondria and chloroplasts, PRORP2 and PRORP3 are located to the nucleus. Each protein possesses a pentatricopeptide repeat domain (PPR) and a metallonuclease domain [2].

Here we provide biophysical and functional data to understand the mode of action of PRORP enzymes [3]. Activity assays and footprinting experiments show that the anticodon domain of tRNA is dispensable, whereas individual residues in D and TψC loops are essential for PRORP recognition. The affinity constant between a minimal functional substrate and a catalytic mutant of PRORP2 determined by microscale thermophoresis and isothermal titration calorimetry is in the range 0.6 - 1 μM. A molecular envelope of PRORP 1 and 2 proteins was derived from small-angle X-ray scattering in solution confirming a two domain architecture (in agreement with the crystal structure of PRORP1). Conserved residues are shown to be involved in the binding of one zinc atom to PRORP. These results led to the elaboration of a model of the PRORP/tRNA interaction. The comparison with the ribonucleoprotein RNase P/tRNA complex suggests that transfer RNA recognition by PRORP proteins is similar to that by ribonucleoprotein RNase P.

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477 Complementary sequence properties of proteins and their cognate mRNAs suggest direct binding as the physicochemical foundation of the genetic code

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The ability to interact with messenger RNA (mRNA) has recently been reported for a number of known RNA binding proteins, but surprisingly also for different proteins without recognizable RNA binding domains, including several transcription factors and metabolic enzymes. Being potentially important in the context of translational auto-regulation, direct binding of a diverse set of proteins to their cognate mRNAs also creates a challenge to search for basic physico-chemical principles behind such interactions. Using experimentally and computationally derived measures of solubility of amino acids in aqueous solutions of pyrimidine analogs together with knowledge-based interaction preferences of amino acids for different nucleobases, we have revealed a statistically significant proteome-wide matching between the composition of coding mRNA sequences and the base-binding preferences of their cognate protein sequences [1, 2]. From an evolutionary prospective, our findings provide strong support for the stereochemical hypothesis of genetic code's origin, whereby the code evolved as a consequence of direct interactions between amino acids and appropriate bases [3]. These results furthermore suggest a possibility of direct binding between mRNAs and their cognate proteins even in present-day cells, not only in coding, but also in untranslated regions. Moreover, matching between complementary sequence properties of RNAs and proteins provides a novel, general principle for rationalizing and predicting interactions between the two in contexts going beyond just mRNAs and their cognate proteins. As a proof of this concept, our computational framework, when applied to archeal, bacterial or eukaryotic ribosomal RNAs (rRNAs), correctly identifies ribosomal proteins that directly interact with rRNA and are important for ribosomal assembly. Finally, functional annotation of predicted direct binders to cognate mRNAs for the human proteome reveals a surprising enrichment of transcription factors, for which such property may be functionally important when it comes to regulatory feedback loops in translation.

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478 Division of labor: separation of loading and unwinding units in an RNA helicase oligomer

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DEAD-box RNA helicases are involved in virtually all aspects of eukaryotic RNA metabolism. These ubiquitous enzymes remodel RNA and RNPs in an ATP-dependent fashion. Several lines of evidence suggest that at least a subset of DEAD-box helicases form oligomers. However, the impact of oligmerization on the function of DEAD-box helicases has not been examined. Here we show that the DEAD-box helicase Ded1p from *Saccharomyces cerevisiae* forms oligomers in vitro and in the cell. We also show that the C-terminal domain of Ded1p is critical for oligomerization, and that interaction of Ded1p with eIF4G, which is mediated by the C-terminus of Ded1p, interferes with the oligomerization of the helicase. To understand the oligomerization of Ded1p on a mechanistic level, we apply ensemble biochemical and single molecule fluorescence approaches. We find that Ded1p acts as a trimer during duplex unwinding, and make the remarkable observation that individual units of Ded1p have clearly separate activities. Two units bind single stranded RNA and load a third unit to the duplex region. Only this unit directly separates the strands. However, the bulk of ATP hydrolysis is associated with the units bound to the single stranded region; yet, ATP hydrolysis, and even ATP binding by these units is dispensable for the unwinding reaction. Our findings provide the first mechanistic description of a DEAD-box helicase oligomer, revealing an unprecedented mechanism where identical enzyme units perform fundamentally distinct roles.

479 Exon Junction Complex (EJC) protein components are recruited at transcription sites independently of splicing in Drosophila melanogaster

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The results of number of studies across organisms show that that pre-mRNA splicing, a strictly nuclear process, affect NMD, a process expected to be strictly cytoplasmic. It has been proposed that this link is mediated by the exon junction complex (EJC), a multiprotein complex deposited during splicing in the nucleus, which remains associated with the mRNA during export to the cytoplasm. Some observations are not consistent with this function attributed to the EJC. Additionally, all of the proteins that constitute the EJC are well conserved in Drosophila, yet these proteins are not required for NMD in this organism. To understand better the function of the EJC, we aimed to visualize its association with nascent RNA at the polytene chromosomes of Drosophila. Notably, we found that EJC components are recruited at transcription puffs corresponding to both intron-containing and intron-less genes. The finding was confirmed in S2 Drosophila cells using chromatin immunoprecepitation coupled to high throughput sequencing (ChIP-seq). Presently, we are investigating, whether EJC proteins are required for the expression of selected genes. We will present our latest results at the meeting.

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480 Investigating the structural basis for Roquin-mediated post-transcriptional gene regulation

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T cell differentiation and the launching of an effective adaptive immune response require the complex interplay of T cell co-receptors and cytokines whose abundance is tightly regulated on the mRNA level. Misbalanced expression of these factors can lead to autoimmune diseases. The protein Roquin plays a central role in the regulation of key steps for the onset of (auto)immunity. It was found to destabilize the mRNAs of the inducible co-stimulator ICOS, the co-receptor Ox40, and the cytokine Tnf. ICOS overexpression was suggested to provoke T cell activation which results in enhanced systemic hypersensitivity, showing a phenotype similar to Systemic Lupus Erythematous (SLE).

Roquin is a 125 kDa protein and belongs to the group of E3-ubiquitin ligases. It is mainly predicted to be unstructured, but - besides a RING domain - Roquin exhibits a zinc-finger domain and an additional N-terminal domain, called ROQ. Evidence is mounting that Roquin itself is an RNA-binding protein via its ROQ domain. Recently, it was shown that Roquin recognizes a conserved class of mRNA stem-loops, termed constitutive decay elements (CDE) suggesting a total of more than 50 vertebrate mRNAs to be targeted. However, the mode of RNA binding, the domains involved and structural details of these interactions are unknown.

We take a divide-and-conquer approach for structural analysis of Roquin by expressing and studying smaller fragments for structural studies. We found that both the RING and zinc finger domains are soluble proteins, suitable for NMR analysis. Moreover, we were able to generate soluble fragments of various lengths of the predicted RNA-binding domain of Roquin (ROQ) that showed binding of a 23-mer RNA in NMR titration experiments. Structural studies of the ROQ domain free and when bound to the 23-mer RNA are underway. The protein-RNA interaction is further studied by small angle scattering experiments and biophysical techniques to investigate the interplay of multiple domains in Roquin for RNA binding and additional interactions.

481 The Nucleic Acid Binding Mechanism of the PWI Motif

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The PWI motif is a highly conserved domain that is named after a nearly invariant Pro-Trp-Ile sequence and is found in several proteins that are known or predicted to be involved in the processing of pre-messenger RNA. PWI motif containing proteins play roles in constitutive and alternative splicing and in the 3′-end cleavage of transcripts. The motif is also found in mammalian homologs of the yeast Prp3p protein, which is associated with the U4/U6 snRNP. Three distinct classes of the PWI motif are definable by sequence homology, the position of the motif in the protein and the presence of an adjacent sequence that is rich in basic amino acids. The PWI motif folds into a four-helix bundle and has little to no affinity for nucleic acids itself. Optimum binding of the motif to both single and double-stranded DNA and RNA requires the adjacent basic region in human SRm160, PRP3 and RBM25 proteins. The individual roles of the PWI motif and the basic region in nucleic acid binding and the mechanism by which the PWI motif containing proteins associate with nucleic acids are still not clear. We are currently using NMR and fluorescence spectroscopies to investigate the mechanism of PWI motif binding to nucleic acids.

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482 Constitutive patterns of gene expression regulated by RNA-binding proteins

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RNA-binding proteins regulate several processes such as synthesis, folding, translocation, assembly and clearance of RNAs. Recent studies reported that a previously unexpected number of proteins are able to interact with RNA, but partners of many RNA-binding proteins are still uncharacterized.

We combined prediction of ribonucleoprotein interactions, based on catRAPID calculations, with the analysis of protein-RNA expression profiles from human tissues. We found strong interaction propensities for positively and negatively-correlated expression patterns. Our integration of *in silico* and *ex vivo* data unraveled two major types of protein-RNA interactions related to *cell cycle control* (positively-correlated patterns) and *survival, growth and differentiation* (negatively-correlated patterns).

Our analysis sheds light on the role of RNA-binding proteins in regulating proliferation and controlling differentiation processes. We hope that our results will be useful to design future experimental studies.

483 Structural studies of organellar RNA processing factors: The PPR proteins

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Numerous recently identified mutations leading to mitochondrial diseases have been shown to alter the RNA processing machineries rather than the respiratory complexes themselves. As for the cytoplasmic mRNAs, the maturation of the mitochondrial mRNAs is a highly regulated process. According to the punctuation model, multiple processing steps are present prior to the "final" mRNA polyadenylation step. The interconnection between these processing steps has a high potential for pharmaceutical treatments. Many of the proteins involved in these maturation steps contain large array of pentatricopeptide repeat or PPR. This protein motif is a 35 amino acids repeat, which folds into two α-helices connected by a short linker, which is almost exclusively found in proteins residing in the organelles. Recently, a series of published atomic models has clarified the "PPR code". However, it appeared that only a few PPR motifs were bound to a nucleobase and that a large part of the protein was used only as a scaffold rather than as a RNA binding site. Important architectural aspects explaining the capacity of PPR proteins to assemble into large ribonucleoprotein complexes containing both their RNA targets and their protein partners are still left unexplained by these studies.

In an attempt to further understand the intrinsic properties of PPR proteins, we have designed new PPR proteins with pre-determined binding specificity. We characterized in vitro their binding properties and solved the atomic models of various engineered PPR proteins. These atomic structures and their associated biochemical characterizations will be presented. They revealed an unexpected modularity within the PPR protein architecture as well as rationalize several hints on the determinant behind their atypical RNA binding properties. These studies will help to understand the logic behind PPR protein association with RNA and how we can use these properties for our own interest. Furthermore, we are determining the atomic structure of the PPR protein complexes involved in the late step of the mitochondrial mRNA maturation pathway. Latest development of these projects will also be presented.

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484 Remodeling of U2 snRNA/Cus2 Complexes by the DEAD-box ATPase Prp5

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The spliceosome assembles *de novo* on each pre-mRNA substrate to remove introns and ligate exons. Besides the individual snRNPs, eight DExD/H-box proteins with RNA-stimulated ATPase activity play important roles in promoting spliceosome remodeling and ensuring the fidelity of splicing. One key player is the DEAD-box ATPase Prp5 that promotes stable U2 snRNP association with the branchpoint during pre-spliceosome formation. While the Prp5 protein is essential for pre-spliceosome assembly, ATP hydrolysis by Prp5 is only required in the presence of the U2 snRNA binding protein Cus2. However, the interplay between U2 snRNA, Cus2, Prp5 binding, and ATP hydrolysis are poorly understood. We are using biochemical and single molecule studies to investigate the role of Cus2 and Prp5 in pre-spliceosome formation. It has previously been reported that the U2 snRNA toggles between two different conformations (stem IIa and stem IIc) during splicing; however, only the stem IIa conformation is competent for efficient U2 snRNP recruitment to the branchpoint[1]. Using an *in vitro* model system, we show that Cus2 displays a clear preference for binding to the stem IIa conformation of the U2 snRNA-indicating a potential role as a placeholder on U2 for restricting the snRNA conformation to favor branchsite association. Preliminary data suggest displacement of Cus2 from the U2 snRNAs coupled with the ATP-hydrolysis activity of Prp5. These data indicate an RNA-protein-remodeling function for Prp5 during U2 snRNP recruitment. In depth biochemical analysis of Prp5′s ATPase activity using nucleotide analogs, helicase, and RNA binding activities suggest that its core properties are reminiscent to those described for other DEAD-box proteins[2,3]. We predict that the specific biochemical attributes of Prp5 are responsible for its role in spliceosome assembly.

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485 RNA binding without motifs explains the large size of the FUS trasncriptome

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FUS (Fused in sarcoma is an important nuclear RNA-binding protein as mutations in FUS cause neurodegenerative diseases including Amyotrophic Lateral Sclerosis. The normal functions of FUS are still not fully understood. RNAs play an active role in FUS functions and many RNA targets have been identified. Using electrophoretic mobility shift assays(EMSA), we find that FUS binds numerous RNAs cooperatively and both the RRM(RNA recognition motif and the zinc finger of FUS contribute to binding to RNAs. Four different laboratories have identified specific FUS-binding motifs. We have tested these proposed motifs side-by-side, and find that they all bind FUS but that many noncoding and coding RNas bind with similar affinity. Furthermore, FUS binds to RNA with a length-dependence predicted for non-specific binding. We propose a model in which this promiscuous binding of FUS allows it to form close-packed arrays along numerous RNAs, which recruits bindings of the CTD(C-terminal domain) of RNA polymerase II.

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486 The core NMD protein UPF1 is required for genome stability in fission yeast

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Up-frameshift protein 1 (UPF1) is a protein conserved across eukaryotes that is required for nonsense-mediated mRNA decay (NMD). NMD is strictly linked to translation thus is believed to happen only in the cytoplasm. However, there is evidence that UPF1 might also have nuclear functions, which might be independent of its role in NMD. The strongest line of evidence for UPF1 having a nuclear function is this lab previous genome-wide chromatin immunoprecipitation (ChIP) experiments in the well-studied eukaryotic model organism Schizosaccharomyces pombe. The results of these experiments have shown selective association of UPF1 with many gene loci (unpublished). There is also evidence that UPF1 might bind DNA polymerase in mammalian cells. In this project, I am investigating the role of UPF1 and two additional NMD factors (UPF2 and UPF3) in DNA metabolism in S. pombe. Using growth spot assays, we found that upf1 and other NMD mutants are hypersensitive to the DNA replication inhibitors hydroxyurea (HU) and methyl methanesulfonate (MMS), suggesting that there is an increase in DNA damage in these strains. Consistent with this interpretation, the NMD mutants showed negative genetic interaction with Rad22, a Rad52 homologues that are known to play a central role in homologous recombination and DNA double-strand break repair in S. pombe. We also found that UPF1 is enriched at highly transcribed rDNA and tRNA gene loci. Therefore, our data suggest that UPF1 is directly involved in maintaining genome stability possibly by coordinating DNA replication with transcription. Presently, I am investigating the mechanism that recruit of UPF1 at these highly transcribed genes and its putative functions in transcription, RNA processing and DNA replication, which could account the observed phenotypes.

487 The PDCD4 mRNA transcript, a well-studied target of the oncomiR-21, is also regulated by the RNA binding protein, HuR

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The RNA binding protein, HuR, binds to AU-rich elements found primarily in the 3′UTRs of target mRNA transcripts. Binding of HuR to a target transcript often results in the stabilization of that message, albeit by an unknown mechanism. A shift in localization of HuR protein from the nucleus to the cytoplasm has been observed in more aggressive breast tumors and is hypothesized to result in an over-stabilization of HuR target mRNAs, which include a number of cell cycle regulators and transcription factors. Preliminary data suggests that HuR may target the mRNA transcript of the novel tumor suppressor, Programmed Cell Death 4 (PDCD4). PDCD4 protein expression is reduced in invasive ductal carcinoma samples compared to non-invasive breast tumors and suppression of PDCD4 in MCF-7 breast cancer cells leads to increased invasiveness, and is therefore an intriguing candidate target of HuR. We performed RNA-IP analyses in MCF-7 cells and observe clear enrichment for the PDCD4 transcript in association with HuR, suggesting that PDCD4 is a target of HuR in these cells. Further RNA-IP analyses using a putative RNA binding mutant of HuR demonstrate a loss of enrichment of PDCD4 mRNA compared to WT HuR, underscoring the requirement of the two N-terminal RNA recognition motifs of HuR in RNA binding. Biotin pulldown assays reveal two independent binding sites for HuR within the first 290nt of the PDCD4 3′UTR. Together, these data strongly suggest that PDCD4 mRNA is a target of HuR. To test the functionality of this interaction, we performed knockdown of HuR and observed a significant reduction of HuR steady-state mRNA and protein levels. Interestingly, preliminary results suggest that knockdown of HuR also results in increased levels of miR-21, a well-studied oncomiR that targets the PDCD4 transcript. The miR-21 binding site is adjacent to the HuR binding sites, suggesting that there may be functional interplay between HuR and miR-21 on the PDCD4 3′UTR, which may have broad implications for the role of HuR in breast cancer pathogenesis.

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488 In vitro assembly of nucleocapsid like particles from recombinant core protein of Dengue virus

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Core protein (CP) is one of the three structural proteins of Dengue virus (DENV). CP binds viral genome RNA to form nucleocapsid, which is surrounded by the membrane bound envelope (E) and M proteins in DENV particle. In this study, we expressed, purified, and characterized recombinant DENV CP and we tried the to assemble the nucleocapsid-like particles in vitro using recombinant CP and synthetic DENV RNA. CP could be purified to near homogeneity by phosphocellulose column chromatography due to the highly basic nature of CP. The far-UV circular dichroism spectroscopy disclosed that CP possessed the structural features of typical a-helical proteins. CP had a broad thermal transition that had a mid point of around 70 ^{o}C. Studies of the oligomeric state of CP in solution determined by the size exclusion column chromatography and the glutaraldehyde cross-linking experiment showed that CP mainly forms dimers. The formation of nucleocapside-like particles between CP and viral genome RNA was observed by electronic microscopy. The presence of low concentration of the non-ionic detergent tween 20 promoted nucleocapside-like particle formation. The hydrophobic residues near the C terminus of CP were found to be important to maintain the alpha helicity and the dimeric state of CP. Moreover, these residues were important for the assembly of nucleocapside-like particles in vitro.

489 Multidomain conformational changes of PABPC1 upon inhibition by Paip2

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PABP consists of four RNA-binding domains (RRM 1-4) followed by a linker region and a conserved C-terminal domain, termed MLLE. The RRM domains are pivotal for the circularization of mRNA through the binding of the poly(A) tail and eIF4F complex that binds the mRNA 5′ cap. By circularizing mRNA and binding exposed poly (A) tail, PABP helps maintain mRNA stability. The displacement of PABP off poly (A) tail is a required step before deadenylation could take place. Thus the dynamics of PABP on and off mRNA is critical to regulation of mRNA decay and translation at the same time. However, little is known about how PABP gets displaced.

PABP-interacting protein 2 (Paip2), namely, interacts with PABP. Paip2 inhibits translation through prevention of PABP from binding poly(A) RNA and destabilization of the circularized mRNA. By suppressing PABP, Paip2 contributes to control of synaptic plasticity and memory, and spermiogenesis in mice. In addition, Paip2 regulates homeostasis of PABP and serves as innate defense to restrict viral protein synthesis to counter virus-induced PABP increase.

Paip2 binds PABP with two sites: PAM1 and PAM2. PAM2 of Paip2 binds the C-terminal MLLE domain of PABP. The second site, PAM1, characterized by the presence of a large number of negatively charged residues, binds RRM domains and prevents PABP from binding poly (A). Our studies of the multi-domain conformational changes between active and inhibited PABP is of significance to deepen our understanding of translation and mRNA metabolism.

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490 Arsenite-activated JNK signaling enhances CPEB4-Vinexin interaction to facilitate stress granule assembly

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Stress granules (SGs) are compartmentalized messenger ribonucleoprotein particles (mRNPs) where mRNAs are translationally repressed and stored when cells encounter environmental stress. Cytoplasmic polyadenylation element-binding protein (CPEB)4 is a sequence-specific RNA-binding protein and translational regulator. In keeping with the results obtained for many other RNA-binding proteins, we found CPEB4 localized in SGs in various arsenite-treated cells. In this study, we identified that Vinexin, a CPEB4-interacting protein, is a novel component of SGs. Vinexin is a SH3-domain-containing adaptor protein and affects cell migration through its association with Vinculin to localize at stress fibers (SFs). Unexpectedly, Vinexin is translocated from SFs to SGs under arsenite-induced stress. The recruitment of Vinexin to SGs depends on its interaction with CPEB4 and influences SG formation and assembly. Arsenite-activated c-Jun N-terminal kinase (JNK) signaling enhances the association between CPEB4 and Vinexin, which consequently facilitates SG localization of Vinexin. Taken together, this study uncovers a novel interaction between a translational regulator and an adaptor protein to influence SG assembly.

491 Three nucleotides form the core Musashi recognition motif

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Musashi (MSI) family proteins control cell proliferation and differentiation in many biological systems. They are over-expressed in tumors of several origins, and their expression level correlates with poor prognosis. MSI proteins control gene expression by binding RNA and regulating its translation. They contain two RRM domains, which recognize a defined sequence element. The relative contribution of each nucleotide to the binding affinity and specificity is unknown. We analyzed the binding specificity of MSI RRM domains using a quantitative fluorescence anisotropy assay. We found that the core element driving recognition is the sequence UAG. Nucleotides outside of this motif have a limited contribution to binding free energy. For mouse MSI1, recognition is determined by the first of the two RRM domains. The second RRM adds affinity but does not contribute to binding specificity. In contrast, the recognition element for *Drosophila* MSI is more extensive than the mouse homolog, suggesting functional divergence. The short nature of the binding determinant suggests that protein-RNA affinity alone is insufficient to drive target selection by MSI family proteins.

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492 MicroRNAs rescue deltaF508-CFTR-dependent inflammation in Cystic Fibrosis

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Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in cystic fibrosis transmembrane conductance regulator (CFTR). The most common mutation, ΔF508, is associated with failure of the mutant CFTR to traffic to the plasma membrane, with concomitant loss of cAMP-activated chloride conductance, and hypersecretion of pro-inflammatory IL-8. Small molecules have been sought which might functionally rescue [ΔF508]CFTR protein. However, functional rescue of this most common CFTR mutation remains a goal.

Our approach has been to study the control of IL-8 expression in CF lung epithelial cells by analyzing pro-inflammatory RNA-binding proteins (RBPs) and microRNAs (miRNAs, miRs) in these cells. The rationale has been that naturally occurring microRNAs, aberrantly expressed in the CF lung, might be novel therapeutic agents by themselves. Recent studies with animals and human clinical trials have shown that single miRs can induce a therapeutic response. We have therefore analyzed miR expression in cultured and ex-vivo CF lung epithelial cells, to identify a drugable miR-based pro-inflammatory mechanism. We have found that miR-155 is elevated in CF lung cells, and that, antagomiR (anti)-155 suppresses IL-8 expression through reduction in AKT1 activation. miR-155, a multifunctional miRNA, has been identified as an important regulator of the immune system and inflammation and is associated with cancer, cardiovascular diseases, and immune disorders. We have also demonstrated that the enhanced processing of the miR-155 precursor is the basis for the aberrant increase in mature miR-155 in CF cells. Additionally, we have identified CF deficient miRs, miR-16 and miR-302a, which can not only suppress miR-155 and IL-8, but also rescue ΔF508-CFTR-trafficking/activity defect. Our findings will ultimately lead to the development of a novel miRNA-based anti-inflammatory therapy for pulmonary disorders.

493 How does a cytoplasmic virus with an AU-rich RNA genome escape cellular ARE-mediated mRNA degradation and translational blockade?

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AU-rich elements mediate of mRNA degradation and translational repression. Several proteins that bind AU-rich sequences have been identified such as HuR, hnRNP D, BRF1, TTP, TIAR. Rotavirus, belonging to the family *Reoviridae*, contains a genome of 11 segments of double-stranded RNA, which is about 65% rich in AU content. Our attempts to express majority of the viral proteins in mammalian cells using the best expression vector systems have met with failure. Though mRNAs transcribed from transfected vectors are readily detected, protein expression is either extremely poor, or undetectable depending on the gene, indicating translational repression of rotaviral mRNAs in gene transfected cells. However, in infected cells, the virus makes copious amounts of the viral proteins, suggesting virus-mediated deregulation of translational repression. Investigations into the mechanism of translational repression using ECFP-roatvirus gene fusion constructs revealed that while ECFP is readily expressed when rotavirus gene ORF is placed downstream of ECFP following the termination codon, no expression of the reporter was detected when the two genes are placed in translational fusion or when rotavirus gene sequence is placed upstream of ECFP ORF, clearly indicating severe translational blockade when the ribosome encounters rotaviral gene sequence. To further understand the molecular events occurring during virus infection, we have examined the time-dependent nucleo-cytoplasmic redistribution of a battery of cellular factors involved in RNA metabolism and translation. Our results reveal nucleo-cytoplasmic relocalization of several proteins including ARE-BPs and hnRNPs, that of PABP and its interacting proteins being very critical for directing translational machinery to viral mRNAs during the very early stages of virus infection. We further demonstrate that several nuclear factors migrating to cytoplasm interact with both structural and non-structural viral proteins suggesting that function as sinks to sequester the migrating nuclear factors to regulate their cytoplasmic functions and suppress formation of stress granules and P-bodies in response to virus-induced stress The significance of this large body of results on the mechanisms of modulation of cellular ARE-mediated translational repression, and inhibition of formation of stress granules and P-bodies by the virus, which does not code for a protease, will be discussed.

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494 Aberrant NEFL mRNA 3'UTR variants in ALS spinal cord tissue

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Amyotrophic Lateral Sclerosis (ALS) is a progressive, adult onset neurodegenerative disease of motor neurons (MN). Tragically, ALS is characterized by a relentless loss of muscle function, ultimately leading to death through respiratory failure within 3 and 5 years from symptom onset. ALS can be viewed as a disorder of RNA metabolism. Evidence includes the observation that MN death is related to the selective suppression of low molecular weight neurofilament (NEFL) mRNA in spinal cord in ALS. Our main goal is to study stability determinants of the NEFL mRNA. Previously, we documented an expression profile alteration of a type of non-coding RNA that regulates the majority of the transcriptome, microRNAs. Our results showed that this profound microRNA dysregulation could be affecting NEFL mRNA levels in ALS. Now, we are interested in studying the main target of microRNAs, mRNA 3′ untranslated region (UTR). mRNA UTRs, often neglected during genetic screening of disease-associated genes, play important roles in the pathogenesis of several neurodegenerative diseases. Using RACE-PCR and sequencing we found different variants for NEFL mRNA 3′UTR in spinal cord control samples. Interestingly, we also found aberrant NEFL mRNA 3′UTR variants in ALS patients; some of them possibly caused by the selection of alternative polyadenylation (APA) sites: an emerging widespread mechanism used to control gene expression. Alterations in the mRNA 3′UTR length could influence the fate of the transcript in several ways, for example, by altering the availability of microRNA recognition elements and RNA-binding protein sites. To build on these findings, we are characterizing NEFL mRNA 3′UTR variants. Ultimately, we are interested in determining whether there is a correlation between the alteration of NEFL mRNA 3′UTR in ALS and the selective vulnerability of MN in this neurodegenerative condition.

495 Characterization of the RNA recognition mode of hnRNP G extends its role in SMN2 splicing regulation

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The protein hnRNP G is an important regulator of gene expression in human cells. It modulates splicing of several pre-mRNAs including Survival of Motor Neuron (SMN), which can be transcribed from two genes: SMN1 and SMN2. Spinal Muscular Atrophy (SMA) is one of the leading genetic causes of death in children and results from the lack of functional SMN1 gene. A promising approach in curing this disease consists in acting on SMN2 exon 7 alternative splicing regulation to increase the level of SMN2 mRNAs containing this exon.

We previously proposed that hnRNP G could be recruited on SMN2 exon 7 by transformer2-beta1 (Tra2-β1) upstream its binding site(1) and together, they activate the inclusion of this exon in SMN2 mRNAs(2). However, the RNA recognition mode of hnRNP G, its binding site on SMN2 and its function in SMN2 exon 7 splicing regulation are still unknown. We recently found that in addition to the C-terminal region of hnRNP G, the RNA Recognition Motif (RRM) and the middle part of the protein containing the Arg-Gly-Gly (RGG) box are important to promote SMN2 exon 7 inclusion. To better understand the mode of action of hnRNP G in this context we determined by NMR the structure of its RRM bound to an SMN2-derived RNA. The RRM interacts with a 5′-AAN-3′ motif and specifically recognizes the two consecutive adenines. By testing the effect of mutations in hnRNP G RRM and in its putative binding sites on the splicing of SMN2 exon 7, we show that it specifically binds to exon 7. This interaction is required for hnRNP G splicing activity and we propose its recruitment to a polyA tract located upstream of the Tra2-β1 binding site. Finally, our data suggest that one important function of hnRNP G as a regulator of SMN2 exon 7 splicing consists in increasing the specificity of exon 7 recognition by the heterodimeric complex Tra2-β1/hnRNP G.

References:
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496 Functional analysis of human Prp8 mutations linked to retinitis pigmentosa

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Retinitis pigmentosa (RP) is hereditary retinal disorder leading to degeneration of photoreceptors. There are approximately 60 eye specific genes that are associated with RP. Surprisingly, RP-related alleles were also found in genes coding ubiquitously expressed pre-mRNA splicing factors (PRPF3, PRPF4, PRPF6, PRPF8, PRPF31 and SNRNP200).

Using BAC recombineering, we prepared five point RP-mutations of human hPrp8, tagged the mutants with GFP and expressed them stably in cultured cells. Under normal condition, wild-type hPrp8 is localized exclusively in the cell nucleus. Here we observed that some mutations affected hPrp8 nuclear localization. hPrp8 is a crucial component of the U5 snRNP. We showed that hPrp8 mutations inhibited maturation of the U5 snRNP and prevented incorporation of additional U5-specific proteins. However, each mutation inhibited U5 snRNP maturation to a different extent. In addition, we observed faster degradation of mutant proteins and the rate of degradation correlated with the effect the mutation had on U5 snRNP maturation. To examine effects of RP mutation on pre-mRNA splicing, we knocked down specifically the endogenous hPrp8 protein and analyzed splicing efficiency in cell lines expressing either wild-type hPrp8-GFP or RP mutants. None of the mutants was able to rescue the splicing defects to the extent observed in wild-type hPrp8 expressing cells.

Taking together, our data show that RP-related mutations of hPrp8 compromise hPrp8 stability, maturation of the U5 snRNP and reduce splicing efficiency. The extent to which individual mutants affect these events correlates with the severity of RP clinical phenotype.

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497 The RNA-binding protein Quaking maintains endothelial barrier function by targeting vascular endothelial cadherin mRNA

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Background: The endothelial monolayer forms a vital barrier between blood and tissue, and its integrity is determined largely by the adherens junction protein vascular endothelial cadherin (VE-cadherin). While many of the receptors and signaling proteins involved in regulating adherens junctions have been identified, surprisingly little is known about their regulation at the post-transcriptional level. The RNA-binding protein Quaking (QKI), known for its function in the nervous system, is as well essential for blood vessel formation.

Observations: We find that QKI is highly expressed in quiescent endothelial cells (ECs) in vivo. In vitro, ECs displayed increased levels of QKI when cultured under laminar atheroprotective flow. Using KLF2 overexpression and a human QKI promoter reporter gene, we found that KLF2 mediates this increase in QKI expression. Subsequently we aimed to investigate the role of QKI in EC vascular integrity. Interestingly, the mRNA of VE-cadherin, the prime adhesion protein in EC adherens junctions, contains a conserved QKI-binding site. Moreover the targeted reduction of QKI resulted in a reduction of VE-cadherin expression. Importantly, we identified a direct role for QKI in regulating VE-cadherin mRNA biology, as RNA immunoprecipitation and luciferase-reporter assays revealed that QKI can directly bind to the VE-cadherin mRNA and induces transcript translation (4 fold ±0.4 $p<0.01$), respectively. This effects was perturbed when the QKI-binding site was mutated. These results suggest that QKI acts to enhance barrier function. Overexpression of QKI markedly increased (1.3 fold ±0.96) the capacity to form a high resistance endothelial monolayer, while silencing of QKI marked impaired EC barrier function (0.65 fold ±0.13; $p<0.05$). To confirm a role for QKI in maintaining EC barrier function in vivo, we measured Bradykinin-induced vascular leakage in QKI viable mice (QKI^{v}), which express decreased levels of the QKI protein. Indeed, QKI^{v} mice displayed a 20% ($p<0.05$) increase in extravascular accumulation of Evans blue-labeled albumin compared to wild type littermates.

Conclusions: We show that QKI binds VE-cadherin mRNA and alters its protein expression. Moreover that reduction of QKI negatively affects endothelial monolayer integrity. These studies provide novel insight into a role for post-transcriptional regulation in the maintenance of vascular integrity.

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498 Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling

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The type I interferon system is integral to human antiviral immunity. However, inappropriate stimulation or defective negative regulation of this system can lead to inflammatory disease. We sought to determine the molecular basis of genetically uncharacterized cases of the type I interferonopathy Aicardi-Goutières syndrome, and of other patients with undefined neurological and immunological phenotypes also demonstrating an upregulated type I interferon response. We found that heterozygous mutations in the cytosolic double-stranded RNA receptor gene *IFIH1* (*MDA5*) cause a spectrum of neuro-immunological features consistently associated with an enhanced interferon state. Cellular assays indicate that these mutations confer a gain of function, characterized not only by an increased level of basal signaling activity in the absence of the exogenous ligand, but also the length range of the ligand that is able to induce interferon signaling was increased. Biochemical assays show that mutant IFIH1 binds RNA more avidly, either by increasing the intrinsic affinity for RNA and/or stabilizing the protein-protein interaction surface. Our results illustrate a novel paradigm in human disease by demonstrating aberrant sensing of nucleic acids as a cause of immune upregulation.

499 Effects of genetic variations on microRNA:target interactions

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Genetic variations within microRNA (miRNA) binding sites can affect miRNA-mediated gene regulation, which may lead to phenotypes and diseases. We perform a transcriptome-scale analysis of genetic variants and miRNA:target interactions identified by CLASH. This analysis reveals that rare variants tend to reside in CDSs whereas common variants tend to reside in the 3¢ UTRs. For common variants, miRNA binding sites in CDSs are under negative selection. Furthermore, an overwhelming majority of genetic variants within or near miRNA binding sites can not only alter the potential of miRNA:target hybridization but also the structural accessibility of the binding sites and flanking regions. These suggest an interpretation for certain associations between genetic variants and diseases, i.e., modulation of miRNA-mediated gene regulation by common or rare variants within or near miRNA binding sites, likely through target structure alterations. Our data will be valuable for discovering new associations among miRNAs, genetic variations and human diseases.

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500 An atypical Leucine–rich domain in the ALS–related protein RGNEF is critical for the proper regulation of its RNA destabilizing activity

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Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive disorder characterized by degeneration of motor neurons. Although the cause of the disease remains elusive, protein aggregate formation, including RNA binding proteins, in motor neurons is a neuropathological hallmark. Recent evidence supports the hypothesis that alterations in RNA metabolism in motor neurons can lead to the development of these aggregates. Previously, we observed that Rho Guanine Nucleotide Exchange Factor (RGNEF) forms cytoplasmic inclusions in ALS spinal motor neurons that co-localize with ubiquitin, p62/Sequestosome-1, and the RNA binding proteins TDP-43 and FUS/TLS and presents genetic alterations in *f*ALS cases. Additionally, we demonstrated that RGNEF is an RNA binding protein that destabilizes low molecular weight neurofilament (NFL) mRNA. Because RGNEF is a novel RNA binding protein involved in the ALS pathology is important to understand the regulation of its RNA destabilizing activity. To achieve this, we developed several RGNEF mutants lacking specific regions of the protein to analyze their roles in RNA destabilizing activity. Using luciferase reporter gene assays we observed that RGNEF's GEF domain is not relevant for its RNA destabilizing activity. Interestingly, we observed that a region containing an atypical Leucine-rich (L-rich) domain in the amino terminal domain of RGNEF is critical for proper regulation of its RNA destabilizing activity. Additionally, we determined the minimum region of the protein necessary to have RNA destabilizing activity. Finally, we observed that deleting the region containing the L-rich domain on mouse isoform of RGNEF (p190RhoGEF) its RNA stability activity is also greatly affected. Our results provide evidence that the amino terminal region of RGNEF, containing the L-rich domain, is critical for proper regulation of its RNA destabilizing activity across different species. Moreover, since L-rich domains participate in protein-protein interactions, this suggests participation of a protein complex in the RNA stability regulation by RGNEF

501 Inhibition of Nonsense-Mediated Decay by Curcumin

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The nonsense-mediated mRNA decay (NMD) pathway is a translation-coupled mRNA surveillance system that recognizes and eliminates transcripts with a premature termination codon (PTC), located more than 50-55 nucleotides upstream of the last exon-exon junction. It has an important role not only in the homeostatic control of splicing factors but also in the degradation of PTC-containing transcripts resulted from ~30% of mutations that cause human genetic diseases. We found that a polyphenolic compound, curcumin, increased the PTC-containing transcripts of serine/arginine-rich splicing factor 1 (SRSF1) in different cell lines, including HEK293T, NSC-34 and dermal fibroblasts from a patient with Spinal Muscular Atrophy (SMA). The effect of curcumin on *SRSF1* splice variants was completely abolished upon knocking down the critical factor hUPF1 in the NMD pathway. Chromatin immunoprecipitation analysis revealed reduced occupation of acetyl-histone H3 and RNA polymerase II at the promoter region of *hUPF1* but not of *GAPDH* upon treatment by curcumin, suggesting specific control of hUPF1 transcription by curcumin. Furthermore, we verified the effect of curcumin on the PTC-containing transcripts in two dermal fibroblast cell lines derived from patients with SMA or Tay-Sachs disease. In SMA cells, curcumin altered the homeostasis of splicing activator SRSF1 and upregulated its protein level by preventing NMD of PTC-containing *SRSF1* splice variants; this increased the proportion of *SMN2* transcripts containing exon 7. In Tay-Sachs disease cells, curcumin increased the PTC-containing transcript of the *Hexosaminidase A* gene harboring a frame-shift mutation. Thus, curcumin inhibits the NMD pathway and stabilizes the PTC-containing transcripts of *trans*-acting splicing factors or of causative genes with *cis*-acting mutations. Curcumin likely has therapeutic potential for human genetic diseases involving NMD.

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502 Unveiling the pharmacological potential of targeting microRNA precursors with chemical tools

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Pre-miRNAs are often expressed at low levels and are generally considered simply as precursors. However, in some cases they appear to have functions beyond that of precursors. Two examples are miR-122 and let-7. MiR-122 is a prominent miRNA with a role in the life cycle of the hepatitis C virus (HCV), in which it stabilizes the 5′UTR of HCV RNA. In clinical trials the anti-mir-122 drug miravirsen lowers circulating virus to negligible levels in HCV patients. Recent independent papers have described how miR-122 precursors play roles independent of their mature miRNAs, for instance during circadian rhythm or regulating HCV life cycle (RNA 2013, 19, 1). This opens the possibility to targeting them with ligands in disease-pathway contexts. Indeed, we demonstrated that miravirsen invades the stem-loop structure of miR-122 precursors in cells and inhibits their processing by Dicer and Drosha (NAR 2014, 42, 609). Its affinity for the stem-loop was less than for mature miR-122 but still in the mid-nanomolar range. In hepatocytes miravirsen accumulates miR-122 precursors as well as sequestering mature miRNA.

The tumor suppressor role of the let-7 family is often disrupted in cancer, one mechanism of which involves its suppressor, oncogenic Lin28. Using a novel assay we showed that Lin28 binds selectively to GNNG motifs in let-7 terminal loops and blocks their processing (NAR 2013, 41, e47; NSMB 2012, 19, 84), thereby depleting cells of these important negative regulators of proliferation/transformation. The Lin28/let-7 binding site is therefore a potential pharmacological target. In proof-of-concept studies we characterized highly-modified oligonucleotides for their affinity, selectivity and activity in vitro and in cells. The oligonucleotides antagonize Lin28 but, crucially, still allow processing of the precursors by Drosha and Dicer. Building on these findings we have developed new synthetic techniques (Angew Chem 2013, 52, 12028) and used them to construct a FRET system to screen for small-molecule antagonists using labeled pre-let-7/Lin28-GFP fusion protein. In this presentation we will report on progress in these projects.

503 The RNA-binding protein QKI suppresses cancer-associated aberrant splicing

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Lung cancer is the leading cause of cancer-related death worldwide. Aberrant splicing has been implicated in lung tumorigenesis. However, the functional links between splicing regulation and lung cancer are not well understood. Here we report that the RNA-binding protein QKI is a key regulator of alternative splicing in lung cancer. We showed that QKI is frequently down-regulated in lung cancer, and its down-regulation is significantly associated with a poorer prognosis. QKI-5 inhibits the proliferation and transformation of lung cancer cells both *in vitro* and *in vivo*. We present evidence that QKI-5 regulates the alternative splicing of *NUMB* via binding to RNA elements in its pre-mRNA, which in turn suppresses cell proliferation and prevents the activation of the Notch signaling pathway. We further showed that QKI-5 regulates alternative splicing in a position-dependent manner, and it inhibits splicing by selectively competing with a core splicing factor SF1 for binding to the branchpoint sequence. Taken together, these findings reveal QKI as a critical regulator of splicing in lung cancer and suggest a novel tumor suppression mechanism involving QKI-mediated regulation of the Notch signaling pathway.

504 Abstract Withdrawn

505 Abstract Withdrawn

506 A Conserved Role for the Zinc Finger Polyadenosine RNA Binding Protein, ZC3H14, in Control of Poly(A) Tail Length

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The *ZC3H14* gene, which encodes a ubiquitously expressed, evolutionarily conserved, nuclear, zinc finger polyadenosine RNA binding protein, was recently linked to autosomal recessive, nonsyndromic intellectual disability. Although studies have been carried out to examine the function of putative orthologues of ZC3H14 in *S. cerevisiae*, where the protein is termed Nab2, and *Drosophila*, where the protein has been designated, dNab2, little is known about the function of mammalian ZC3H14. Work from both budding yeast and flies implicates Nab2/dNab2 in poly(A) tail length control while a role in poly(A) RNA export from the nucleus has been reported only for budding yeast. Here we provide the first functional characterization of ZC3H14. Analysis of ZC3H14 function in a neuronal cell line as well as *in vivo* complementation studies in a *Drosophila* model identify a role for ZC3H14 in proper control of poly(A) tail length in neuronal cells. Furthermore, we show here that human ZC3H14 can functionally substitute for dNab2 in fly neurons and can rescue defects in development and locomotion that are present in *dNab2* null flies. These rescue experiments provide evidence that this zinc finger-containing class of nuclear polyadenosine RNA binding proteins plays an evolutionarily conserved role in controlling the length of the poly(A) tail in neurons. Furthermore, this study demonstrating the functional conservation of this class of zinc finger polyadenosine RNA binding proteins validates the use of dNab2 mutant Drosophila as a model to study the molecular defects that contribute to brain dysfunction in the patients with intellectual disability.

507 Novel Alternatively Spliced KLK7 mRNAs Expressed in Breast Cancer Patients from Bahawalpur, Pakistan

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At least 15 % of the mutations resulting in development of genetic diseases are known to affect pre-mRNA splicing. Alternative splicing commonly occurs in cancer cells and many cancer specific splice variants have been reported as potential candidate biomarkers of the disease. Breast cancer is considered as the third most frequent type of cancer and the leading cause of death among females worldwide. Pakistan unfortunately has the highest prevalence of breast cancer in Asia and on the average ~40,000 Pakistani females die annually due to this disease. We focused our studies on human tissue Kallikrein 7 (KLK7) mRNAs expression profile in breast cancer patients of our region. Earlier studies have reported differential expression of KLK7 mRNAs in breast cancer patients. We optimized touch down nested PCR assay to amplify KLK7 mRNAs and observed an unusual splicing event consisting of E3 truncation at 3′ end (by 124 nucleotides), E4 exclusion and E5 truncation at 5′ end (by 33 nucleotide) in a patient suffering from mammary dysplasia. Moreover 3 other KLK7 mRNAs (KF963190, KF963191, and KF963193) expressed in breast cancer patients of our study were noticed to exhibit SNPs. It was interesting to explore the effect of these variations which were uniquely observed in breast cancer patients of our population and not reported worldwide. Employing various bioinformatics tools we analyzed KLK7 mRNAs to get an overview of the potential effect of these mutations on encoded protein sequences and other characteristics of proteins. We have observed that alternatively spliced mRNA (KF963192) will potentially encode a truncated and non-functional protein lacking histidine and aspartic acid residues of the classical catalytic triad (histidine : aspartic acid : serine) of serine proteases. Similarly although encoded proteins have considerable homology with normal hK7 protein, SNPs seem to cause great variations in pIs, structures and molecular weights of encoded proteins. We propose that this pilot study must be extended further to characterize these population specific mutations and their possible role in the pathogenesis of breast cancer.

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508 Germline and somatic mutations of the spliceosomal DEAD-box helicase *DDX41* lead to bone marrow neoplasms

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Recent exome sequencing of myeloid malignancies have revealed frequent somatic mutations and/or deletions of several spliceosomal factors including *SF3B1*, *U2AF1*, *SRSF2*, *PRPF8* and others. These mutations are often single heterozygous missense mutations at highly recurrent sites. We have recently found similar mutations in the spliceosomal C complex DEAD-box helicase *DDX41* (*Abstrakt*). *DDX41* is located at the distal tip of chromosome 5q, a region frequently deleted in myeloid neoplasms. Remarkably, several families with a highly penetrant genetic pattern of AML show germline frame shift mutations in one copy of *DDX41* combined with the same somatic missense mutation in the other copy of *DDX41* as seen in sporadic cases. Analysis of patient cohorts shows that *DDX41* mutations and deletions are associated with significantly worse clinical outcomes.

To further investigate these effects, we performed knock down and overexpression experiments in leukemia cell lines. Knock down of *DDX41* by 80% in K562 cells led to enhanced growth rate while overexpression in U937 cells, which express very little *DDX41*, led to significant growth inhibition. *DDX41* knock down in normal bone marrow cells led to enhanced colony formation. Preliminary results of mouse xenograft experiments also show enhanced *in vivo* growth of *DDX41* knock down tumor cells.

Analysis of RNA-seq data from tumors with *DDX41* mutations or deletions shows widespread alterations in alternative splicing. An analysis of alternative exons genome wide showed both enhanced exon inclusion and exon exclusion in hundreds of genes compared to controls with wild type *DDX41* expressed at normal levels.

We conclude that *DDX41* is a new spliceosomal tumor suppressor gene that acts through effects on the alternative splicing of downstream genes to promote cell division and/or inhibit proper differentiation in myeloid progenitor cells.

509 Identification and Characterization of a Downstream Auxiliary Element that Mediates Dux4 mRNA 3′end formation

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The importance of accurate and efficient processing of the 3′end of the mRNA is evident in several diseases, one of which is Facioscapulohumeral Dystrophy (FSHD). FSHD is an autosomal dominant muscle dystrophy attributed to the inappropriate expression of Double homeobox 4 (Dux4) in muscle cells. Dux4 is generally suppressed in somatic tissue and its pathogenic expression has been shown to promote apoptosis contributing to muscle atrophy. The inappropriate expression of Dux4 in FSHD patient skeletal muscle cells is facilitated by the creation of a nonconsensus polyadenylation signal (PAS) due to a single nucleotide polymorphism occurring in a transcriptionally derepressed environment. The Dux4 PAS is surprisingly active despite utilizing a suboptimal cleavage site and the lack of any well-defined DSE. Taken together, Dux4 cleavage and polyadenylation represents an intriguing biological context to investigate *cis* regulatory elements of cleavage and polyadenylation.

To interrogate the elements that contribute to Dux4mRNA cleavage and polyadenylation we designed a reporter system in which the Dux4 3′ end processing elements are removed from their epigenetic context. The reporter system utilizes transcriptional read-through as a measure of cleavage and polyadenylation. We have validated our reporters using the well-studied SV40PAS in HeLa cells and have demonstrated sensitivity of our reporter to mutations within *cis* elements mediating cleavage and polyadenylation. We demonstrate that, not only as previously shown, is the PAS necessary for the stabilization of the transcript, we identify a novel 3′ end formation *cis* element that is required for the efficient polyadenylation of the DUX4 mRNA. The small element is located surprisingly, ~200 nucleotides downstream of the Dux4 PAS. We observe that the absence of this element impairs cleavage and polyadenylation and that variation in the position of this element can be tolerated provided that it is appropriated orientated downstream of the Dux4 PAS. We favor a model were this downstream auxiliary element mediates RNA polymerase II pausing to facilitate recognition of the poorly defined cleavage and polyadenylation sequences to allow for Dux4 mRNA production.

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510 Expression of the RNA-binding protein KSRP in skeletal muscle is regulated by Heparin: Implications for novel therapeutics for Duchenne muscular dystrophy

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The RNA-binding protein KSRP is known to be a key regulator of ARE-directed mRNA decay. In this context, recent work from our lab showed that KSRP regulates post-transcriptional expression of utrophin A in skeletal muscle through p38 activation and miR-206. In fact, KSRP knockdown results in an increase in utrophin A expression in dystrophic mdx mice (Amirouche et al. 2013). These findings are of great relevance for developing novel therapeutics for Duchenne Muscular Dystrophy (DMD) since up-regulation of utrophin A in dystrophic muscle can attenuate the extent of the pathology. Therefore, it's important to identify compounds that activate pathways involved in regulating utrophin A in muscle. Our lab has recently shown that treatment of dystrophic mdx mice with heparin leads to an increase in utrophin A expression in diaphragm muscle through p38 activation (Amirouche et al. 2013). The objective of the present study is to build upon these findings and determine the impact of heparin on expression of KSRP and utrophin A in several muscles, and to examine whether heparin can rescue damaged dystrophic fibers.

To this end, we treated 6 week-old mdx mice with 20iU/kg or 500iU/kg of heparin daily, for 4 weeks. Our results indicate that heparin treatment of mdx mice causes a significant 1.5 to 3-fold increase in utrophin A protein expression in diaphragm, EDL and TA muscles. Additionally, we observed a large decrease (up to 2.5-fold) in KSRP protein expression in heparin-treated diaphragm, EDL and TA muscles. In agreement with these observations, immunofluorescence experiments show an accumulation of ß-dystroglycan along the plasma membrane of heparin-treated diaphragm and TA muscles compared to control, suggesting an important reassembly of the dystrophin-associated protein complex. Moreover, intracellular IgM staining is reduced in fibers of heparin-treated muscles indicating an improvement in the structural integrity of the sarcolemma. Overall, the data indicate that activation of p38 by heparin reduces KSRP expression which in turn, leads to increases in levels of utrophin A in dystrophic muscle. These changes are accompanied by an attenuation of the dystrophic phenotype thereby revealing the therapeutic potential of heparin as a novel pharmacological agents for DMD patients.

511 MicroRNAs in limb muscles of patients with chronic obstructive pulmonary disease

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RATIONALE: Limb muscles of patients with chronic obstructive pulmonary disease (COPD) are affected by biochemical and structural dysfunctions. Loss of muscle mass and satellites cells senescence are components of these dysfunctions. Satellite cells receive signals from growth factors such as insulin growth factor-1 (IGF-1) leading to their proliferation and differentiation during muscle growth and regeneration. Muscle-specific microRNA (myomiR) such as miR-1, miR-133a and miR-206 can modulate IGF-1 expression. The aim of this study is to characterize myomiRs and IGF-1 expression in limb muscles of patients with COPD.

OBJECTIVE: To assess levels of miR-1, miR-133a, miR-206 and IGF-1 mRNA in muscle tissue of patients with mild to severe COPD and healthy controls of similar age. Our hypothesis was that levels of myomiRs will increase and IGF-1 mRNA will decrease as the severity of lung disease progresses.

METHODS: Patients with mild (n=28), moderate to severe (n=9) COPD and healthy controls with normal lung function (n=21) were recruited. A biopsy of the *vastus lateralis* was obtained as routinely performed in our laboratory. MyomiRs (miR-1, miR-133a, miR-206) and IGF-1 mRNA expression were quantified by RT-qPCR.

RESULTS: Levels of miR-1 and miR-206 were respectively, 3.33 fold ($p \leq 0.001$) and 1.72 fold ($p \leq 0.01$) lower in patients with mild COPD when compared to patients with moderate to severe COPD. MiR-133a level was 1.27 fold ($p \leq 0.05$) lower in patients with moderate to severe COPD when compared to healthy controls. IGF-1 mRNA level tend to increase 1.29 fold ($p=0.14$) in patients with mild COPD compared to patients with moderate to severe COPD.

CONCLUSION: MyomiRs expression are lower in patients with mild COPD when compared to patients with moderate to severe COPD. Reductions in miR-1 and miR-206 with a tendency to higher IGF-1 levels in mild disease are consistent with a positive muscle mass regulation in this population, in comparison to moderate to severe COPD. Further investigations will be conducted to evaluate whether satellite cells behavior is modified by these variations in myomiRs and IGF-1 expression and to better understand the biological processes involved in muscle mass regulation in the context of COPD.

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512 The RNA-binding protein Staufen1 impairs myogenic differentiation via a c-myc-dependent pathway

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Recent work has shown that Staufen1 plays key roles in skeletal muscle yet, little is known about its pattern of expression during embryonic and post-natal development. Here, we first show that Staufen1 levels are abundant in mouse embryonic muscles and that its expression decreases thereafter, reaching low levels in mature muscles. A similar pattern of expression is seen as cultured myoblasts differentiate into myotubes. Muscle degeneration/regeneration experiments revealed that Staufen1 increases following cardiotoxin injection before returning to the low levels seen in mature muscles. We next prevented the decrease in Staufen1 during differentiation by generating stable C2C12 muscle cell lines overexpressing Staufen1. Cells overexpressing Staufen1 differentiated poorly as evidenced by reductions in the differentiation and fusion indices, and by decreases in MyoD, myogenin, MEF2A and MEF2C independently of Staufen Mediated mRNA Decay (SMD). However, levels of c-myc, a factor known to inhibit differentiation, were increased in C2C12 cells overexpressing Staufen1 through enhanced translation. Collectively, our results show that Staufen1 is highly expressed during early stages of differentiation/development and that it can impair differentiation by regulating c-myc thereby highlighting the multifunctional role of Staufen1 in skeletal muscle.

513 Characterization of PKR activation by RNAs identified during metabolic stress

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Protein kinase RNA-activated (PKR) is an interferon-inducible serine/threonine kinase that is activated by double-stranded RNA (dsRNA). A key component of the innate immune system, it acts as a cytoplasmic sensor to detect the presence of viral dsRNA during an infection. More recently, however, PKR has been shown to be activated in response to metabolic stress, such as genetic and diet-induced obesity.[1]Further, experiments using mouse embryonic fibroblast cells treated with palmitic acid (PA) to mimic a high fat diet, show that metabolic activation of PKR is dependent on a functional dsRNA-binding domain (dsRBD).[1]RNA immunoprecipitation followed by high-throughput sequencing (RIP-Seq) results from our lab identified potential endogenous RNA ligands involved in the observed metabolic activation of PKR. Both coding and noncoding RNAs were enriched in PKR immunoprecipitates in a dsRBD- and PA-dependent manner. Surprisingly, snoRNAs comprised the majority of noncoding RNAs bound to PKR after PA treatment. To provide an in vitro correlate to this result we utilized an in vitro autophosphorylation assay. We found that in vitro transcribed snoRNAs can efficiently activate purified, recombinant PKR. Representatives of both major classes of snoRNA, box H/ACA and box C/D, ranging in size from 72 to 137 nucleotides, could activate PKR in vitro. Further, we found that even snoRNAs not identified in the RIP-Seq experiments could activate PKR in vitro, and to date, all snoRNAs tested can activate PKR in vitro. However, there were significant differences between different snoRNAs in the level of PKR activation for a given concentration of RNA. For example, PKR (100 nM) was activated to 80% or 63% with 500 nM snoRD7 or snoRD113, respectively. Possibly these differences arise from variability in snoRNA structure. Taken together, our results indicate that snoRNAs represent a novel class of RNA capable of activating PKR in vitro, and possibly in vivo.

1. Nakamura, T. *et al.* Double-Stranded RNA-Dependent Protein Kinase Links Pathogen Sensing with Stress and Metabolic Homeostasis. *Cell* **140,** 338–348 (2010).

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514 Splicing factor hnRNP A2 activates the Ras-MAPK-ERK pathway by controlling A-Raf splicing in hepatocellular carcinoma development

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In recent years it has become clear that splicing factors play a direct role in cancer development. We showed previously that splicing factors SRSF1, SRSF6 and hnRNP A2/B1 are up-regulated in several cancers and can act as oncogenes when up-regulated. Here we examined the role of splicing factors hnRNP A1/A1b and hnRNP A2/B1 in hepatocellular carcinoma (HCC). We show that the splicing factors hnRNP A1 and hnRNP A2 are up-regulated in HCC tumors derived from inflammation-induced liver cancer mouse model. Overexpression of hnRNP A1 or hnRNP A2, but not the splicing isoform hnRNP B1, induced tumor formation of immortalized liver progenitor cells, while knockdown of these proteins inhibited anchorage-independent growth and tumor growth of human liver cancer cell lines. In addition, we found that cells overexpressing hnRNP A2 showed constitutive activation of the Ras-MAPK-ERK pathway. In contrast, knockdown of hnRNP A2 inhibited the Ras-MAPK-ERK pathway and prevented ERK1/2 activation by EGF. Moreover, we found that hnRNP A2 regulates the splicing of *A-Raf*, reducing the production of a short dominant-negative isoform of A-Raf and elevating the full-length *A-Raf* transcript. Taken together, our data suggest that hnRNP A2 up-regulation in HCC induces an alternative splicing switch that down-regulates a dominant-negative isoform of A-Raf leading to activation of the Raf-MEK-ERK pathway and cellular transformation.

515 Region-specific gene expression changes in TDP-43 transgenic mice displaying impaired memory

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TDP-43 is an RNA-binding protein important for many aspects in RNA metabolism including pre-mRNA splicing, snRNP formation, miRNA biogenesis, and RNA granule transport. TDP-43 abnormally accumulates in affected neurons and glial cells in some neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with TDP-43 proteinopathy (FTD-TDP). To generate animal models for these neurodegenerative diseases, we had developed transgenic mice expressing human TDP-43 in neurons and glial cells under prion promoter. The TDP-43 transgenic mice displayed impaired memory in fear conditioning test at 8-month old, indicating that the mice recapitulate some phenotype of FTD patients. To investigate a region-specific effect of TDP-43 on mRNA expression and splicing in the mice, mRNA from different brain region including motor cortex, hippocampus, amygdala and cerebellum were extracted and analysed using exon array (Affymetrix). Although expression of most genes remained the same, the expression of several genes crucial for neuronal function, including a potassium channel binding protein, was down-regulated in the TDP-43 transgenic mice. Moreover, the expression level of a molecule that could enhance the production of reactive oxygen spices was up-regulated. The changes in these genes might cause neuronal dysfunction in the TDP-43 transgenic mice.

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516 A 3-dimensional, genome-wide assessment of post-transcriptional events orchestrated by the RNA-binding protein Quaking that determine monocyte fate

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The post-transcriptional events that enable monocytes to home to sites of vascular injury, and subsequently differentiate into macrophages within atherosclerotic lesions are poorly understood. We discovered that the mRNA and protein levels of the RNA-binding protein Quaking (QKI) are augmented in $CD14^+$ myeloid cells extracted from advanced human atherosclerotic plaques (4.2-fold increase vs. early lesions; $p<0.01$). This prompted us to investigate how the RNA-binding properties of QKI could influence monocyte fate.

Our approach was to examine, at a genome-wide level, how altering the expression levels of QKI could impact pre-mRNA splicing (x-axis), mRNA expression (y-axis), and (pre-)mRNA localization (z-axis) upon differentiation of human monocytes into macrophages. For this, we employed both array-based gene expression analyses as well as next-generation sequencing techniques (RNA-seq) of THP-1 monocytic cells as well as $CD14^+$ monocytes derived from the peripheral blood of a unique, QKI haploinsufficient subject (with sibling control). Despite low expression levels of QKI in monocytes, the abrogation of QKI in these cells perturbed cellular adhesion and the ensuing establishment of the cytoskeletal architecture. Interestingly, our investigation of post-transcriptional events that are associated with the conversion of the monocyte to a macrophage, uncovered: 1) 536 alternative splicing events that are directly mediated by binding of QKI proximal to the splice site ($p<0.05$); and 2) 1214 differentially expressed genes (min. ± 1.5-fold; $p<0.05$) that indicate that QKI modulates monocyte activation and differentiation by regulating inflammation, cell growth and survival and RNA editing. Finally, subcellular fractionation of monocytes and macrophages enabled us to identify (pre-)mRNAs that are specifically transported within the cell by Quaking, generating novel insight into a role for Quaking in regulating (pre-) mRNA trafficking during monocyte to macrophage differentiation.

Collectively, our data have uncovered in 3D the post-transcriptional events that drive monocyte to macrophage differentiation, and identify the RNA-binding protein QKI as an orchestrator of this disease response.

517 A novel RNA Helicase associates with *Tb*ZFP3 and coregulates surface protein transcripts in *Trypanosoma brucei*

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Post-transcriptional gene regulation is essential to eukaryotic development. This is particularly emphasized in trypanosome parasites where genes are co-transcribed in polycistronic arrays but not necessarily co-regulated. The small CCCH protein, *Tb*ZFP3, has been identified as a *trans*-acting post-transcriptional regulator of Procyclin surface antigen expression in *T. brucei*. (Walrad et al., *PLoS Pathogens*, 2009; *NAR*, 2012). Mass-spectroscopy of eight separate anti-*Tb*ZFP3 immunoprecipitation experiments isolated a novel DEAD-box RNA Helicase, RH70. We verified this association via immunoprecipitation of an endogenously tagged TY-RH70, which specifically isolates *Tb*ZFP3. Reduction of RH70 levels via RNAi in bloodstream form parasites specifically upregulates *ep procyclin* transcript levels versus control transcripts. Further to this, preliminary data indicates reduced RH70 levels result in early EP procyclin expression during parasite lifecycle progression. While *Tb*ZFP3 stabilises *ep procyclin* mRNA thus promoting its translation, RH70 counteracts this and destabilises *ep procyclin* levels. This data indicates a functional linkage where co-precipitating proteins display opposite regulation of surface protein expression. Current experiments are underway to determine whether the *Tb*ZFP3:RH70 association is mRNA-dependent and verify this surface antigen regulation by RH70 helicase.

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518 Comprehensive analysis of RNA targets regulated by wild-type and mutant hnRNP A2/B1 in the nervous system

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hnRNPA2B1 encodes an abundant and multifunctional RNA binding protein, with widespread expression across tissues. hnRNP A2/B1 was previously shown to be reduced in Alzheimer's disease brains. Recently, mutations affecting the low complexity domain of hnRNP A2/B1 and related proteins were identified in families with multisystem proteinopathy (MSP) and amyotrophic lateral sclerosis (ALS). Despite the clear link between hnRNP dysfunction and neurological disease, a comprehensive survey of the RNA targets of hnRNP A2/B1 has not been performed in the central nervous system. In addition, the effects of damaging mutations in hnRNPs on RNA processing are not well understood. To elucidate the mechanism underlying pathogenesis, we identified transcriptome-wide RNA targets of hnRNP A2/B1 in spinal cords of wild-type mice using crosslinking and immunoprecipitation, followed by sequencing. Using antisense oligonucleotide technology, we depleted hnRNP A2/B1 in the mouse spinal cord, followed by splicing sensitive microarray analysis, which revealed several hundred alternative cassette events with a bias towards exon skipping upon depletion. Expression analysis by high throughput sequencing also revealed about 800 differentially expressed transcripts, with a strong bias towards reduced expression after depletion. To study the mechanism of disease progression in human cells, we used patient derived cells and microarray analysis to examine the RNA targets of hnRNP A2/B1 in humans. We compared splicing changes from fibroblasts from a family with MSP cause by an hnRNP A2/B1 D290V mutation with splicing changes induced by depletion of hnRNP A2/B1. We have also used reprogramming and differentiation technology to derive iPSCs and motor neurons (MNs) from hnRNP A2/B1 D290V patients and unaffected controls, which we have subjected also to genome-wide RNA processing studies. In summary, our data reveals thousands of functional A2/B1 targets relevant to degenerative diseases, and suggests that the D290V mutant likely results in a mostly gain-of-function and only a partial loss-of-function of A2/B1 activity.

519 U1 snRNP components are present in Gems, are essential for their integrity, and are required for normal motor axons in zebrafish

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Mutations in the RNA-binding protein FUS are a cause of the motor neuron disease ALS. We previously showed that U1 snRNP and the SMN complex are among the factors that associate with FUS and that FUS interacts directly with SMN. The SMN complex localizes in the cytoplasm and in nuclear Gems, and we found that Gem levels were decreased in ALS patient fibroblasts containing FUS mutations and in FUS knockdown HeLa cells. However, at present, the components and function(s) of Gems are not known. Here, we report that FUS interacts directly and robustly with the U1 snRNP-specific protein U1-70K. In light of this observation and that FUS is required for normal Gem levels, we asked whether the U1 snRNP-specific proteins affect Gems. This analysis revealed that knockdown of U1-70K, U1A, or U1C results in a striking loss of Gems, and the SMN complex components become diffuse in the nucleus. We also found that all three of the U1 snRNP-specific proteins are Gem components and co-IP with SMN from nuclear extract. Moreover, we found that U1A and U1C are required for the presence of U1-70K in Gems. To investigate a role for U1 snRNP in motor neuron disease, we used AMOs targeting U1-70K or U1 snRNA and found a motor neuron defect in zebrafish. Together, our data show that U1 snRNP components are present in Gems, are required for their integrity, and are essential for normal motor axons. The tight links between FUS, SMN, Gems, and motor neuron defects observed with loss of U1 snRNP components raise the possibility that U1 snRNP components are candidates for causing or contributing to ALS.

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520 Uncovering parallel ribosome biogenesis pathways during pre-60S subunit maturation

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Ribosome biogenesis is a highly complex process that in the yeast S.cerevisiae requires more than 200 ribosome maturation factors. Two previously identified paralogs found to be involved in yeast ribosome biogenesis are the protein pair Ssf1 and Ssf2 (94% sequence homology). Ssf2, the non-essential paralog, is believed to replace the essential Ssf1 in case of its absence from cells, and depletion of both proteins was shown to lead to severely impaired cell growth. Our study reveals that contrary to previous belief, upon alteration of levels of Nop7, Ssf1 and/or Ssf2, the Ssf proteins manifest a non-redundant function. Moreover, our results suggest that Ssf1 and Ssf2 cannot fully compensate for the depletion of one another, as they are both, independently, required along parallel pathways during pre-60S subunit maturation. Interestingly, the severely impaired cell growth observed in Nop7-depleted/SSF2Δ cells is probably caused by the appearance of a novel class of mature ribosomes containing truncated rRNA. Besides, Ssf1 and Ssf2, there are several other known paralog pairs, such as Fpr3 and Fpr4 as well as Rai1 and its weakly related paralog Dxo1, found among ribosome biogenesis factors, all of which are believed to play functionally redundant roles during ribosome maturation. However, our results raise the question as to whether these are truly functional paralogs, or, instead, fulfil functionally separate tasks along alternative maturation routes, that remain yet to be determined, interacting with distinct factors along the way.

521 An initial spliceosomal assembly event probed by reversibly constraining a 12-subunit U1 snRNP:pre-mRNA complex through a site-specific disulfide bond

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Pre-mRNA splicing, an essential step in mammalian gene expression, is catalyzed by the spliceosome - the very large and highly dynamic nuclear assemblage composed of 5 ribonucleoprotein particles or U snRNPs. Spliceosomal assembly occurs through the ordered addition of the U snRNPs onto a pre-mRNA. This assembly process is initiated by recognition of a pre-mRNA 5′ splice site by the 11-subunit U1 snRNP. The U1 snRNP remains stably associated with a 5′ splice site sequence until its displacement, just prior to the formation of the catalytically competent spliceosome. U1 snRNP recognition of a pre-mRNA is mediated by: (1) base-pairing between the 5′ end of the RNA subunit of U1 snRNP and a 5′ splice site, forming an RNA duplex; and (2) recognition of this duplex by the U1 snRNP protein subunit U1-C. Beyond these interactions, it remains unclear to what extent the U1 snRNP recognizes a pre-mRNA. To further investigate the pivotal U1snRNP:pre-mRNA interaction, we have engineered a disulfide crosslink between an engineered U1-C cysteine residue and a thiol modified pre-mRNA providing a reversible constraint between a pre-mRNA and U1-C alone and U1-C in context of the U1 snRNP particle. This strategy will allow us to assess the extent of the U1 snRNP:pre-mRNA interaction, providing valuable insight into the initiation of spliceosomal assembly.

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522 Abstract Withdrawn

523 Structure and semi-sequence-specific RNA binding of Nrd1

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In *Saccharomyces cerevisiae*, the Nrd1-dependent termination pathway plays an important role in surveillance and processing of non-coding RNAs. The termination and subsequent 3′ end processing is dependent on the Nrd1 complex that consists of two RNA-binding proteins Nrd1 and Nab3 and Sen1 helicase. It is established that Nrd1 and Nab3 proteins cooperatively recognize specific termination elements within nascent RNA, GUA[A/G] and UCUU[G], respectively. However, it is still not clear how the Nrd1 complex selects the RNA to be degraded/trimmed. Furthermore, some transcripts do not require GUA[A/G] motif for transcription termination *in vivo* and binding *in vitro*, suggesting the existence of alternative Nrd1-binding motifs.

Here we studied the structure and RNA-binding properties of Nrd1 using NMR, fluorescence anisotropy, and phenotypic analyses *in vivo*. We determined the solution structure of a two-domain RNA-binding fragment of Nrd1, formed by an RNA-recognition motif and helix-loop bundle. NMR and fluorescence data show that not only GUA[A/G] but also several other G-rich and AU-rich motifs are able to bind Nrd1 with affinity in a low micromolar range. The broad substrate specificity is achieved by adaptable interaction surfaces of the RRM and helix-loop bundle domains that sandwich the RNA substrates. Our findings have implication for the role of Nrd1 in termination and processing of many non-coding RNAs arising from bidirectional pervasive transcription.

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524 The crystal structure of human SFPQ reveals coiled-coil mediated polymerisation that links RNP granule formation, nucleic acid binding and gene regulation activity

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SFPQ, also known as PSF, is a vertebrate-specific tumor suppressor protein that regulates many important transcriptional functions of the cell nucleus including coordination of long non-coding RNA molecules into ribonucleoprotein granules termed paraspeckles. Here we describe the first crystal structures of SFPQ, from human, revealing a highly unusual and striking coiled-coil polymerization domain that, when mutated, results in mislocalization, abrogated molecular interactions and reduced function in transcriptional regulation. This propensity for functional aggregation via the coiled-coil polymerization domain thus explains how SFPQ contributes to manifold aspects of cellular nucleic acid metabolism and RNP granule formation.

525 Characterization of factors required for SMN complex formation and function using fission yeast as model organism

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The snRNPs are essential components of the eukaryotic splicing machinery. The biogenesis of these particles follows a complex pathway and is dependent on the SMN protein, the product of the Spinal Muscular Atrophy (SMA) disease gene SMN1. SMN homologues have been identified in higher eukaryotes as well as in the fission yeast *Schizosaccharomyces pombe*. We previously constructed a temperature-degron allele of SMN (tdSMN) and showed that tdSMN cells display differential snRNP assembly and splicing defects using a genome-wide approach. Our results are consistent with the notion that splice site selection and spliceosome kinetics are highly dependent on the concentration of core spliceosomal components (Campion et al., 2010).

In order to uncover factors involved in the SMN-dependent assembly pathway, we used an epistatic miniarray profile (E-MAP) approach which allows measurement of genetic interactions between all pairwise combinations of viable fission yeast deletion mutants. Using the tdSMN query strain, our analysis uncovered genes whose deletion results in synthetic lethality at the permissive temperature. As expected, our screen allowed the identification of critical regulators of snRNP assembly as for exemple the ICln gene coding for a subunit of the methylosome involved in early steps of snRNP biogenesis (Barbarossa et al., 2014). In addition, we identified genes linked to transcription by RNA polymerase II, consistent with a close functional interplay between splicing and the transcription machinery. We are currently investigating the roles of other factors in the assembly and function of the SMN complex.

Campion Y et al. (2010) EMBO J 29:1817; Barbarossa A et al. (2014) Mol Cell Biol 34:595.

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526 Modeling of human Splicing Factor 3b complex structure using PyRy3D software

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Determination of structures and mechanism of action of macromolecular complexes is one of major challenges in molecular biology. However, experimental structure determination for macromolecular assemblies is very difficult. For this reason a hybrid computational approach is used to incorporate spatial information from a variety of experimental methods into a modeling procedure.

We developed PyRy3D (see http://www.genesilico.pl/pyry3d) - a computational tool that applies hybrid approach in order to build low-resolution models of large macromolecular complexes. The model building procedure applies a Monte Carlo approach to sample the space of solutions. Spatial restraints are used to define components interacting with each other, and a simple scoring function is applied to pack them tightly into contours of the entire complex (e.g. cryoEM density maps).

Splicing Factor 3b (SF3b) is a protein complex responsible for recognition of the intron's branch site in U2- and U12-dependent introns. Human SF3b complex consists of seven proteins (SF3b155, SF3b145, SF3b130, SF3b49, SF3b14a, SF3b14b and SF3b10). However, so far, only fragments of some SF3b components have been determined experimentally. For others computational models are available [1]. Additionally electron density map for the whole complex was solved with 9.7 Å resolution, and the positions of RRM domains of SF3b14a and SF3b49 proteins were proposed [2]. Despite intensive research on SF3b, its complete structure and mechanism of action remain unknown.

We applied hybrid modeling approach implemented in PyRy3D software in order to build ensembles of structural models of the human SF3b complex, that fulfill currently available experimental and theoretical data. Our model recapitulated the predicted positions of RRM domains of SF3b14a and SF3b49 proposed by the Stark group and identified likely positions of other proteins that form the SF3b complex: SF3b155, SF3b145, SF3b130, SF3b14b and SF3b10.

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527 Towards understanding elongation arrest in prokaryotes

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The signal recognition particle (SRP) is a ribonucleoprotein complex that plays an essential role in co-translational targeting of membrane proteins. It is found in all three domains of life and exhibits a high diversity regarding composition and structure. In most organisms, SRP can be divided into two functional domains. The *S* domain mediates recognition and transport of ribosome-nascent chain complexes to the translocation channel, while the *Alu* domain stalls elongation until the complex has been faithfully delivered. In mammals, the mechanism underlying elongation arrest is well established. The *Alu* domain that is composed of a part of SRP RNA and the heterodimer formed by the SRP9/14 proteins competes with aminoacyl-tRNA/elongation factor binding, thus stalling translation. In prokaryotes, no structural or functional homologues of SRP9/14 have been identified and secondary structure predictions suggest a more complex fold of the *Alu* RNA raising the question whether elongation arrest is purely RNA-driven in prokaryotes. Progress towards understanding elongation arrest in prokaryotes and the evolution of *Alu* RNAs will be presented.

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528 PABPC4 Plays an Essential Role in Erythroid Differentiation

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Post-transcriptional controls play a major role in normal erythroid differentiation. Thus, defining post-transcriptional determinants of erythropoiesis has the potential to expand upon our understanding of normal erythroid differentiation and to highlight defects in this process that underlie certain hematologic disorders. We recently demonstrated an essential role for the minor poly(A) binding protein isoform, PABPC4, in erythroid differentiation. Selective depletion of PABPC4 from mouse erythro leukemia (MEL) cells was found to impede terminal differentiation and block (induction or repression) of well-defined gene sets critical to the terminal differentiation pathway. By combining high throughput sequencing of PABPC4-crosslinked mRNA fragments (HITS-CLIP) with RNA-seq studies we were able to define a set of mRNAs that are selectively targeted by PABPC4 in the MEL cell transcriptome (PABPC4-RNA 'interactome') and functionally impacted by these interactions. Combined analyses of HITS-CLIP data sets along with sequence analyses of 3′UTRs of mRNAs co-IP'ed in PABPC4 RNP complexes, revealed a high confidence AU-rich binding site motif that appears to underlie PABPC4-specific RNA recognition. Analysis of poly(A) tail kinetics on a subset of these mRNAs correlated PABPC4 binding with selective stabilization of mRNAs with critically-shortened poly(A) tails. These studies point to novel and essential functions of the minor cytoplasmic PABP isoform, PABPC4, in a mammalian pathway of cellular differentiation has set the stage for defining mechanisms of action(s) that distinguish PABPC4 activities from those of the structurally similar but far more abundant PABPC1.

529 Elucidating the Role of the uncharacterized mRNA maturation factor Yhr127p in *S.cerevisiae*

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Gene expression requires the action of various RNA binding proteins (RBPs), which package mRNA into ribonucleoprotein particles (RNPs) to protect, guide and mediate the maturation of nascent RNA Pol II transcripts. RNPs are heterogeneous with frequent rearrangement of protein composition through dynamic and transient interactions, which change as a function of the maturity of the mRNA. RBPs are important effectors of key mRNA maturation events by recruiting and forming processing machineries for splicing, 3′ end processing and polyadenylation. Quality control mechanisms ensure that only properly processed mRNA and formed RNPs are exported through the nuclear pore to the cytoplasm for translation. While key events and factors of mRNA maturation have been described, there is a lack of understanding of underlying regulatory mechanisms and the emerging roles of accessory proteins involved in mRNP maturation. This project focuses on the study of the uncharacterized nuclear RBP Yhr127p in *S.cerevisiae*. Through affinity purification mass spectrometry, we have identified Yhr127p as a component of mRNPs, showing co-transcriptional recruitment and association with mRNAs all the way to their export through the nuclear pore. Proteomics data also suggests a role for Yhr127p in ribosome biogenesis through its association with pre-ribosomal maturation proteins. While non-essential, YHR127W deletions strains show hypersensitivity to the TORC1 inhibitor rapamycin and an increase in ribosomal protein gene transcripts. In order to gain a greater understanding of the function of this protein, we use a variety of cutting-edge technologies to dissect the cellular function of Yhr127p.

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530 ATP modulates the RNA binding and phosphorylation of UPF1

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Nonsense-mediated mRNA decay (NMD) controls the quality of gene expression by detecting and rapidly degrading aberrant mRNAs that often contain a premature termination codon (PTC). However, the molecular mechanism for how NMD targets are discriminated from other transcripts is not fully understood. A central NMD factor is the ATP-dependent RNA helicase UPF1, which in human cells preferentially associates with mRNA 3′- untranslated regions (3′ UTRs) in a way that correlates with 3′ UTR length and the presence of a 3′ UTR exon-junction complex. We show here using immunoprecipitation of human-cell lysates that UPF1 variants lacking ATP or UPF2 binding or helicase activity fail to discriminate between mRNAs that are or are not NMD targets. Ensemble *Förster resonance energy transfer experiments reveal* that addition of ATP to purified UPF1 results in the release of UPF1 from RNA. Human transcriptome-wide RNA-footprinting of sequences that co-immunoprecipitate with phosphorylated UPF1 (p-UPF1) demonstrates that the presence of p-UPF1 significantly discriminates NMD targets from other transcripts. RNA immunoprecipitation of NMD reporter transcripts shows that, like steady-state UPF1, p-UPF1 is also enriched on the 3′ UTRs, but unlike steady-state UPF1, the location of p-UPF1 is more focused. Since hyper-phosphorylated UPF1 variants co-immunoprecipitate with abnormally high levels of SMG5, SMG6, SMG7 and protein phosphatase 2A, we conclude that p-UPF1 binding to the 3′UTR of NMD targets provides a binding platform for mRNA degradative activities and present a model for the dynamics of nonspecific vs. productive UPF1 binding to cellular RNAs.

531 A Conserved RNA Motif Required for 30S Ribosome Assembly

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Conserved motifs such as the A-minor, kink-turn, and right angle motifs, are found throughout ribosomal RNA. One interesting question is whether some RNA sequence motifs are conserved because they form metastable structures during ribosome assembly. The bacterial 16S rRNA encodes a highly conserved right angle motif between helices 5 and 6; however, those helices do not form a right angle in 30S crystal structures, as helix 6 interacts with helix 15.

Mutations that abolish the ability of this junction to form a right angle perturb 30S biogenesis *in vivo*. *E. coli* strains expressing the mutant rRNA in a background of WT rRNA have a slow growth phenotype, and produce perturbed polysome profiles with a broad 30S peak containing immature mutant rRNA. Decreasing the growth temperature exacerbates assembly and processing defects. The overexpression of an assembly factor rbfA improves the slow-growth phenotype for the mutants, while a chromosomal deletion of rbfA produces mutants with difficulties coming out of stationary phase.

SHAPE chemical probing experiments of the mutant 5′ domain RNAs shows a secondary structure similar to WT with some localized perturbations near the sites of the mutations. In order to correlate the structure of the assembly intermediates with changes in 30S biogenesis, a new method of purifying the mutant ribosomal RNA from WT background after *in vivo* hydroxyl radical footprinting was developed. The results will illuminate the importance of this individual sequence motif, the role of metastable structures during ribosomal assembly, and hopefully elucidate the structure of an assembly intermediate.

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532 Structural and functional analysis of the spliceosomal RNP remodeling enzyme, Brr2

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Splicing entails the removal of non-coding intervening sequences from eukaryotic pre-mRNA and the ligation of the neighboring coding regions and is carried out by the spliceosome. An active spliceosome evolves on the substrate by a stepwise assembly that is driven by members of the superfamily 2 (SF2) of ATPases/RNA helicases. Major conformational and compositional RNP remodeling is required to convert an initially inactive complex into an active spliceosome. The Brr2 protein is the key player in this catalytic activation process. Brr2 belongs to a unique group of SF2 helicases, whose members are exceptionally large and contain two expanded helicase units (cassettes) fused in tandem. Unlike other spliceosomal helicases, Brr2 requires strict regulation. Recently, we determined the crystal structure of an active, 200 kDa portion of Brr2, showing that its two helicase cassettes intimately interact and functionally cooperate [1]. The C-terminal cassette, while inactive in ATP hydrolysis and RNA duplex unwinding, strongly stimulates the N-terminal helicase via the extensive interaction interface between the two cassettes. We furthermore showed how Brr2 is regulated by a C-terminal Jab1/MPN domain of the spliceosomal Prp8 protein, which also requires the inactive C-terminal cassette [2]. However, it is not clear how the two helicase cassettes of Brr2 functionally communicate. Using pre-steady state kinetics, we probed the nucleotide binding preferences and worked out possible nucleotide binding mechanisms of each cassette. We also observed that the nucleotide binding rates of the active N-terminal cassette are affected when we introduce mutations in the intercassette interface. Our results further elucidate possible communication lines between the helicase cassettes.

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533 Quantitative Measurement of RNA Thermodyanamics Using Chemical Mapping

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The nearest neighbor model of RNA secondary structure thermodynamics underlies nearly all efforts to predict and design RNA structures. We seek to expand the experimental basis of these models by leveraging experiments that can make thousands of simultaneous quantitative measurements. Chemical mapping measurements, read out through sequencing, have been shown to offer powerful portraits of thermal melting behavior, but these method's accuracy have not been shown to be comparable to that offered by classic experiments using optical absorbance. In pilot systems with two-state and more complex behavior, we show that carbodiimide chemical mapping gives r delta-G measurements within this error compared to optical experiments. Further, the thermodynamics of particular motifs can be measured with this precision even within the context of a larger RNA, which may enable development of nearest-neighbor-like models for pseudo knots and tertiary contacts.

534 Surveillance of spliceosomal snRNP assembly by sequestration of incomplete particles in Cajal bodies

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Spliceosomal snRNPs are essential splicing factors, comprised of small, structured RNAs complexed with specific proteins. Here we identified a surveillance mechanism that controls final steps of snRNP assembly in the cell nucleus and sequesters incomplete particles in Cajal bodies (CBs). CB retention requires the TPR repeat-containing protein SART3, which bridges incomplete snRNPs and coilin, the scaffolding protein of CBs. Remarkably, inhibition of snRNP assembly lead to the de novo formation of CBs in cells that normally lack these compartments. Based on these data, we propose that CB nucleation is triggered by an imbalance in the snRNP assembly pathway, which leads to sequestration of stalled snRNP assembly intermediates by SART3 and coilin in CBs and thereby allows cells to maintain snRNP levels in the nucleoplasm in homeostatic balance.

535 Ro60, A new factor for P53 dependent P21 control

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The human RNA-binding protein Ro60 was discovered in 1984 but already achieved medical attention at the end of the 1960's, where it was discovered that Ro60 is a common autoantigen connected to rheumatic diseases ('sjögren's-sydrome' and 'systemic lupus erythematosus'). Orthologs of the protein have been discovered in various animals (e.g. primates, fish, nematodes) and even in bacteria, showing the evolutional conservation of Ro60. In humans four different isoforms of the Ro60 protein have been described, arising by alternative splicing from one gene locus. The crystal structure of *Xenopus laevis* Ro60 revealed, that the protein folds into a characteristic ring shaped form. Furthermore it could be shown that the protein can bind small non-coding and/or misfolded RNAs (e.g. Y RNA, U2-snRNA, 5S RNA). Therefore it was speculated, that Ro60 functions in RNA quality control, due to its ability to bind misfolded RNAs. Additionally it emerged, that Ro60 contributes to cell survival upon UV-stress in bacteria, nematodes and murine cells.

Here we provide data suggesting a role for Ro60 in P53 dependent activation of the cell cycle inhibitor P21. Our studies show that a depletion of Ro60 by RNAi results in an increase of P21 at mRNA- and protein levels. Accordingly, we observed a severe decrease of cell proliferation and viability. Additionally we provide evidence, that the observed cellular phenotypes are independent of Checkpoint kinase 1 and Checkpoint kinase 2, which are major facilitators of P53 regulation. Taken together we propose a model, where Ro60 functions as suppressor of P21 and therefor influences cell cycle arrest and viability.

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536 Proteomic analysis of *Entamoeba histolytica in vivo* assembled pre-mRNA splicing complexes

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The genome of the human intestinal parasite *Entamoeba histolytica* contains nearly 3000 introns and bioinformatic predictions indicate that major and minor spliceosomes occur in *Entamoeba*. However, except for the U2-, U4-, U5- and U6snRNAs, no other splicing factor has been cloned and characterized. Here, we HA-tagged cloned the snRNP component U1A and assessed its expression and nuclear localization. Because the snRNP-free U1A form interacts with polyadenylate-binding protein, HA-U1A immunoprecipitates could identify early and late splicing complexes. To avoid the parasite's endonucleases and ensure the precipitation of RNA-binding proteins, *Entamoeba* cultures were UV cross-linked prior immunoprecipitations with HA antibodies and precipitates were subjected to tandem mass spectrometry (MS/MS) analyses. To discriminate their nuclear roles (chromatin-, co-transcriptional-, splicing-related), MS/MS analyses were carried out with proteins eluted with MS2-agarose from nuclear extracts of an MS2 aptamer-tagged Rabx13 intron amoeba transformants. Thus, thirty-six Entamoeba proteins corresponding to 32 cognate splicing-specific factors (among them 13 DExH/D helicases required for all stages of splicing) were probed. In addition, another 12 splicing-related helicases were identified. Furthermore 50 other proteins, possibly involved in co-transcriptional processes were identified, revealing the complexity of co-transcriptional splicing in *Entamoeba*. Some of these later factors were not previously found in splicing complex analyses.

537 eIF4A1 modulates the slicing activity of AGO1 in plant RNA silencing

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In *Arabidopsis thaliana*, microRNAs (miRNAs) are loaded in ARGONAUTE 1 (AGO1) as part of RNA induced silencing complexes (RISCs) to regulate target messenger RNAs (mRNAs) via base-pairing. AGO1 possesses an intrinsic endonuclease activity responsible for the 'slicing' of mRNA targets, an activity that is abrogated when bulges or mismatches face nucleotides 10-11 of the miRNAs. Such mismatches promote in turn translational inhibition and/or decay of the target mRNAs. Intriguingly, the fact that in *Arabidopsis* most of the miRNAs regulates their target mRNAs via perfect or near-perfect complementarity has contributed to the widespread belief that plant miRNAs, unlike their animal counterparts, exert their effect mostly through target mRNA slicing. Yet, a previous work notably carried out in our lab suggests that these miRNAs can concurrently slice and transnationally inhibit a given pool of mRNAs. This raises the fundamental question of how slicing is avoided during translational inhibition? One possibility is that translational repressor proteins associate to AGO1 to change further the fate of the mRNA targets. Following this hypothesis, we have isolated eIF4a1 as a direct cofactor of AGO1. This protein biochemically and genetically interacts with AGO1; it does not affect the miRNAs biogenesis, nor the stability of RISC factors, but clearly impacts the mode of action of AGO1. Indeed, in an *eif4a1* background, the slicing activity of AGO1 is greatly improved, favouring the cleavage of the target mRNAs. Surprisingly, this property also gives to the mutant plants a stronger resistance upon infection by the *Tobacco rattle virus* (TRV). Together, our results show the existence of an AGO1 cofactor that directly modulates the slicing activity of AGO1. As multiple pools of AGO1 co-exist in *Arabidopsis*, we propose that eIF4a associates with AGO1 in a protein complex where an alternative form of target repression (i.e. translational repression) is favoured over slicing.

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538 Understanding microRNA function in animal germline

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Many crucial decisions, such as timing of cell division and cell-fate determination are made during early embryogenesis, a time in development when there is often little or no transcription. Therefore, gene expression relies on maternally supplied mRNAs. In the germline, 3′UTR-mediated post-transcriptional regulation is the primary mechanism controlling gene expression. microRNAs are short 21-23 nucleotides long non-coding RNA molecules which regulate their target mRNAs post-transcriptionally by forming a complex with the Argonaute proteins called miRISC. A recent study from our lab has shown that the microRNA pathway is important for the control of germ cell biogenesis in *C. elegans* (*Bukhari et al, Cell Research, 2012*). However, the mechanism by which microRNAs regulate the expression of target genes in germline remains largely unknown.

In *C. elegans*, the Argonautes ALG-1 and ALG-2 are involved exclusively in the miRNA pathway. Since the absence of Argonaute protein in somatic cells is associated with an increase of target mRNAs, we first decided to compare the level of mRNAs found in gonads of wild-type and *alg-1* mutant animals. Surprisingly, we observed that the level of mRNAs bearing predicted microRNA-binding sites is significantly decreased in the absence of ALG-1, suggesting that the miRISC protects mRNAs rather than induces their degradation in animal germline. This led us to investigate the function of microRNA-dependent regulation in germ cells. To address this issue, we designed a in vivo reporter of germline microRNA activity to study the effect of microRNA on the targeted mRNA. We will also purify and characterize the germline-specific miRISC to gather new insights on microRNA function in animal germline. Recent progresses on this project will be presented during the meeting.

539 Role of arginine methylation on AGO1 biology and the silencing pathway in *Arabidopsis thaliana*

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ARGONAUTES (AGOs) are one of the main components of the small RNA (sRNA) silencing pathway. They are the core members of the RNA-induced silencing complex (RISC), which silence target genes at the chromatin or at the mRNA level. Among the 10 AGO paralogs of the model plant *Arabidopsis thaliana* (AGO1-10), AGO1 is the main effector of the micro RNA (miRNA) pathway and is also associated with various classes of small interfering RNAs (siRNAs) acting post-transcriptionally, including transgene-, virus-derived and trans-acting siRNAs (tasiRNAs). Despite its importance in plant physiology and defense, scarce information is available regarding the modulation of AGO1 activity via post-translational modifications (PTMs). One such PTM has been documented in the case of AGO-related metazoan proteins called PIWIs. Indeed, N-terminal arginine residues of PIWIs are symmetrically methylated by protein methyltransferase 5 (dPRMT5), a process possibly involved in accurate subcellular localization and stability of the PIWI. Sequence alignment between PIWIs and Arabidopsis AGOs has unraveled arginine residues in AGO1 that are possibly targeted for methylation. We have generated an AGO1 mutant, in which putative methylated arginines are replaced by alanines ($AGO1^{met-}$). This mutant version together with the wild type AGO1 ($AGO1^{wt}$) were used to complement *ago1* knock out plants. These plants are being analyzed in order to better understand the impact of arginine methylation on AGO1 biology.

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540 A Novel Role for Symplekin in the Biogenesis of Endogenous Small Interfering RNAs

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Small RNAs (siRNAs) participate in numerous cellular processes to control gene expression including posttranscriptional silencing, translational repression, and induction of heterochromatin formation to regulate protein production within the cell. Errors in metabolism of these small RNAs can lead to developmental defects and disease, including cancer. Because siRNAs are ubiquitously involved in overall cellular function, it is important to study their maturation. siRNAs are derived from both exogenous (exo-) and endogenous (endo-) sources. Exo-siRNAs have been extensively studied in *Drosophila* since the late 1990s, however, endo-siRNAs have only recently been discovered. Endo-siRNAs are found in *Drosophila* and mice, and silence both mobile elements and protein coding genes. Maturation of these endo-siRNAs from their precursor dsRNAs requires specific proteins such as Dicer-2 (Dcr-2) and Loquacious (Loq), however other factors involved remain uncharacterized. Recent data from our lab suggests that one of these uncharacterized factors may be the scaffold protein Symplekin. Symplekin binds several other proteins (such as CPSF73 and CPSF100) forming a core cleavage complex responsible for correct processing of the 3′ end of both histone and canonical mRNAs. An immunoprecipitation assay (IP) using an antibody specific to Symplekin followed by mass spectroscopy revealed not only the suspected binding partners of Symplekin, but also novel proteins such as Dcr-2. The Symplekin-Dcr2 interaction was then confirmed via co-immunoprecipitation (co-IP) followed by western blotting. To investigate Symplekin's potential role in endo-siRNA biogenesis, northern blots probing for a specific endo-siRNA (esi2.1) were performed. This experiment revealed that when Symplekin is RNAi-depleted, levels of esi2.1 also decrease, suggesting a role for Symplekin in its production. To further investigate this novel role of Symplekin, our lab performed High Throughput Sequencing (HTS) on small RNA libraries that have been RNAi-depleted of Symplekin. Preliminary results show global decreases in small RNA levels, specifically with respect to endo-siRNAs. It should be noted however, that data analysis has yet to be completed. These data suggest that in addition to Symplekins role in 3′ end processing of mRNA, it may also have a role in the biogenesis of endo-siRNAs.

541 MicroRNAs are essential for sex chromosome stability during mammalian spermatogenesis

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Small RNAs play a variety of essential roles in mammalian spermatogenesis. Piwi-interacting RNAs (piRNAs), a class of small RNAs abundant in germ cells, function in developing spermatocytes to restrict the movement of transposable elements. MicroRNAs (miRNAs), a second class of small regulatory RNAs, also play an essential role in the germ line. However, it is unknown which specific processes miRNAs regulate during meiosis. MicroRNAs are produced from sequential cleavage of hairpin-structured transcripts, first by the miRNA-specific Drosha/Pasha endoribonuclease complex and then by a second endoribonuclease, Dicer; Dicer is also essential for small interfering RNA (siRNA) biogenesis. We, and others, have examined germline conditional mouse knockouts (cKO) of Dicer, Drosha, and Pasha, revealing essential roles for miRNAs in spermatogenesis - such mice produce few to no sperm and are infertile. We chose to more carefully phenotype Dicer and Pasha cKO mice to elucidate the specific roles of miRNAs in meiosis. We discovered that these knock-out mice display frequent sex chromosome abnormalities during pachytene, including ring chromosome formation and fusion to autosomes. Aberrant DNA-damage repair responses have been previously linked to genomic instability, including telomere fusion. We therefore conducted immunofluorescence analysis of DNA-damage repair proteins, which revealed normal localization of MDC1, a mediator of DNA damage complex assembly, but aberrant localization of RNF8, a ubiquitin-protein ligase recruited by phosphorylated MDC1. To characterize potential miRNA-mRNA target relationships that might explain the observed phenotypes, we performed whole-transcriptome sequencing of mRNAs and miRNAs from purified leptotene/zygotene and pachytene spermatocytes. Although RNF8 and MDC1 transcript levels appeared unchanged, levels of ATM, the kinase which phosphorylates several DNA damage repair proteins including MDC1, were upregulated ~3 fold during leptotene/zygotene in Dicer and Pasha cKOs. Importantly, ATM is targeted by miR-18a, a miRNA with spermatocyte-enriched expression, and mir-18a levels were negatively correlated with ATM in leptotene/zygotene spermatocytes. From our work, we conclude that miRNAs play a role in maintaining the genomic stability of sex chromosomes, and have identified a miRNA-target relationship which may underlie the observed chromosome fusion phenotypes. Ongoing experiments are directly examining the role of miR-18a, and regulation of ATM by miR-18a, during male meiosis.

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542 Identification, expression and molecular evolution of microRNAs in "living fossil" *Triops cancriformis* (*tadpole shrimp*)

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MicroRNAs (miRNAs) are identified and analysed in various model species such as human and fly. To understand the whole picture of miRNA evolution, investigation of miRNAs in non-model species has become an important task. Therefore, we focused on non-model species, living fossil *Triops cancriformis* (tadpole shrimp), which their mature form has been the same for almost 200 million years. Meanwhile, *T. cancriformis* undergo dramatic morphological change during the early developmental stages. Despite these characteristics, almost no genomic or transcriptomic data was accumulated. To elucidate the miRNA evolution, we performed comparative analysis of miRNAs and their machinery components. Firstly, deep sequencing analysis of small RNA libraries was performed on six developmental stages of *T. cancriformis* (egg, 1st to 4th instar larvae, and adult). In parallel, deep sequencing analysis was also performed on genomic DNA to obtain reliable information of miRNA sequences and their machinery components involved in miRNA processing. From our analysis, 87 conserved miRNA families and 93 novel miRNA candidates were identified. Furthermore, comparative analysis between *T. cancriformis* and *D. melanogaster* revealed the inconsistency among the expression patterns of conserved miRNAs, suggesting that although evolutionary conserved miRNAs possess very similar sequences across species, they may play different roles in each species. We have also predicted miRNA related protein coding genes: DICER1, AGO1-3, PIWI and AUB, from the genome sequence. Especially, *T. cancriformis* possessed only single DICER protein containing the DEAD domain, which was different from that of most arthropods. These results suggest that *T. cancriformis* miRNA machinery seems to have evolved in a unique way. This is the first study of comprehensive miRNA analysis of fresh water crustacean, and this study provided insight into the molecular basis of miRNA evolution.

543 Characterization of *NEFM* mRNA targeting microRNAs in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron degeneration disease characterized by muscle weakness which leads to paralysis and ultimately death. Neurofilaments (NF), composed of low, medium and high molecular weight neurofilament subunits (NFL, NFM, and NFH respectively, encoded by *NEFL, NEFM* and *NEFH*), are an important structural and functional component of motor neurons. Stoichiometric imbalance of NFL, NFM and NFH subunits can lead to neurofilament aggregate formation, therefore regulated expression of neurofilaments is vital for normal structure and function. We have previously reported that miRNAs regulate the expression of *NEFL* mRNA (levels of which are known to be reduced in degenerating motor neurons in ALS), and that the expression of these miRNAs are also altered in ALS in a manner predicted to result in a loss of *NEFL* mRNA. We hypothesize that miRNA might play a significant role in expression and stability of *NEFM* transcripts and thus aimed to identify miRNAs regulating *NEFM* expression. These miRNAs may impact the expression of NF by disrupting the stoichiometry of NF subunits produced from mRNA. We first examined the *NEFM* 3′UTR lengths expressed in sporadic ALS (sALS; n=7) and neuropathologically normal control samples (n=5) by using 3′ Rapid Amplification of cDNA ends (3′RACE). Three *NEFM* 3′UTR lengths were detected: 487 bases in all spinal cord samples from sALS patients and controls; 300 bases and 853 bases which were found in some control and some sALS samples. MiRNA binding sites were determined using Miranda and Targetscan, as well as manual screening for recognition sites of at least seven nucleotides for miRNAs we have identified to be altered in ALS. MiRNAs having binding sites in the *NEFM* 3′UTR are being assessed for their functional relevance to *NEFM* stability and expression using luciferase reporter gene assay. This is the first report describing alternate 3′UTR lengths of *NEFM* in ALS and control spinal cords, and suggests that alternative 3′UTRs may be instrumental in dictating miRNA regulation of expression of NF mRNA species.

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544 Functional characterization of the PIWI domain of microRNA-specific Argonaute in Caenorhabditis elegans

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The microRNA pathway is an important conserved mechanism that contributes to precisely orchestrated gene expression in animals. At the core of this regulatory pathway, microRNA-mediated silencing complex (miRISC), a small ~21-nt RNA-loaded Argonaute protein, specifically interacts with partially complementary sequences in the 3′ untranslated region of mRNA. Afterward, the miRISC recruits and interacts with other proteins such as GW182 proteins to negatively regulate translation and/or induce degradation of the target mRNAs. While the nature and consequences of the interaction between the miRISC and its binding partners as yet to be characterized, the recent crystal structures of human Argonaute1 and 2 have identified two tryptophan-binding pockets (Trp-binding pockets) on the exterior of the PIWI domain, with which GW182 proteins interact (Nakanishi et al., Cell Rep., 2013: Schirle et al., Science, 2012).

Here we used Caenorhabditis elegans as in vivo animal model to elucidate the impact of the corresponding pockets on ALG-1, a microRNA-specific Argonaute. Alteration of the pockets does not impair interaction with microRNAs but leads to microRNA-related defects that are more prominent than ones caused by the complete loss of ALG-1. Furthermore, in contrast with C. elegans lacking ALG-1, the transgenic worms expressing the Trp-binding mutants still produce microRNAs from precursor microRNAs. Interestingly, the expression of the Trp-binding mutants in ain-1 (the GW182 homolog) null mutants causes severe phenotypes that are not observed in the ain-1; alg-1 double mutant. Taken together, our data suggest thus far that the presence of only one microRNA-specific Argonaute unable to interact with GW182 proteins can severely alter microRNA-mediated gene regulation in animals.

545 RNA as a Boiling-Resistant Anionic Polymer Material to Build Robust Structures with Defined Shape and Stoichiometry

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RNA is a polyribonucleic acid belonging to a special class of anionic polymers, holding a unique property of self-assembly that is controllable in the construction of structures with defined size, shape, and stoichiometry. We report here the use of RNA as polymers to fabricate boiling-resistant triangular nano-scaffolds, which were used to construct patterned hexagonal arrays. The RNA triangular scaffolds demonstrated promising potential to construct fluorogenic probes and therapeutic agents as functionalization with siRNA, ribozyme, folate, and fluorogenic RNA aptamers revealed independent functional activity of each RNA moiety. The ribozyme was able to cleave hepatitis genomic RNA fragments, the siRNA silenced the target genes, and all fluorogenic RNA aptamers retained their fluorescence emission property. The creation of boiling temperature-resistant RNA nanoparticles opens a new dimension of RNA as a special polymer, feasible in industrial and nanotechnological applications.

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546 RNA detection by ligating two DNA oligos annealed to a complementary RNA splint using Chlorella virus DNA ligase

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Ligation of two adjacent DNA oligonucleotides splinted by complementary RNA has historically been difficult to achieve. We have discovered that Chlorella virus DNA ligase (PBCV-1 DNA ligase) is much more efficient in this ligation than T4 DNA ligase, which was traditionally used in this application. When these enzymes were compared using the same RNA:DNA substrates the Chlorella virus enzyme achieved complete ligation with 10X less enzyme but 15X faster than the T4 DNA ligase [1].

We have taken advantage of this efficient RNA splint mediated ligation to develop a miRNA detection method. By using DNA oligos of different lengths we found efficient ligation can be achieved with only a 4-6 base pair overlap between one of the DNA oligos and the miRNA. Because the ligase is sensitive to a single nucleotide mismatch, we designed DNA probes that were specific for individual members of the mammalian let-7 family. The ligated DNA probes contained terminal sequences that allowed them to be amplified with PCR primers. Using qPCR with SYBR green we were able to amplify and detect sub-picogram amounts of miRNA. The efficient and rapid ligation of RNA:DNA hybrids by the Chlorella virus DNA ligase should allow it to be used in a wide range of RNA detection methods for both cellular and viral RNAs.

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547 Pokeweed Antiviral Protein is Regulated by a Small RNA

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Plants have evolved many strategies to evade pathogen attack and glycosidases are one group of enzymes thought to be involved in defense. Glycosidases remove purine bases from RNA, thereby inhibiting their translation. Pokeweed antiviral protein (PAP), synthesized by the pokeweed plant *Phytolacca americana*, inhibits the replication of several plant and animal viruses by removing purines from viral RNAs. Most of the studies investigating PAP's mode of action have relied on its expression in heterologous systems. Very little is known about the regulation of PAP in the native plant. We report here that PAP mRNA is regulated by a small RNA that targets its open reading frame (ORF). By primer extension and 5′ RACE, we show that PAP mRNA is cleaved 192 nt from its 5′ end. Electrophoretic mobility shift assay indicated the presence of a small RNA complementary to the region spanning the cleavage site. qRT-PCR showed that by scrambling the small RNA target site, PAP mRNA levels increased 23-fold compared to the wild type target site. Subsequent sequencing of the small RNA pool of pokeweed confirmed that it aligned completely with the target site within the PAP ORF. Work is underway to confirm the *in vivo* significance through a small RNA sponge. As well, an Argonaut pulldown will test the link between the small RNA and the silencing pathway in plants. We are currently investigating the physiological relevance of the small RNA and our initial results suggest that it is down-regulated by jasmonic acid. Jasmonic acid is a plant hormone that has been implicated in controlling plant response to stress. This down-regulation would likely increase PAP levels and supports our hypothesis that PAP defends against pathogens. This is the first report of a plant defense protein being regulated by a small RNA.

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548 bmo-let-7 cluster miRNAs coordinately control the development and metamorphosis of *Bombyx mori* (silkworm)

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Bombyx mori (silkworm) has been domesticated for silk production for about 5,000 years and is emerging as an ideal molecular genetic resource for solving a wide range of biological problems. So far, more than 480 miRNAs have been identified in the silkworm but very few of them have been functionally determined. miR-2795 is a silkworm-specific miRNA and forms one miRNA cluster with the conserved miR-100 and let-7 in the silkworm genome. bmo-let-7, let-7*, miR-100 and miR-2795 were temporally and spatially co-expressed in the silkworm under control of the same promoter. The stage transitions of silkworm development were seriously delayed or inhibited when the expression levels of these cluster miRNAs were changed at the molting and prepupal stages but not at the instar larval, mounting and pupal stages. The roles of these cluster miRNAs in this species were further confirmed by transgenic approaches. The targets were systematically identified by means of transcriptome and proteome analyses combined with prediction and dual luciferase reporter assay. In a word, this work tried to reveal the molecular mechanism of silkworm development and metamorphosis involved by let-7,and its cluster members.

549 Computational and evolutionary analysis of bacterial small RNAs using synteny information

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In bacteria, small RNA (sRNA) is one of the post-transcriptional regulators by base-pairing with target mRNA. The sRNAs of *Escherichia coli* have been well studied but their nucleotide conservation seems to be limited within closely-related species even inγ-proteobacteria. Therefore, the molecular evolution of these sRNAs is one of the big topics in the field.

To efficiently find sRNA genes in many bacterial species and to reveal its evolution, we focused on gene loci and conserved adjacent genes around each sRNA. This synteny analysis could enable to find and extract sRNA homologous sequences for an additional group of bacterial species. In this research, we analyzed the 442 γ-proteobacteria genomes and 18 *E. coli* sRNAs. First, BLAST analysis of each sRNA was performed and showed that sRNAs were conserved only in specific group of species that are closely related with *E. coli*. Next, based on the multiple alignment of intergenic regions obtained from the synteny analysis, a new set of sRNA candidates are found for 9 out of 18 sRNAs. Especially, when the conserved adjacent genes are involved in the same regulatory system with the corresponding sRNAs, high efficiency of the detection of novel sRNA candidates are observed. For example, the relationships between sRNA genes and their transcription factors are found to be very useful to extract the candidates; OxyR for *OxyS* sRNA, GcvA for *GcvB* sRNA, and SgrR for *SgrS* sRNA. The evolution of the 18 sRNAs including the novel candidates will be discussed at the nucleotide sequence level.

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550 Lin28a controls neuronal differentiation by inhibiting biogenesis of brain-specific miR-9

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MicroRNAs (miRs) shape the identity and function of cells by regulating gene expression. In spite of the great effort to understand the various biological roles of individual microRNAs there is a huge void of knowledge about the regulation of their own biogenesis. It is known that several tissue-specific microRNAs are under transcriptional control; however, it is unknown whether post-transcriptional processes contribute to establishing their levels.

Here, we show that brain-specific miR-9 is regulated transcriptionally and post-transcriptionally during the neuronal differentiation. We demonstrate that miR-9 is more efficiently processed in differentiated than undifferentiated cells. We reveal that Lin28a inhibits the processing of miR-9 by inducing the degradation of its precursor through a uridylation-independent mechanism. Furthermore, we show that constitutive expression of untagged but not GFP-tagged Lin28a causes a severe neuronal differentiation phenotype, which coincides with reduced miR-9 levels. Using an inducible Tet-On 3G system we demonstrate that Lin28a can also reduce miR-9 levels in differentiated cells.

Altogether, we shed more light on the role of Lin28a in the neuronal differentiation as well as show novel, substrate-dependent mechanism of Lin28a action. Finally, our results provide evidence that transcriptionally controlled microRNAs can undergo extensive and complementary post-transcriptional regulation.

551 miR-19 and miR-155 inhibit the SOCS1-p53 axis in leukemias

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MicroRNAs (miRNAs) are upregulated in various leukemia suggesting they can play an oncogenic role. Indeed, miR-19, from the miR17-92 cluster, seems to contribute to progression of DL-BCL (diffuse large B cell lymphoma), follicular lymphoma and CML (chronic myelogenous leukemia). In addition, miR-155 is found overexpressed in B cell lymphoma and CLL (chronic lymphocytic leukemia). They both target numerous tumor suppressors, which includes PTEN and SOCS1. Our work has shown that SOCS1 can interact with both ATM and p53 resulting in the phosphorylation and activation of the latter to mediate cellular senescence. The latter is characterized by a cell cycle arrest in the G1/S phase and is thought to impede cancer progression.

In order to study the role of the oncogenic miRNAs in leukemia, we used microRNA sponges targeting miR-19 and miR-155. Sponges consist of multiple tandemly cloned binding sites for a given miRNA and act by titrating their levels. Our results show that sponges against miR-19 or miR-155 stabilized SOCS1 and enhanced p53 activation as shown by measuring p53 phosphorylation at serine 15 and its transcriptional target p21. Although the development of therapeutics to induce miRNA down-regulation is currently underway, our work suggests that inhibition if miR-19 and miR-155 can reactivate endogenous tumor suppressors in leukemia.

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552 Charged amino acid residues are behind the RNA annealing and chaperone activity of Hfq

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Hfq is an essential co-factor for post-transcriptional gene regulation in bacteria. Hfq simultaneously binds small non-coding RNAs (sRNA) and un-translated regions (UTR) of target mRNAs, stabilizing the sRNA-mRNA complex and increasing the rate of RNA base pairing by 100-fold. Highly conserved arginines on the outer rim of the Hfq hexamer form active sites where helix initiation takes place. Unexpectedly, conserved acidic residues line a channel from the inner pore to the arginine patch in which the sRNA and other U-rich RNAs are thought to bind. Fluorescence anisotropy and gel mobility shift assays showed that substitution of four acidic residues to alanine (D9A, E18A, E37A, or D40A) resulted in stronger binding of U-rich RNA oligonucleotides, with no discrimination between U-rich and A-rich oligonucleotides in annealing reactions. By contrast, substitution of an acidic residue weakened binding of structured sRNAs. RNase footprinting results showed that unlike WT Hfq, the mutants cannot destabilize sRNA secondary structure. In vivo reporter assays and in vitro gel mobility shift assays showed that this correlated with an inability of the Hfq mutants to facilitate up-regulation of *rpoS* translation by structured sRNAs like DsrA. Nonetheless, the Hfq mutants supported *rpoS* regulation by less structured sRNAs like ArcZ56. Overall, our results suggest that acidic residues on the proximal face of Hfq partially melt the secondary structure of the sRNA, so that the seed region can interact with the arginine patch. This interaction with the arginine patch is necessary for base pairing with target mRNAs and the regulation of gene expression.

553 Engineering the scaffold region of natural and artificial small RNAs to enhance their gene regulation abilities

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Non-coding small RNAs (sRNAs) regulates their target gene expression by base pairing with the target mRNA, thereby modulating the mRNA stability, elongation or the translational efficiency. Many natural *trans*-encoded sRNAs contain a "scaffold" that allows binding of the RNA chaperone protein Hfq, which is required for these *trans*-encoded sRNAs since Hfq-binding enhances the stability of sRNAs by protecting them from ribonuclease degradation.

Artificial sRNAs can be designed based on the complementary sequence of their target mRNA sequence and have been described as efficient genetic tools to regulate target gene expression. To improve the gene regulation abilities of sRNAs, elongating the antisense sequence or to target different mRNA region is the conventional method, although altering antisense sequence may affect the specificity of sRNA against target sequences. Therefore, an alternative strategy to improve sRNA gene regulation will be valuable if it can be applied to wide range of sRNAs.

In this research, the gene regulation abilities of sRNAs were improved by directly fusing the *Escherichia coli trans*-encoded sRNA-derived scaffold sequences to the 3′-end. The sRNAs of transcriptional regulator (attenuators) (Qi *et al. Nucleic Acids Research*, 2012) and post-transcriptional regulator (riboregulators) (Isaacs *et al. Nature Biotechnology*, 2004, Abe *et al. Biotechnology Letters*, 2014) were selected as the target to improve their gene regulation abilities. The fusion of scaffold improved the gene regulation abilities of both transcriptional and post-transcriptional regulators. To further improve, mutations were introduced into the best scaffold and mutations predicted to stabilize the secondary structures of the scaffolds dramatically increased their abilities. From northern blot analysis, scaffold-fused sRNAs were more stable compared to the sRNAs without the scaffold and exhibited lower gene regulation abilities in Δ*hfq* strain. These results indicate that Hfq binds to the scaffold-fused sRNAs and enhances their stabilities, resulting in increased gene regulation abilities (Sakai *et al. ACS Synthetic Biology*, DOI: 10.1021/sb4000959). This mutational approach was also applied to well-characterized *E. coli*-derived sRNAs and mutating the scaffold region of sRNAs increased their gene regulation abilities without altering the antisense region. We believe our engineered sRNA scaffolds may present a valuable strategy to regulate target gene expression strongly.

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554 Transcriptome and transposable elements dynamics during PIWI-mediated regulation in Drosophila ovarian cell cultures

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A conserved function for Piwi proteins and Piwi-interacting RNAs (piRNAs) is to repress transposable elements (TEs) from mobilizing in gonadal cells. To determine the spectrum of piRNA-regulated targets that may extend beyond TEs, we conducted a genome-wide survey for transcripts associated with PIWI and for transcripts affected by PIWI knockdown in Drosophila OSS (Ovarian Somatic Sheet) cells, a follicle cell line with functional Piwi pathway. Despite the immense sequence diversity amongst OSS cell piRNAs, TE transcripts were still the major transcripts associated with and regulated by PIWI. Interestingly, the coding genes that were regulated by PIWI tended to have de novo TE insertions that generated a nascent TE transcript nearby to the expressed gene. Some de novo inserted TEs also stimulated expression of novel long non-coding RNAs that associate with PIWI and influenced the expression of nearby genes. However, we noticed that PIWI-regulated genes in OSS cells greatly differed from the regulated genes in a related follicle cell culture, OSCs (Ovarian Somatic Cells). Therefore, we characterized the distinct TE landscape dynamics across four OSS and OSC genomes, and discovered that TE landscapes in gonadal cultures may exist in a highly metastable state. Our analyses of OSS and OSC cells demonstrate that despite having a Piwi pathway that suppresses endogenous TEs, TE insertion landscapes can still dramatically change and result in rewiring the transcriptomes of gonadal cells.

555 The small RNA content secreted by benign (MCF-10A) and malignant (MCF-7) mammary epithelial cell lines in different extracellular fractions

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Cancer cells are known to secrete high levels of extracellular vesicles, namely exosomes (30-100nm; derived from multivesicular bodies) and ectosomes or microvesicles, which are larger in size and derived from plasma membrane budding. Once thought as a vehicle for excretion of toxic compounds, extracellular vesicles are now known to exert different functions in respect to intercellular communication. The discovery that mammalian extracellular vesicles contain active mRNAs and microRNAs (Valadi et al. 2007) attracted great attention because it implies that cells are able to control gene expression of neighboring or even distal cells. This has obvious implications in pathologies such as cancer, where tumor-induced modifications in stromal cells are described. We thought to characterize the small RNA content in a breast cancer cell line (MCF-7) and a benign mammary epithelial cell line (MCF-10A) by next-generation sequencing. We purified three different fractions from the conditioned media of serum-free cultures: exosomes, ectosomes, and ribonucleoproteins (corresponding to 100.000g supernatants, which are known to contain microRNAs; Arroyo et al. 2011). Three biological replicates were sequenced for each cell line and extracellular fraction, together with the intracellular small RNA transcriptome. A variety of small RNAs were detected and characterized, including microRNAs, and fragments of tRNA, rRNA, and to a lesser extent, snRNAs and snoRNAs. Remarkably, tRNA 5′ halves showed great enrichment in the extracellular milieu, especially in the ribonucleoprotein fraction. The microRNAs adjusted well to a theoretical model of passive secretion. However, a few microRNAs showed significant enrichment in the extracellular milieu and are candidates to be actively selected for secretion. Care was taken to detect and obliterate sequencing reads derived from cross-contamination. As we have recently described (Tosar et al. 2014), unawareness of these effects would have yielded a different and artificial scenario. Overall, we present a description of how different cell lines secrete different small RNAs in different extracellular fractions, what will help to undercover new roles for small RNAs in intercellular communication pathways and biomarker research.

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556 NUFIP/R2TP and ZNHIT3 interact with the SMN complex to promote assembly of U4-specific proteins

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Assembly of RNPs is often a complex process that involves dedicated machineries. We showed previously that the NUFIP/R2TP machinery and a new factor ZNHIT3 are implicated in the assembly of box C/D snoRNPs. Here, we report that this machinery is also involved in the formation of U4 snRNP. This particle is assembled with Sm proteins using the SMN complex and in addition contains specific proteins such as 15.5K and Prp31. 15.5K is common to both box C/D snoRNPs and U4 snRNP, and it binds to a K-turn structure found in these two types of RNAs. Prp31 is structurally related to Nop58 and like it, it interacts with 15.5K and contains a Nop domain.

Here, we report that Prp31 interacts with NUFIP/R2TP and ZNHIT3. We showed that a mutant protein Prp31 A216P, which causes retinitis pigmentosa, specifically loses its interaction with ZNHIT3. RNase protection experiments with U4 mutated in its K-turn domain, which specifically binds 15.5K and PRP31, showed that surprisingly, recruitment of both these proteins on U4 occurs independently of this domain. We found an association of NUFIP, ZNHIT3 and Prp31 with the SMN complex in cell extract, and NUFIP was shown to directly interact with Gemin-3 and Gemin-6 in vitro. Furthermore, depletion of NUFIP leads to accumulation of Prp31 in Cajal Bodies, a phenotype that arise when assembly of the U4/U6:U5 tri-snRNP is perturbed.

We propose a model in which the SMN complex provides a plateform that interacts with NUFIP/R2TP and free Prp31, thereby allowing assembly of Prp31 and formation of the tri-snRNP. This could occur in Cajal bodies on newly synthesized snRNP, but it could also involve pre-existing snRNP and facilitate their recycling. SMN was long ago proposed to facilitate snRNP regeneration, but the mechanisms remained unclear. A role of SMN in the assembly of snRNP-specific proteins would provide a molecular basis for its requirement in snRNP recycling.

557 The role of the Arabidopsis exosome complex in silencing of heterochromatic loci and regulation of smRNA producing loci

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The exosome functions throughout eukaryotic RNA metabolism and has a prominent role in gene silencing in yeast. In Arabidopsis, exosome regulates expression of a ''hidden'' transcriptome layer from centromeric, pericentromeric, and other heterochromatic loci that are also controlled by small (sm)RNA-based de novo DNA methylation (RdDM). However, the relationship between exosome and smRNAs in gene silencing in Arabidopsis remains unexplored. To investigate whether exosome interacts with RdDM, we profiled Arabidopsis smRNAs by deep sequencing in exosome and RdDM mutants and also analyzed RdDM-controlled loci. We found that exosome loss had a very minor effect on global smRNA populations, suggesting that, in contrast to fission yeast, in Arabidopsis the exosome does not control the spurious entry of RNAs into smRNA pathways. When we analyzed the minor group of smRNA producing loci using genome-wide clustering method, we found a minor population of smRNA producing loci (3-4%) is dependent on exosome core subunits RRP4, RRP41 and catalytic subunit RRP44a for smRNA production with subunit specificity; these minor group of clusters are densely localized in centromeric and pericentromeric regions.

Exosome defects resulted in decreased histone H3K9 dimethylation at RdDM-controlled loci, without affecting smRNAs or DNA methylation. Exosome also exhibits a strong genetic interaction with RNA Pol V, but not Pol IV, and physically associates with transcripts produced from the scaffold RNAs generating region. Our data suggest that Arabidopsis exosome may act in parallel with RdDM in gene silencing, by epigenetic effects on chromatin structure, not through siRNAs or DNA methylation. We also propose that the Arabidopsis exosome may coordinate the transcriptional interplay of different RNA polymerases to modulate repression of some repetitive sequences. Results of further examination of exosome involvement in gene silencing and siRNA producing loci in Arabidopsis will be presented.

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558 The *C. elegans* Argonaute CSR-1 promotes germline gene expression

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Small RNA-mediated gene silencing pathways are key regulators of gene expression in a variety of organisms. These pathways play critical roles in defending the genome against foreign nucleic acids, and in regulating gene-expression throughout development. Argonaute proteins (AGOs), the main effectors of these pathways, bind small-RNAs that guide the protein/small-RNA complexes via sequence complementarity to target transcripts and induce effects such as mRNA degradation and translational inhibition to elicit "gene silencing". While most small-RNA pathways studied to date have negative regulatory effects on their target transcripts, we recently described a positive role for a C. elegans AGO in promoting or "licensing" the expression of germline transcripts. CSR-1 is an essential C. elegans AGO, that has the capacity to target nearly all germline expressed transcripts. csr-1 homozygote mutant animals are embryonic lethal, making it difficult to study the transgenerational consequences of loss of csr-1. As such, we developed an in vivo RNA tethering assay where CSR-1 is artificially tethered to a nascent transcript. We have shown that CSR-1 counteracts the epigenetic silencing initiated by the piRNA pathway, by establishing /maintaining an epigenetic memory of previous germline transcription.

The mechanism(s) by which the CSR-1 pathway establishes/maintains this epigenetic memory and how this extends to endogenous CSR-1 targets remains poorly understood. We hypothesize that the CSR-1 pathway functions to establish unique combinations of histone post-translational modifications at its targeted genomic loci, that are reinforced each generation by continuous recruitment of CSR-1/small-RNA complexes and specific histone modifying enzymes. In support of this, global analysis of histone post-translational histone modifications has demonstrated that the CSR-1 pathway is required for the appropriate accumulation of several histone modifications associated with chromatin environments that are permissive to transcription.

While small-RNA pathways have previously been implicated in the formation of heterochromatin and transcriptional silencing, our findings suggest that the CSR-1 pathway licenses transcripts in part by influencing the accumulation of epigenetic marks associated with active transcription. These findings open an exciting new frontier of small-RNA mediated regulatory mechanisms that can act in a positive manner to counteract silencing pathways and maintain a balance of gene expression.

559 MicroRNA regulation of neonatal immunity

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Unlike adults, neonates can contract the same disease multiple times due to developmental differences in their adaptive immune systems. While naive CD8+ T cells in both neonatal and adult mice are able to form the effector cells necessary for combating infection, only adults efficiently create a memory population of CD8+ T cells that are capable of responding to re-infection. Because the T cell immune response is dependent on Dicer, we hypothesized that differential microRNA expression underlies this immunological deficiency. To investigate this possibility, we profiled both mouse and human microRNAs present in the naive CD8+ T cells of adult and neonates. We also profiled the microRNAs expressed in mouse effector cells after infection. We found that while adults and neonates switch on the same complement of microRNAs in their effector cells, they differentially express several microRNAs in their naive cells. We investigated the role of miR150, one of the microRNAs that was significantly differentially regulated in both human and mouse naive cells. To do so, we transferred CD8+ T cells from a miR150 knockout mouse into a wild-type mouse, enabling us to assay their response to infection in an otherwise normal animal. The miR150 knock-out cells behaved similarly to wild-type prior to infection, yet during infection, they preferentially created memory-like cells instead of effector cells. These data suggest that miR150 contributes to the establishment of a gene regulatory network in naive cells that controls the proportion of effector and memory cells created during infection. We are currently measuring transcriptome differences between the wild-type and miR150 knockout mice to determine which genes are targets of miR150. Additionally, we sequenced the 3′ termini of mRNAs in naive and effector cells and discovered substantial differential cleavage and polyadenylation patterns between them. These results suggest that although miRNA expression does not differ between adults and neonates in effector cells, differential 3′UTR isoforms could alter which genes are able to be regulated by miRNAs. Together, these results indicate that miR150, perhaps in concert with other microRNAs, helps to control adult and neonatal T response and suggest widespread roles for post-transcriptional gene regulation in T cells.

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560 Abstract Withdrawn

561 RNase L is involved in some microRNAs biogenesis

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MicroRNAs are a family of small noncoding RNAs that regulate gene expression. Biogenesis, processing and expression regulation of miRNAs are very complicated and the exact mechanism of these process is not fully understood now. The maturation of miRNA is usually processed by Drosha and Dicer. Our study found that RNase L is involved in the generation of miRNA under specific conditions. RNase L overexpression in HepG2 cells increased some mature miRNA level and siRNA-mediated RNase L knockdown in HeLa cells also decreased some miRNA level. RIP was performed with anti-flag-RNase L antibody and we found that RNase L can bind pri-miRNAs. The results of this study contribute to a better understanding of the complexity of miRNA biogenesis.

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562 MicroRNA inhibitors specifically associate with target microRNAs in the context of Argonaute displacing the target-miRNA regulated mRNA transcript

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MicroRNAs, the genomically-encoded small RNA molecules that post-transcriptionally regulate gene networks, have crucial roles in cell physiology and aberrant microRNA expression can lead to disease. Chemically modified oligonucleotides offer the promise of modulating microRNA levels as a therapeutic intervention in disease. Although microRNA-based therapeutics are advancing to the clinic, their mechanism of action has not been fully elucidated. Here we show evidence of direct physical interaction between microRNA inhibitors and Argonaute proteins *in vitro* and *in vivo.* The Argonaute/microRNA/inhibitor association takes place only in the context of the cognate target microRNA, is mediated by the seed region of the miRNA while the placement of chemical modifications inadvertently affects this interaction. Target miRNA levels remain unaffected and target miRNAs continue to be associated with Argonaute. On the contrary, previously Argonaute-associated mRNA transcripts are no longer targeted by Argonaute and increase in abundance. Our data support a model of microRNA inhibitor mechanism of action where microRNA inhibitors associate specifically with target microRNAs in Argonaute leading to the displacement of the microRNA-regulated transcript, which is subsequently stabilized.

563 Elucidating the mechanism by which CELF2 regulates LEF1 alternative splicing during T-cell activation

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Alternative splicing is an important process by which T-cells regulate RNA expression according to signaling cues. One example of a splicing change upon T-cells activation is the induced inclusion of exon 6 of the LEF1 transcription factor (LEF1-E6). This increase in LEF-E6 inclusion generates an isoform of LEF1 that is preferentially active in promoting transcription of the alpha chain of the T-cell receptor. Previous work in the lab has shown that CELF2 binds two conserved elements (USE and DSE) in the upstream and downstream introns flanking exon 6 to drive this signal-responsive splicing event. Importantly, CELF2 knockdown results in a reduction of both LEF-E6 inclusion and TCRα mRNA.

To understand the mechanism by which CELF2 regulates LEF1 exon 6 inclusion via the USE/DSE we have first used standard minigene assays. We show that stimulation-induced exon inclusion is sensitive to the specific sequence identity and location of the USE and DSE. In isolation, neither sequence alone is sufficient to confer signal-responsive splicing. However, the USE functions as a basal splicing silencer, while the DSE promotes exon inclusion regardless of the presence of the USE.

To complement the functional studies, we have investigated how stimulation alters the association of CELF2 with the USE and DSE. UV crosslinking has shown that CELF2 binding to both elements is stronger in extracts from stimulated cells than from resting cells. Moreover, RNA mobility shift assays demonstrate that CELF2 exhibits enhanced coopertivity in its binding to both the USE and DSE upon stimulation. Interestingly, mass spec, 2-D gel analysis and western blots have demonstrated a potential change in both post-transcriptional modification and CELF2 co-associated proteins upon stimulation. Analysis of the functional implications of these stimulation-induced changes to CELF2 are in progress.

Taken together, we propose a model where the occupancy of CELF2 on the USE and DSE controls the level of inclusion of LEF1-E6. Upon stimulation, the levels of occupancy change, favoring the DSE and resulting in an increase of LEF-E6 inclusion. We hypothesize that this change in occupancy could be a result of a change in CELF2′s PTM landscape or a change in co-associated proteins.

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564 Genome-wide intron mapping by spliceosome footprinting

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We recently reported that the abundant U2·U5·U6 snRNA complexes observed in logarithmically growing *Schizosaccaromyces pombe* are intron lariat spliceosomes (ILS)[1]. These intron product complexes are remarkably stable and protect long stretches of nucleotides at intron ends from nuclease digestion. RNA-Seq revealed these spliceosome footprints to consist of ~19 nt length downstream of 5′SS and ~40 nt upstream of the branch site (BS). We have used these footprints to identify dozens of new *S. pombe* introns, correct previously annotated splice sites and discover new sites of alternative splicing. This demonstrates the utility of spliceosome footprinting for both definitive mapping of introns and for studying alternative splicing.

1. Chen, W., Shulha, H.P., Ashar-Patel, A., Yan, J., Green, K.M., Query, C.C., Rhind, N., Weng, Z. & Moore, M.J. Endogenous U2·U5·U6 snRNA complexes in S. pombe are intron lariat spliceosomes. *RNA published online January 17, 2014*

565 The Interactions of NTC and Cwc2 with the 5′ Splice Site in Formation of the Active Spliceosome

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The spliceosome is a large and dynamic ribonucleoprotein complex, assembled *via* sequential binding of five small nuclear RNAs, U1, U2, U4/U6 and U5, and protein factors to the pre-mRNA. After binding of all snRNAs, the spliceosome undergoes a major structural change to release U1 and U4, and reorganize RNA base pairings in the catalytic core to form the active spliceosome. The NTC is required for stabilizing the association and promoting specific base pairings of U5 and U6 with the pre-mRNA, which also includes an interaction between around +30 of the intron sequence and the Lsm binding site of U6. To understand the function of NTC components in mediating these interactions, we performed site-specific crosslinking to examine the interactions of proteins with the 5′ splice site using 4sU-labeled pre-mRNA. Ntc85/Cef1 and Ntc77/Clf1 were found to crosslink to the intron near the 5′ splice site primarily between +20 to +29, and Ntc30/Isy1 to crosslink primarily between +25 to +38, suggesting a role of the NTC components in stabilizing the interaction between the intron sequence and the 3′ end of U6. These interactions sustain throughout the spliceosome cycle. Strong crosslinks of Ntc30 were also observed at +9 and +12, but specifically only during the first catalytic reaction. Cwc2 was also found to crosslink at +9 and +12 throughout the spliceosome cycle, in agreement with previous report by Rasche *et al.*, showing crosslinking of Cwc2 to +15 of the 5′ splice site in yeast ACT1 pre-mRNA, but only in the activated spliceosome. Cwc2 binds to the spliceosome and functions only in the presence of NTC, and is also required for specific interactions of U6 with the 5′ splice site, suggesting a role of Cwc2 in spliceosome activation to orchestrate the structure of the catalytic core. Depletion of Cwc2, although does not affect the association of NTC with the spliceosome, abolishes crosslinking of Ntc85 and Ntc30, and weakens that of Ntc77 to the intron sequence. Our results demonstrate how NTC components and Cwc2 interact with the 5′ splice site to orchestrate the catalytic core of the spliceosome.

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566 The Ll.LtrB group II intron from the gram-positive bacterium Lactococcus lactis excisses as circles and generates double-stranded head to tail DNA junctions in vivo

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Group II introns are large ribozymes that require the assistance of intron-encoded or free-standing maturases to splice from pre-mRNAs *in vivo*. The branching pathway, releasing group II introns as lariats, is the major and most studied splicing pathway. However, group II introns can also splice through secondary pathways like hydrolysis and circularization that are not as well characterized as branching. The Ll.LtrB intron from the gram-positive bacterium *Lactococcus lactis* is the best model to study group II intron splicing and mobility.

Here, we assessed splicing of the Ll.LtrB group II intron in *L. lactis* by amplifying the splice junction of its ligated exons and released introns. Ligated exons revealed that different mutants of Ll.LtrB undergo alternative splicing using the same remote 5′ and 3′ alternative splice sites. We also found that the maturase activity of LtrA controls 5′ and 3′ splice site selection and the balance between accurate and alternative splicing. The study of excised introns revealed the presence of lariats, circles and alternatively spliced products for all Ll.LtrB variants studied albeit at different ratios. Removal of the branch point residue prevented Ll.LtrB excision through the branching pathway but did not hinder circle formation and alternative splicing. A number of circular and alternatively spliced introns were found harboring extra non-encoded nucleotides at the splice junction. The presence of identifiable mRNA fragments at the junction of some intron RNA circles provides insights into the circularization mechanism of group II introns. Complete intron RNA circles were found associated with LtrA but forming inactive RNPs. Traces of double-stranded head to tail intron DNA junctions were also detected from *L. lactis* total RNA and nucleic acid extracts. This work unveils that Ll.LtrB excises not only as lariats but also as circles *in vivo* and shed light on the circularization pathway of group II introns.

567 Analysis of the interaction of splicing factor SF1 with U2 snRNP components

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Splicing factor 1 (SF1) functions at the onset of spliceosome assembly in the recognition of sequence elements at the 3′ splice site. During the formation of complex E it interacts with the large subunit of the U2 snRNP auxiliary factor (U2AF65) followed by cooperative binding of the proteins to the intron branch site and the polypyrimidine tract, respectively. In the following step, the formation of complex A, U2AF65 recruits the U2 snRNP through an interaction with a U2 snRNP-associated protein, and the U2 snRNA now base pairs with the branch site. Although stable binding of the U2 snRNP to the spliceosome only occurs at the time of A complex formation, it has been reported that it is weakly associated with complex E in a branch site-independent fashion.

By co-immunoprecipitation coupled with mass spectrometry and in yeast two-hybrid (Y2H) screens we found that SF1 interacts with SF3a120, an essential component of the U2 snRNP, and two additional proteins loosely associated with the U2 snRNP. Results from the Y2H screens suggested binding of SF1 to SURP domains of these proteins, which we confirmed in in vitro binding assays. We furthermore mapped the SURP-interaction domain (ID) of SF1. Immunodepletion of SF1 from HeLa nuclear extracts strongly reduces A complex formation, which can be rescued by addition of recombinant SF1 containing the SURP-ID. Only partial rescue is observed with SF1 lacking the SURP-ID. Together, these results suggest that SF1 may initially recruit the U2 snRNP to the spliceosome at the time of E complex formation, whereas the U2AF65-U2 snRNP interaction stabilizes the association of the U2 snRNP with the spliceosome at later assembly stages. In addition, our results shed new light on the function of SURP domains as protein-protein interaction modules.

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568 Mechanistic and structural studies of U4/U6 annealing by Prp24

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The spliceosome is a highly dynamic molecular machine comprised of five small nuclear RNAs (U1, U2, U4, U5, and U6) and numerous associated proteins. U6 RNA undergoes significant structural changes, existing as U6 snRNP, U4/U6 di-snRNP, and U2/U6 di-snRNP during subsequent steps in the spliceosome cycle. A constituent of the U6 snRNP, Prp24, actuates the transition from U6 snRNP to U4/U6, acting as a chaperone to convert the intramolecular base pairs of the U6 internal stem loop (ISL) to intermolecular base pairs with U4, forming U4/U6 stems I and II. We have previously shown that stabilizing mutations in the ISL result in a cold-sensitive growth phenotype in yeast and reduce levels of U4/U6 in vivo. Cis¬-acting suppressor mutations in U6 and trans-acting suppressor mutations in both Prp24 and U4 RNA have been identified. Understanding the mechanism of these suppressing mutations will allow us to propose a mechanism of annealing.

We are investigating the mechanism by which Prp24 anneals the U4 and U6 snRNAs. These studies are guided by our previous genetic results and our recently determined high-resolution crystal structure of the U6 RNA-Prp24 complex (see Montemayor et al. abstract). By following the kinetics of U4/U6 formation on native gels, we have determined annealing rates for wild-type RNAs and various combinations of mutations and their suppressors. We find that the in vitro annealing assay can recapitulate some of the defects observed for mutations that cause cold-sensitive growth phenotypes in vivo. Stabilizing ISL mutations decrease both the observed rates and extents of annealing in vitro. Our current understanding of the mechanism of Prp24-stimulated annealing of U4/U6 will be presented.

In addition to investigating the mechanism of U4/U6 annealing, we are determining the solution structure of a 92-nucleotide U4/U6 RNA construct that corresponds to the central core of the U4/U6 di-snRNA interaction, using a hybrid NMR/SAXS approach. Our progress in determining the NMR/SAXS structure of this U4/U6 complex will be presented.

569 Splicing Inhibitors - Tools to dissect the Spliceosome

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We are identifying and characterizing chemicals tools that affect human spliceosomes. Such compounds are important because spliceosomes play a central role in eukaryotic gene expression, but deciphering the function of individual components has been challenging due to the highly dynamic and complex nature of the spliceosome. Additionally, several spliceosome components have been linked to human disease, and small molecule splicing inhibitors may represent a pathway to improve treatments. For example, SF3B1 is a core spliceosome protein that has been consistently identified with specific mutations in several cancers. Notably, SF3B1 is the target of potent natural products that were first identified as chemotherapeutic leads. We are using one of these compounds, pladienolide B, to study SF3B1 and are making progress in deciphering its role during spliceosome assembly. However, there are still over 100 protein components in the spliceosome for which we have almost no functional information, and we are lacking tools target these proteins. To address this deficiency, we recently used a high-throughput splicing assay to screen a unique library of over 100,000 natural products from geographically distinct marine bacteria that can be cultured in lab. The library is structured as pre-fractions of bacterial lysates, which each contain ~20 compounds. The compounds exhibit high structural diversity on a variety of chemical scaffolds. In an initial screen, we identified 23 pre-fractions that inhibit *in vitro* splicing in a dose dependent manner and interfere with spliceosomes assembly at different stages. A cytological profiling assay reveals that these pre-fractions produce different classes of cellular phenotypes. To identify the active compound in the pre-fractions, we used automated HPLC-based fractionation to generate "one-compound-per-well" peak libraries for secondary screening. For 18 pre-fractions, we identified single wells with splicing inhibitory activity. Currently, we are focusing on the most promising hits and are in the process of large-scale production of the active molecules from the source organism for further analyses and structural elucidation. These new splicing inhibitors have the potential to improve our mechanistic understanding of the spliceosome and to determine how alterations in spliceosome function relate to aberrant situations like cancer.

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570 The Roles of Intrinsic and Extrinsic Factors on the Interactions between snRNPs and RNAs

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Small nuclear ribonucleoproteins (snRNPs) are complexes of snRNAs and proteins that form the building blocks of the spliceosome. Portions of the snRNA are used to recognize specific RNA sequences in pre-mRNAs or in other snRNAs by duplex formation. In addition to these basepairing interactions, snRNAs engage their targets through a number of other protein/protein, protein/RNA, and RNA/RNA interactions. Our goal is to understand how different types of non-covalent molecular interactions lead to snRNP recruitment to specific RNA locations using recruitment of the U1 snRNP to 5′ splice sites as a model system. Using well-characterized, purified U1 snRNPs from S. cerevisiae, we are analyzing how the particle engages small, unstructured RNA targets using a single molecule binding assay. Our results reveal that binding and unbinding of RNAs from the snRNP is an inherent property of the particle and does not require the presence of other RNA binding proteins or helicases. These assays using purified snRNPs provide a valuable tool for investigating snRNA/RNA duplex formation and the roles of various snRNP and buffer components and RNA sequence in this process. Using colocalization assays in whole cell extract, we are complementing these experiments to study snRNP binding to full-length pre-mRNAs and the influence of extrinsic snRNP factors on this process. We are particularly interested in the roles that other commitment complex components (BBP/Mud2 and the Cap Binding Complex) play in U1 binding. By varying the pre-mRNA sequence as well as the genetic background from which the whole cell extract is prepared, we can dissect how each of these components contributes to snRNP recruitment. Our results highlight how both branchsite binding proteins (BBP/Mud2) and 5′ cap binding proteins can contribute in different ways to snRNP association and often work synergistically. In sum our experiments using binding assays in both purified model systems and whole cell extract highlight how single molecule approaches can inform on both the thermodynamic and kinetic features of snRNP/RNA interactions.

571 SRSF3 promotes Exon 9 skipping of pro-apoptotic caspase-2 through exon 8

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Alternative splicing plays an important role in gene expression by producing different proteins from a gene. Caspase-2 pre-mRNA produces anti-apoptotic Casp-2S and pro-apoptotic Casp-2L proteins through exon 9 inclusion or skipping. However, the molecular mechanisms of exon 9 splicing are not well understood. Here we show that knockdown of SRSF3 (also known as SRp20) with siRNA induced significant increase of endogenous exon 9 inclusion. In addition, overexpression of SRSF3 promoted exon 9 skipping. Thus we conclude that SRSF3 promotes exon 9 skipping. In order to understand the functional target of SRSF3 on caspase-2 pre-mRNA, we performed substitution and deletion mutagenesis on the potential SRSF3 binding sites that were predicted from previous reports. We demonstrate that substitution mutagenesis of the potential SRSF3 binding site on exon 8 severely disrupted the effects of SRSF3 on exon 9 skipping. Furthermore, with the approach of RNA pulldown and immunoblotting analysis we show that SRSF3 interacts with the potential SRSF3 binding RNA sequence on exon 8 but not with the mutant RNA sequence. In addition, we show that a deletion of 26nt RNA from 5′ end of exon 8, a 33nt RNA from 3′ end of exon 10 and a 2225nt RNA from intron 9 did not compromise the function of SRSF3 on exon 9 splicing. Therefore we conclude that SRSF3 promotes exon 9 skipping of caspase-2 pre-mRNA by interacting with exon 8. Our results reveal a novel mechanism of caspase-2 pre-mRNA splicing.

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572 Genetic screen to identify factors that modulate function of C-rich exon motifs in yeast

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To better understand how the substrate is selected and positioned at the spliceosome's catalytic center, we identified several classes of exon motifs that compensate for the defects of the adjacent 5′ splice site in yeast. C-rich exon motifs strongly suppress defects of a 5′SS-G5a mutation, which destabilizes 5′SS interactions with both U1 and U6 snRNAs, leading to the inhibition of spliceosome assembly and the first step. Whereas the C-rich motifs improve the first step of splicing for G5a introns, they inhibit the second step for other intron mutants - U2a, A3c, BS- and 3′SS mutants. Thus, these motifs generally act by improving the first step but inhibiting the second.

To identify alleles that modulate the function of C-rich exons, we carried out a genetic screen in yeast. A *cup1*Δ strain carrying an *ACT1-CUP1* reporter with a suboptimal C-rich exon and the G5a intron was UV irradiated, and mutants with an improved copper tolerance were selected. Some of the obtained mutant strains suppressed G5a defects regardless of exon identity, whereas others required the presence of suboptimal C-rich exons for their effect.

To identify the selected alleles, we confirmed that the functional mutation resides in the genome, subjected the strains to genomic DNA sequencing, and identified SNPs relative to the starting strain. The screen yielded several suppressors linked with early spliceosome assembly events, including mutants in U1 and U2 snRNAs and their associated proteins (Prp40, Hsh49, Spp41), as well as Prp28, the ATPase that modulates the transition of the 5′SS interaction from U1 to the spliceosome. Interestingly, we also obtained mutants in transcription, mRNA stability and transport factors (e.g. Snf2, Cth1, Npl6, Npl3, Mtr10). We are currently in the process of validating the functionality of these mutations. Several of the sequenced strains did not reveal any obvious mutations; these are likely to contain some genomic deletions, insertions, or rearrangements not detectable by our current bioinformatic analysis. Because our screen did not yield any mutant factors implicated in the catalytic phase of splicing, we consider carrying out another screen, in the context of one of the identified alleles that improve early assembly events.

573 A hierarchical clustering approach to single molecule FRET analysis: dissecting pre-mRNA dynamics during spliceosome assembly and catalysis

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Spliceosomes are multi-megadalton ribonucleoprotein (RNP) complexes responsible for catalyzing the removal of noncoding introns from eukaryotic precursor messenger RNA (pre-mRNA) transcripts and ligating the flanking coding exon sequences to produce a mature messenger RNA (mRNA). Spliceosome assembly and catalysis require a highly dynamic coordination of protein and RNA elements with the purpose of identifying optimal substrates and producing the mature mRNA that can be properly transcribed by the ribosome. Perhaps not surprisingly, it is estimated that up to 50% of all mutations leading to human disease act through disruption of the splicing code.

For the past 30 years, genetic and biochemical techniques have been the preferred tools utilized to identify and characterize splicing complexes present throughout spliceosome assembly and catalysis. Unfortunately, these techniques fail to capture the inherent dynamic and heterogeneous nature of the process, a characteristic perhaps most exemplified by the fact that both chemical steps of splicing are reversible processes. In addition, these techniques often focus on the role of the spliceosome and fail to capture the conformations of the pre-mRNA and the vital role of pre-mRNA dynamics in efficient splicing.

Single molecule fluorescence microscopy has recently emerged as an alternative approach to study pre-mRNA splicing. Using a dual-labeled, fluorescent substrate we are able to observe time and ATP-dependent conformational rearrangements of single pre-mRNA molecules. Unfortunately, the complex behaviors of thousands of molecules interconverting between multiple conformational states progressing along the splicing pathway in a highly asynchronous manner limits our ability to assign dynamics to specific stages of the assembly process. In an effort to refine this approach and completely understand the sets of pre-mRNA dynamics unique to splicing, we have utilized a number of substrate and extract mutations that allow us to stall assembly at specific stages and enrich for the conformational behaviors characteristic to a particular stage of splicing. In addition, we have developed a clustering analysis tool that allows us to first classify these diverse behaviors and then assign dynamics to specific complexes along the splicing pathway.

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574 Brr2 Retinitis Pigmentosa Mutations Reduce Helicase Processivity

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Brr2 is a member of the DExD/H family of RNA-dependent ATPases that unwinds the extensively base paired U4/U6 snRNAs during pre-mRNA splicing. This reaction is stimulated by the C-terminus of another spliceosomal protein, Prp8. Prp8 has been shown to bind between Brr2′s tandem repeat of helicase domains, only the first of which has catalytic activity[1]. The lengthy base pairing interaction between U4 and U6 suggests Brr2 must act processively to unwind them. However, there is currently little mechanistic detail describing how Brr2 unwinds the U4/U6 snRNAs.

We are performing a biochemical characterization of the Brr2 helicase activity using a minimal *in vitro* system. Single turnover ensemble helicase assays demonstrate that the C-terminal fragment of Prp8 increases the overall fraction of U4/U6 snRNAs unwound. However, the Prp8 fragment does not increase the affinity of Brr2 for U4/U6, suggesting Prp8 may increase Brr2′s low inherent helicase processivity. Like other helicases, the rate of Brr2 unwinding U4/U6 is highly dependent upon the monovalent cation concentration, decreasing exponentially as the concentration of salt increases. Brr2 is able to unwind model duplex RNAs with over 30 base pairs, although increasing the duplex length leads to decreasing fraction unwound. Mutations within the putative “unwinding ratchet” of Brr2 that cause Retinitis Pigmentosa in humans result in a lower extent of total duplex RNA unwound. We predict that these mutations decrease the processivity of Brr2 and result in more aborted attempts at unwinding.

[1]Santos, K.F. et al., Proc Natl Acad Sci U S A 109 (43), 17418-17423.

575 Elucidation of hnRNP A1 binding to ssA7 of HIV-1

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Alternative splicing of the HIV-1 genome is necessary for translation of the complete viral proteome. Host proteins, such as hnRNP A1, are used to regulate splicing at the various donor and acceptor sites along the viral genome. One such site regulated by hnRNP A1 is the conserved 3′ acceptor splice site A7 (ssA7). Silencing of splicing at this site is necessary in order to retain the Rev Responsive Element (RRE) in the adjacent tat/rev intron. The RRE is responsible for nuclear export of unspliced and partially spliced transcripts.

Our research seeks to clarify the binding determinants of hnRNP A1 on ssA7 by developing a structural model that seeks to correlate ssA7 structure to its splicing function. Initial experiments have used isolated domains of both ssA7 and hnRNP A1. For ssA7, SL3 (ESS3) of the three stem loop ssA7 structure is examined as this contains a high affinity UAG binding site for hnRNP A1. We previously solved the 3D solution structure of ESS3 by NMR and found the UAG is located in a terminal heptaloop. Structural studies have been done using the protein UP1, which consists of the two RNA recognition motifs (RRM) of hnRNP A1. Crystallization experiments have yielded a structure of UP1 bound to an AG dinucleotide corresponding to A15 and G16 in the heptaloop of ESS3. This structure shows the two nucleotides are bound in a pocket formed by aromatic residues of RRM1 and the linker between the two RRM domains. NMR experiments with the UP1:ESS3 complex have shown similar results, revealing NOEs between the nucleotides of the terminal heptaloop of ESS3 and the aromatic residues of UP1.

Full length hnRNP A1 has recently been cloned and purified. When the solution structure of ESS3 was solved, the biochemical profile of its binding by the domains of UP1 was analyzed. Identical experiments are being done with full length hnRNP A1 to determine if the presence of the C-terminal glycine rich domain alters the binding of the RNA.

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576 Choosing between Human or yeast? Co- and post-transcriptionally, *Ustilago maydis* has it all!

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Alternative splicing is catalyzed by the spliceosome and originates multiple protein isoforms from a single pre-mRNA in most eukaryotic organisms. After splicing, mRNAs could go through NMD not only as a quality control to avoid the production of aberrant proteins, but also to regulate the ammount of messenger available. The molecular regulatory mechanisms for splicing and NMD are similar for all metazoans, but some differences between yeasts and humans have been documented. *Ustilago maydis* is a basidiomycete that causes the carbon disease in corn commonly known in Mexico as “cuitlacoche”. Interestingly, splicing is a rather common mechanism occurring in *Ustilago maydis*, given that around 50% of its genes are interrupted. Moreover, all types of alternative splicing described for vertebrates also occur in *U. maydis*, being intron retention the prevailing event. Regulatory cis-elements for most of the *U. maydis* introns are very similar to those described for human pre-mRNAs both in sequence composition and relative position. When comparing *Ustilago* and human protein sequences, identity for most putative splicing factors ranges between 40% and 60%. The most conserved factors were the core of snRNPs (Sm and Lsm proteins), as well as some components of snRNP U1 (U1-70K), snRNP U2 (SF3b complex) and snRNP U5. Other conserved proteins involved in splicing regulation include CBP80, U2AF35 and U2AF65. Alternative splicing regulators were also identified in *U. maydis*, but while some SR proteins are highly similar, hnRNP proteins are barely conserved. In silico experiments showed that the putative fungal factors could replace the function of the human homolog. As it was observerd for alternative splicing, the NMD machinery in *U. maydis* resembles the one described for human. RT-PCR experiments showed that NMD also regulates mRNA expression in *U. maydis*. In silico and mutational analysis demonstrated how the interaction between the fungal Upf core proteins occur. Confocal microscopy showed the cellular localization for Upf proteins in *U. maydis*. Together, our results suggest that this fungus could be considered as a new fungal model to study the complex regulation of human splicing and NMD events.

577 Intron Circles Are the Main Splicing Products in *Entamoeba histolytica*

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Splicing is a crucial step in gene expression and precise intron removal depends on the proper recognition of splicing signals. In the protozoan parasite *Entamoeba histolytica* only the 5′ splice site (ss) and the 3′ss have been determined but not the amoebic branch point (BP) sequence. In this work, first we focused in identifying the BP nucleotide in the amoebic introns of RabX13, ClcB (intron 2), rpL12, and rpS14 genes, by means of divergent-primers PCR. In all cases, the products amplified corresponded to 5′ss-3′ss-ligated (circular) introns and surprisingly no lariat structures were detected. After Actinomycin D RNA polymerase II inhibition and self-splicing reactions we concluded that RabX13 circularized introns are produced during pre-mRNA processing and not from self-splicing reactions. In vivo second step of splicing inhibition with boric acid slightly diminished both spliced variant and intronic circles formation, and we were able to identify lariat structures (canonical and alternative ones) suggesting that intronic circles arise after the lariat has been released from the lariat-3′ exon intermediate in a fast circularization reaction possibly by a third nucleophilic attack from the 3′ss to the 5′ss. From the lariat structures identified, we observed that the RabX13 and rpS14 canonical BP nucleotides are localized 6 and 11 nt upstream their respective annotated 3′ss, and that the polypyrimidine tract in which they are embedded is not fully conserved between them nor with the 95 Rab gene family introns. These data confirm that the BP sequence in *E. histolytica* introns is not well defined but possibly other sequence features, such as a polyA-rich tract around 20 nt upstream from the 3′ss might be involved in its recognition.

To our knowledge this is the first work in which the BP in *E. histolytica* has been experimentally identified. Also this is the first report on the full-length *E. histolytica* introns are circularized during the splicing process.

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578 Brr2-facilitated unwinding of U4/U6 is promoted by a mutually exclusive stem loop in U4

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Before U6 performs its catalytic activity during splicing, it is kept inactive in a complex with U4. Activation of the spliceosome therefore requires separation of two intermolecular helices between U4 and U6, called stem I and stem II, before U6 can assume its catalytic conformation. Unwinding of U4/U6 is mediated by the RNA dependent ATPase Brr2, which is a 3′-5′ DExH/D-box helicase. Brr2 could formally unwind U4/U6 by moving 3′ to 5′ on U6 or U4, but biochemical results strongly suggest that Brr2 translocates on U4.

To gain further insight into how Brr2 functions we used a weakened brr2 mutant (R1107L), which is orthologous to a human mutation leading to autosomal dominant Retinitis Pigmentosa (adRP), and asked if U4 mutations in either stem I or stem II that destabilize the U4/U6 interaction could suppress this mutant by alleviating the workload on Brr2. No genetic interaction was observed between mutant brr2 and mutations in stem II. Surprisingly, mutations that disrupted stem I did not suppress the mutant brr2; instead they exacerbated the growth phenotype. These mutations not only destabilized stem I but also a mutually exclusive intramolecular stem loop in U4 (SL*). To test if disruption of SL* was responsible for the exacerbation, complementary mutations were introduced in U4 that restored base pairing in SL*. The enhancement was indeed suppressed when SL* in U4 was restored. Furthermore, when SL* was repaired so the original mutation exclusively destabilized stem I, we observed suppression of brr2-R1107L. This suppression of stem I but not stem II mutations supports translocation along U4 rather than U6, as suggested by published biochemical data. Further, our data suggests that formation of SL* is important for efficient U4/U6 unwinding by stabilizing an intermediate before U4 release. In the minor spliceosome, an intermediate in which stem I is unwound but stem II is paired was observed by crosslinking, suggesting that an intermediate is a conserved feature of U4/U6 unwinding.

579 High-throughput sequencing of *SMN1* exon 7 reveals low intrinsic splicing error rate and patterns of splicing errors

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Alternative splicing diversifies mRNA transcripts in human cells. It has previously been shown by quantitative real-time PCR that the spliceosome pairs exons with a high degree of accuracy. However, pre-mRNA splicing error rates have not been analyzed at the nucleotide level to determine the quantity and identity of splicing errors across splice junctions. Using a high throughput approach, two intron/exon and two exon/exon junctions generated through splicing were sequenced at a depth of 54 millions reads. After background corrections to errors introduced by amplification, and sequencing steps, pre-mRNA splicing maintains a very low overall error rate of less than 0.005%. The majority of splicing errors were deletions. These errors never occurred in tandem, suggesting that splicing errors at a splice site do not contribute to an increased error rate at the adjacent splice site. Our analysis also shows a propensity for insertions at splice sites to occur in 1, 3, 4, and 6 nucleotide increments, with very few 2 or 5 nucleotide insertions. These results underscore the impressive fidelity of pre-mRNA splicing and uncover a three-nucleotide splice selection bias that is established during the second step of splicing.

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580 Structural studies of the core spliceosomal component Prp19

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The NineTeen Complex (NTC) is an essential building block of the spliceosome and it is evolutionarily conserved from yeast to humans. NTC is recruited to the spliceosome during the formation of the spliceosomal catalytic centre and it remains associated to the spliceosome until the final stages of disassembly [1]. Prp19 is an essential NTC component that plays a pivotal role in the formation of the spliceosomal catalytic center [2]. It contains three domains: an N-terminal U-box domain, a central coiled-coil domain and a C-terminal WD40 domain. *In vivo* and *in vitro* Prp19 forms a tetramer and its quaternary structure is essential for its function [3]. We were able to produced stable fragments corresponding to the tetrameric stalk of Prp19, comprising the coiled-coil and four Ubox domains. Small-angle X-ray scattering of the stalk and of the WD40 domains showed an elongated shape for the former and the typical globular shape for the latter. Our analysis of the stalk is consistent with the previously reported EM images of the full-length Prp19 [3], where four WD40 domains are flexibly tethered to the very elongated stalk. Currently we focus our efforts on the crystallization of the Prp19 stalk. Furthermore, we were able to crystallize and determine the crystal structure of the WD40 domain from two orthologs of Prp19. Notably, only one of the them exhibits the atypical WD40 shape that was previously reported [4]. Taken together, our data brings new information about the structure of the core spliceosomal protein Prp19 and paves the way towards the elucidation of Prp19 quaternary structure at atomic resolution.

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581 Genetic screen for factors modulating branch site selectivity during spliceosome assembly

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U1 and U2 snRNPs are principal participants in intron and exon definition. Interaction between these snRNPs is critical for ultimate juxtaposition of 5′SS and branch site. We previously characterized a U1–U2 snRNP interaction network, the components of which are conserved from *S. pombe* to humans – however, its critical parts have not been identified in *S. cerevisiae*. Splicing of introns containing branch-flanking mutations is strongly improved by *prp5* mutants, either slow in ATPase activity or impaired in SF3b-interaction[1,2]. We hypothesized that other impairments of U1–U2 interaction would have similar effects. Thus, to identify components of this network in *S. cerevisiae*, we screened genome-wide using UV mutagenesis for improved splicing of reporters sensitive to *prp5* mutants. We identified 33 candidate strains confirmed to improve splicing of branch-flanking mutations.

Plasmid shuffling experiments identified 7/33 mutants as alleles of *prp5*. Synthetic lethality with cs *prp5* alleles allowed us to screen genomic library plasmids, which identified 6 strains rescued by *PRP9*-containing plasmids; all 6 strains carried the same *prp9*-R341K mutation. Genomic DNA of the remaining strains were sequenced using Illumina Hi-Seq, and we subsequently identified SNPs relative to the parent strain. This approach yielded several classes of suppressors linked with early spliceosome assembly events, including U2 snRNP SF3a component Prp9 (ySF3a3); SF3b components Hsh155 (ySF3b1), Cus1 (ySF3b2), and Rse1 (ySF3b3); and Msl5 (BBP/SF1).

We are particularly interested in comparing the molecular mechanisms of these mutations to those of alleles found in hematopoietic diseases. We anticipate to elucidate a network of interactions needed to differentially recognize a variety of branch sites and, thus, to understand the molecular mechanisms of altered splicing found in MDS and other diseases. Collectively, these mutations identify a network of components important for branch-region–U2 snRNP interactions and stability during the joining of U2 snRNP to the branch region. We will continue to investigate the mechanism of these effects and the relationship of these mutations to those identified in human diseases.

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582 The N-terminal domain of the unusual SR protein, hPrp38, is a protein-protein interaction hub in the spliceosome

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The removal of introns from eukaryotic pre-mRNA is a crucial step in transcript maturation, which is carried out by the spliceosome. For each splicing reaction, a spliceosome is assembled *de novo* on its substrate by the stepwise recruitment of five small nuclear ribonucleoprotein particles (snRNPs) and many non-snRNP proteins. Initially, the spliceosome is assembled as an inactive particle that undergoes major conformational and compositional rearrangements upon conversion to a catalytically active complex. Spliceosome activation in yeast requires the Prp38 protein [1]. While yeast Prp38 is a stable subunit of the U4/U6-U5 tri-snRNP, the human counterpart (hPrp38) joins the spliceosome independent of the tri-snRNP [2]. In addition to a yeast-like N-terminal domain (NTD), hPrp38 contains a C-terminal RS domain, a signature of splicing regulatory SR proteins. While in canonical SR proteins the RS domain is associated with one or more RNA-binding domains [3], the function of the Prp38 NTD is unknown. We determined the crystal structure of the hPrp38 NTD and used a comprehensive yeast 2-hybrid (Y2H) library of human spliceosomal proteins [4]to identify twelve splicing factors, including several proteins implicated in alternative splicing decisions, that interact with it. We mapped four distinct interaction sites on the hPrp38 NTD using a surface-scanning Y2H approach. Our results suggest that hPrp38 is an unusual SR protein, whose NTD serves as an interaction hub that recruits splicing regulatory proteins to influence the functional pairing of splice sites.

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583 Stemloop 4 of U1 snRNA is essential for splicing and interacts with the U2 snRNP specific SF3A1 protein

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Spliceosomal interactions that pair the 5′ and 3′ splice sites across an intron must occur with a high degree of specificity and fidelity to allow expression of functional mRNAs. Here, we report involvement of the stem-loop 4 of the U1 snRNA (U1-SL4) in these interactions. Using an *in vivo* U1 snRNP complementation assay, we found U1-SL4 is important for splicing. In vitro analyses showed that addition of free SL4 to a splicing reaction inhibits splicing and blocks complex assembly prior to formation of the prespliceosomal A complex, indicating a role for U1-SL4 contacts in splice site pairing and commitment to splicing. Using a combination of Stable Isotopic Labeling of Amino Acids in Culture (SILAC), biotin/Neutravidin affinity pull down, and mass spectrometry, we found interactions between the U2 snRNP specific SF3a complex and U1-SL4. To identify the interacting protein, we used UV-crosslinking and immunoprecipitation and found that U1-SL4 makes a direct contact with the SF3A1 protein. We find that this contact occurs in the prespliceosomal E complex but not in the A complex, indicating that it may play a role in transition of E to A complex.

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584 Single Molecule Dynamics Analysis of Prespliceosome Assembly and Stability

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Splicing of intron-containing pre-mRNAs is carried out by a spliceosome, a dynamic RNA-protein machine that must assemble anew on each pre-mRNA molecule. Using Colocalization Single-Molecule Spectroscopy (CoSMoS) (1) in yeast whole cell extract, we can directly observe the real-time dynamics for association of U1, U2 and U5 snRNPs with surface-tethered pre-mRNA molecules (2, 3). Using this system, we recently demonstrated that productive spliceosome assembly can be initiated by both U1-first and U2-first pathways (4). More comprehensive analysis of the data, including all possible intermediates on the spliceosome assembly pathway, confirms that the dominant forward pathway for the spliceosome assembly is formation of a pre-mRNA-U1-U2 complex (the prespliceosome) followed by the tri-snRNP recruitment. Now we are exploring the kinetics of pre-mRNA-U1-U2 complex formation in greater detail. In particular, we are interested in assessing whether the presence of bound U1 or U2 accelerates and/or kinetically stabilizes binding of the other snRNP. Understanding the dynamics of prespliceosome formation will contribute to a better grasp of the mechanisms underlying splice site selection and pairing.

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585 The 3′-end of U6 snRNA is required for spliceosome disassembly and fidelity

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After the catalytic phase of pre-mRNA splicing, the spliceosome releases the mRNA and then releases the excised intron and disassembles to recycle components for subsequent rounds of splicing. Although a number of protein factors that participate in product release and disassembly have been identified, such as the DExD/H-box proteins Prp22, Prp43, and Brr2, as well as Snu114, Ntr1 and Ntr2, the mechanism of disassembly, and in particular the role of snRNAs, remains unclear.

We have discovered a critical role for nucleotides at the 3′-end of U6 in excised intron release. Consistent with a previous report, we find that truncation of the 3′-end of U6, including the Lsm binding site and nucleotides involved in U2/U6 helix II, results in a striking accumulation of excised intron in yeast extracts. We have ruled out a requirement for the 3′-end in Prp22-mediated mRNA release or in recruitment of Prp43, Ntr1, or Ntr2, and we find no evidence that intron release requires rebinding of the Lsm2-8 ring, which dissociates from the U6 3′-end during assembly. We observe RNase H protection of nucleotides at the 3′-end of U6 in endogenous spliceosomes poised for disassembly, suggesting a protein factor directly involved in intron release engages these nucleotides at the stage of disassembly. We also find that the U6 3′-end is involved in promoting splicing fidelity via discard of suboptimal splicing intermediates. Because Prp43 is involved in both disassembly and discard, these data strongly implicate Prp43 as the factor directly associated with, and dependent upon, the 3′-end of U6. We also investigated the dependency of disassembly on U6 3′-end nucleotide composition by substituting stretches of U6 with DNA, either at the extreme 3′-end or at a segment upstream. Surprisingly, both substitutions also result in defective intron release. This indicates a role for sequential 2′-hydroxyls and suggests a mechanism by which Prp43 translocates along U6 snRNA to promote disassembly. These results thus define a previously unknown direct role for U6 snRNA in spliceosome disassembly and fidelity, and potentially Prp43 function, and could provide a model for understanding how ATPases facilitate RNA rearrangements in the spliceosome.

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586 A structural framework for the association of U1 snRNP with alternative splicing factors

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Five Uridine-rich small nuclear ribonucleoproteins (U snRNPs) function in the critical eukaryotic mRNA processing step of splicing, the removal of non-coding sequences from precursor-mRNA. The five U snRNPs are U1, U2, U4, U5, and U6. Consisting themselves of RNA and protein, the U snRNPs assemble onto a pre-mRNA to form a dynamic macromolecular machine, the spliceosome. The U1 snRNP initiates splicing upon its recognition of a pre-mRNA at the key 5′ splice site. Additional regulation occurs at this stage in metazoans, as U1 snRNP is aided by transiently associated proteins known as alternative splicing factors that function to guide U1 snRNP to specific 5′ splice-site(s). In *S. cerevisiae*, the 800 kDa, 18 subunit U1 snRNP has, as integral protein members, subunits that function as alternative splicing factors in higher eukaryotes, producing their highly diversified metazoan proteome. Towards understanding the structure-function relationship of the yeast U1 snRNP, its 3D structure has been determined by electron microscopy and individual subunits are being localized with a novel gold-labeling method. The working model of the yeast U1 snRNP provides insight into the function of the yeast protein subunits and furthers our understanding of the association of metazoan U1 snRNP with alternative splicing factors.

587 FUS mediates an interaction between U1 snRNP and RNAP II and functions in coupling transcription to pre-mRNA splicing

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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease with no treatment. Recent insight into understanding ALS has come from the observation that several proteins that cause the disease share two features, the capacity to bind RNA and a propensity to aggregate due to the presence of prion-like domains. A prototypical example is the RNA binding protein FUS, which has a prion-like domain and is mutated in ALS. We and others showed that RNAP II and U1 snRNP associate abundantly with FUS (e.g. refs. 1, 2). Using an antisense morpholino (AMO) that base pairs to U1 snRNA and blocks splicing, we obtained several lines of evidence that U1 snRNP functions in coupling transcription to splicing. Using the AMO, we show that efficient splicing requires recruitment of U1 snRNP to the pre-mRNA during transcription. In light of the observation that FUS associates abundantly with both U1 snRNP and RNAP II, we also investigated the role of FUS in coupling transcription to splicing. To do this, we made FUS knockdown nuclear extracts (NEs) using our new method for preparing small-scale NEs that are fully functional in our coupled RNAP II transcription/splicing system[3]. We show that splicing is inhibited when transcription is carried out in FUS knockdown NE, but is efficient when transcription is carried out in normal NE and spliced in FUS knockdown extract. These data indicate that FUS must be recruited to the pre-mRNA during transcription. Moreover, we show that FUS is required for the interaction between RNAP II and U1 snRNP, and the U1 AMO blocks the interaction between U1 snRNP and FUS, and between U1 snRNP and RNAP II. Together, our data reveal that U1 snRNP and FUS function in coupling transcription to splicing, and our findings provide important new insights into the functions FUS, which contribute to understanding ALS disease mechanisms.

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588 Structural and functional analysis of the N-terminal helicase-associated region of the spliceosomal Brr2 protein

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Nucleic acid helicases are required at essentially every step of gene expression and regulation. Apart from NTPase/helicase domains, many helicases contain helicase-associated domains or regions, whose functions are frequently poorly understood. For example, pre-mRNA splicing is carried out by the spliceosome, a multi-megadalton ribonucleoprotein machinery. For each round of splicing, a spliceosome is assembled *de novo* from five small nuclear ribonucleoprotein particles (snRNPs) and many non-snRNP proteins. Neither any of the spliceosomal subunits nor an initial assembly containing all snRNPs is capable of carrying out the two transesterification reactions of a splicing event. Instead, following initial assembly the spliceosome's active site is generated anew on every substrate in a process termed catalytic activation. After splicing catalysis, the post-splicing complex is disassembled in an ordered fashion. Spliceosome assembly, activation, catalysis and disassembly are characterized by repeated, profound remodeling of the underlying spliceosomal RNA-RNA, RNA-protein and protein-protein interaction networks. These RNP remodeling events are driven and controlled by at least eight highly conserved superfamily 2 RNA helicases/RNP remodeling enzymes. Among these enzymes, the Brr2 protein mediates the disruption of the U4/U6 di-snRNP during spliceosome catalytic activation. While recent crystal structures provided insights into the molecular architecture and regulation of the helicase region of Brr2[1,2,3], little is known about the structure and function of its large helicase-associated N-terminal part comprising ca. 400 residues. Here, we present results from crystal structure and functional analyses that clarify the organization of this N-terminal region in Brr2 and its role in Brr2 activity and pre-mRNA splicing.

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589 Identification of Novel Type of Splicing Induced by CLK Inhibitor Using Second and Third Generation Sequencer

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Among a numbers of factors involved in splicing machinery, CDC-like kinase (CLK) family is known to be crucial for exon selection in the splicing reaction and considered attractive targets of small molecule inhibitors to study molecular mechanisms of splicing as well as to study potential therapeutic values. Here we report comprehensive splicing profile analyses based on the whole mRNA-sequence results of second generation sequencing (SGS) and third generation sequencing (TGS) and report splicing alterations induced by a small molecule CLK inhibitor (CLKi). SGS using HiSeq showed that the CLKi induced multiple type of splicing including skipping exon type event. Surprisingly, some kinds of read-through type of splicing were significantly induced by treatment with CLKi in colorectal cancer cell line, HCT-116. In addition, we identified slightly expressed read-through splicing events in normal induced pluripotent stem (iPS) cells, implying that read-through type splicing is a naturally occurring regulatory event in cells. While the high read count of SGS allows for accurate quantitative analysis, the short length of the reads cannot directly detect full-length transcripts, and hence the identification of gene isoforms must rely on assumptions. Therefore, we used recently developed TGS for further validation, which has enabled us to generate and analyze much longer reads than SGS. TGS using PacBio RS was able to comprehensively detect full-lengh alternative splicing and splicing alteration by CLKi. A high throughput quantitative RT-PCR, Fluidigm system assay, confirmed the significant induction of read-through splicing by CLKi. This system also detected other types of splicing events such as exon skipping and mutually exclusive exon. Combination of SGS and TGS analysis may provide a more powerful tool to understand the molecular mechanism of pre-mRNA splicing regulation. Taken together, CLK inhibition induces splicing alteration in a wide variety of genes and this is the first evidence that the small molecule compound induces read through type of splicing.

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590 Genome scale analyses of pre-mRNA alternative splicing in human cells submitted to heat shock or oxidative stress reveals how splicing participates to adaptation to stresses

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When human cells are exposed to various stresses such as heat shock, UV, heavy metal, or oxidative stresses, the transcription of Satellite III (Sat III) RNAs is activated [1] [2]. These RNAs are transcribed by RNA polymerase II from tandem arrays of Sat III DNA located in the pericentromeric heterochromatin of chromosomes 9, 12 and 15 [3] [4]. Sat III RNAs remain associated with sites of transcription leading to nuclear stress bodies (nSBs) formation. The function of nSBs still remains to be characterized, but they are thought to participate in rapid, transient and global reprogramming of gene expression through different types of mechanisms including chromatin remodeling and trapping of transcription and splicing factors. Indeed, a subset of splicing factors including SR proteins (SRSF1, SRSF5 and SRSF7) and hnRNP (HAP, M) are efficiently recruited to nSBs by direct or indirect interaction with Sat III RNAs. Thus nSBs and Sat III RNAs are at the convergence of several important aspects of cell biology such as epigenetic control of gene expression, and control of RNA splicing activities.

To better characterize the impact of Sat III RNAs expression on global changes of gene expression in the course of the stress response, we conducted a genome-wide transcriptome analysis on HeLa cells subjected to either heat shock (2 h at 42°C followed by 1 h at 37°C) or oxidative stress (500 µM H_2O_2 for different times). mRNA splicing and miRNAs were analyzed with Human Junction ArraYs (HJAY) and GeneChip miRNA 3.0 Arrays from Affymetrix. Our results highlight alternative splicing variations of pre-mRNAs coding several proteins involved in the control of mRNA fate. Deep analyses of the effects of these variations on protein isoforms production and their functions are underway. In parallel, we detected variations of UsnRNA steady state levels. The present data on the effects of heat shock and oxidative stresses on alternative splicing and their possible cellular implications will be presented.

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591 Functional analysis of the RNA-binding protein ZRANB2

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Zinc finger Ran-binding domain-containing protein 2 (ZRANB2) is an SR-like protein that is widely expressed across many tissues and across organisms ranging from plants to animals. It contains two N-terminal RanBP2-type zinc fingers, which enable the protein to bind in a sequence-specific manner to AGGUAA motifs on single-stranded RNA. Various studies have shown co-localization of ZRANB2 with spliceosome-related proteins and also an ability to alter splicing in minigene assays. These published data indicate a role for ZRANB2 in mRNA processing; however, the details of this role are not yet clear.

Our aim is to interrogate the function of ZRANB2. Two pathways were followed: first, ZRANB2 was knocked down in HEK293 cells using siRNA and subsequently RNA-seq was conducted to determine direct effects of this knockdown on the transcriptome in human cell culture. Second, a ZRANB2 knockout mouse model was studied to elucidate the effects of the lack of ZRANB2 on mammalian development. The morphology of KO mouse embryos was analysed and mouse embryonic fibroblast (MEF) cell lines were generated. RNA-seq was conducted on these MEF cells.

The results and implications for ZRANB2 function will be presented here.

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592 PTB regulates the alternative splicing of the apoptotic gene *BCL-X*

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Alternative splicing (AS) allows generation of multiple mRNA variants that contribute to fine-tuning of cellular processes. Apoptosis, or programmed cell death, is a biological process tightly regulated by AS. Events of programmed cell suicide have a key role in many cellular processes, such as organogenesis and tissues remodelling during development, or in elimination of irreparably damaged cells and of lymphocytes that target self-tissues. The inappropriate regulation of apoptotic events is implicated in many pathological conditions, as neurodegenerative disease, autoimmune disorders and cancer. Numerous apoptotic genes are regulated by AS and the resulting isoforms often play antagonistic roles. A key example is represented by the *BCL-X* gene. Alternative usage of the 5′ splice sites located in exon 2 promotes the generation of two splice variants, the anti-apoptotic long variant (BCL-XL) and the pro-apoptotic short variant (BCL-Xs). Here, we identified the hnRNP I (PTB) protein as a new regulator of *BCL-X* splicing. PTB was isolated by RNA chromatography as one of the splicing factors that associate with the alternatively spliced region of exon 2. Overexpression of PTB in HEK293T cells modulates 5′ splice site selection in *BCL-X* exon 2, thereby favouring the pro-apoptotic BCL-Xs variant. Conversely, depletion of PTB promotes the BCL-XL variant. Crosslink immunoprecipitation (CLIP) experiments showed that PTB directly binds *BCL-X* exon 2 and restricted its binding to a polypyrimidine tract located between the two alternative 5′ splice sites. Site-directed mutagenesis showed that this *cis*-regulatory element is required for PTB splicing activity on *BCL-X* gene. Moreover, our results showed that PTB antagonizes the activity of SRSF1 in 5′ splice site selection. We are currently addressing the possibility of a more general mechanism of PTB in regulation of splicing in presence of alternative 5′ splice sites. Thus, our results identify *BCL-X* as a new splicing target for PTB and uncover a potential novel role for this RNA binding protein in the regulation of apoptosis.

593 The polypirimidine tract-binding protein contributes to drug-resistance in pancreatic cancer cells by regulating the alternative splicing of pyruvate kinase

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive and incurable cancer. PDAC cells rapidly acquire resistance to treatments, becoming insensitive to therapy. Thus, there is a strong need of novel approaches targeting more directly the molecular aberrations of this disease. Recent data suggest that short-term treatment with gemcitabine, the currently used chemotherapeutic agent for PDAC, triggers a pro-survival mechanism that relies on upregulation of the splicing factor SRSF1. To further investigate the mechanisms involved in the acquisition of drug-resistance, we exposed two PDAC cell lines (Pt45P1 and PANC1) to chronic treatment with gemcitabine and selected drug-resistant (DR) subpopulations of cells. Analysis of a cohort cancer-related splicing factors in the DR subpopulations revealed that the polypirimidine tract-binding protein (PTB) was markedly upregulated in DR-PDAC cells. PTB upregulation was accompanied by the modulation of specific splicing events in DR-PDAC cells, most notably the cancer-specific PKM2 isoform of the *PKM* gene. Importantly, knockdown of PTB switched *PKM* splicing to the PKM1 isoform and almost completely reverted the DR phenotype. Moreover, modulation of *PKM* splicing in favour of PKM1 by an antisense oligonucleotide (ASO) approach also increased sensitivity of DR-PDAC cells to gemcitabine, suggesting that the protective role of PTB involves the promotion of PKM2 splicing isoform. Thus, our findings indicate that upregulation of PTB and regulation of PKM alternative splicing confer a DR phenotype to PDAC cells and suggest two novel suitable targets for this disease.

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594 PSF promotes exon7 inclusion of SMN2 pre-mRNA by contacting exon7

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Spinal muscular atrophy (SMA) is an autosomal recessive genetic disease and a leading cause of infant mortality. Deletions or mutations of SMN1 cause SMA, a gene that encodes an SMN protein. SMN is important for the assembly of Sm proteins onto UsnRNA to UsnRNP. SMN has also been suggested to direct axonal transport of β-actin mRNA in neurons. Humans contain a second SMN gene called SMN2 thus SMA patients produce some SMN but not with sufficient levels. The majority of SMN2 mRNA does not include exon 7. Here we show that increased expression of PSF promotes inclusion of exon 7 in the SMN2 and SMN1, whereas reduced expression of PSF promotes exon 7 skipping. In addition, we present evidence showing that PSF interacts with the GAAGGA enhancer in exon 7. We also demonstrate that a mutation in this enhancer abolishes the effects of PSF on exon 7 splicing. Furthermore we show that the RNA target sequences of PSF and tra2β in exon 7 are partially overlapped. These results lead us to conclude that PSF interacts with an enhancer in exon 7 to promote exon 7 splicing of SMN2 pre-mRNA.

595 Esrp1 regulates pre-mRNA alternative splicing in pluripotent stem cells and enhances reprogramming

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Advancing our understanding of the mechanisms that regulate somatic cell reprogramming is essential in the development of induced pluripotent stem cells (iPSCs) as safe options for regenerative medicine. Also, the low efficiency of iPSC induction is a current limitation of this bio-technology and methods to improve it are wanting. The mesenchymal to epithelial transition (MET) is an essential step in the reprogramming of mouse embryonic fibroblasts (MEFs) into iPSCs. The induction of epithelial specific gene expression, associated with MET, can be observed at an intermediate phase of reprogramming as well as in mature iPSCs and embryonic stem (ES) cells. The epithelial splicing regulatory proteins 1 and 2 (Esrp1 and Esrp2) are among the genes that are most highly upregulated during the MET phase and we hypothesized that they play an important role in reprogramming and/or pluripotency. Using MEFs from targeted transgenic mice expressing rtTA and tet-ON-OKSM as well as an Oct4-NEO reporter, we validated that Esrp1/2 are up-regulated at the MET phase of reprogramming and this is associated with the splicing switches of known Esrp targets. Ectopic expression of Esrp1 concomitant with induction of OKSM yielded markedly enhanced efficiency of reprogramming based on alkaline phosphatase staining, Oct4 induction/NEO resistance, and Nanog positivity. In order to characterize the genome wide splicing program that is induced by Esrp1 during reprogramming and in ES cells we have conducted mRNA-seq over a time course of reprogramming using SSEA1 MACS purification and in Esrp1$^{flox/flox}$/Esrp2$^{-/-}$ ES cells using Cre transfection to ablate Esrp1. Using these data we plan to test the epithelial and ES cell-specific splice isoforms, individually or in combination, that recapitulate the enhanced reprogramming induced by ectopic Esrp1. We are also currently testing the effect of Esrp1/2 knockdown and/or ablation on reprogramming and pluripotency. Esrp independent splicing switches that occur during reprogramming may also be revealed by this analysis. Identifying the key regulators of pluripotency and reprogramming, in an isoform specific manner, will be essential to fully understand the biology of these states/processes including the impact of pre-mRNA alternative splicing, as well as lead to improvements in iPSC technology.

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596 A comprehensive screen of RNA binding proteins to identify novel splicing factors

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Over 400 RNA binding proteins (RBPs) with canonical RNA binding domains or motifs exist in the human genome but the functions of many of these proteins, including pre-mRNA splicing regulatory activity, have not been defined. We hypothesize that novel splicing factors, including tissue-specific examples, reside within this set of genes and our broad goal is to discover them. Greatly confounding the achievement of this goal is the task of determining which RBPs to test on which transcripts. We are therefore using protein/mRNA tethering via the lambda-N/BoxB system to recruit RBPs downstream of a test exon in an EGFP reporter and screen for their ability to activate splicing in co-transfection assays. We have tested about 250 different RBP-lambda-N fusion proteins in this manner identifying many examples of known splicing factors such as ELAV1, TIA-1, and several HNRNP's as well as candidate novel splicing factors. A mutant BoxB, which abolishes tethering, is also being employed as a secondary screen to eliminate factors with off target effects. We have also constructed a splicing reporter that contains the BoxB upstream of the test exon and can be used to screen for splicing silencing, which we have validated via co-transfection with ESRP1 and MBNL1 lambda-N fusions. Two other reporters are currently being generated to screen for splicing activation and silencing from the non-canonical positions relative to the test exon. This screen will greatly expand the list of RBPs which exhibit pre-mRNA splicing regulatory activity. Several of the factors that activated splicing in our screen, thus far, exhibit tissue/cell type specific expression patterns in, for example, testes, placenta, and T-cells and future work will be aimed at using SELEX-Seq to establish novel RNA binding motifs, mRNA-seq to identify target transcripts, and functional analysis in those tissues or cell types.

597 Permanent alterations of alternative splicing by transiently depleting RNPS1

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How alternative splicing profiles evolve to confer new and adaptive properties that allow eukaryotic cells to adapt remains an open question. Genomic instability can possibly accelerate the evolution of splicing profiles through mutations. Our hypothesis is that genomic instability has the potential to permanently modify alternative splicing profiles. One way to promote genomic instability is by depleting proteins involved in RNA processing, such as RNPS1. We predict that splicing units normally regulated by RNPS1 would be more likely to sustain R-loop-mediated genomic instability and hence acquire mutations that may permanently affect alternative splicing. To determine if permanent splicing alterations can occur preferentially in units normally regulated by RNPS1, we depleted RNPS1 using inducible shRNA in the HCT116 cell line. We find that the depletion of RNPS1 specifically enhances the frequency with which permanent changes occur in splicing units normally regulated by RNPS1, with little impact on units that are not normally controlled by RNPS1. Preliminary data using an allele swapping assay indicate that the change in splicing is caused by mutations. The results of this study support the view that genome instability triggered by changes in RBP levels can accelerate gene evolution by imposing permanent and specific changes in alternative splicing.

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598 Identification of Quaking-regulated Alternative Splicing events in Glia cells

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Background: The *quaking viable (qk^v)* mouse represents an animal model with CNS myelination defects. The *quaking (qkI)* gene encodes 3 major alternatively spliced isoforms that are RNA-binding proteins expressed mainly in glia cells, modulating RNA splicing, mRNA export and stability. These RNA binding proteins play key roles in brain and oligodendrocyte development as well as in myelination. The **hypothesis** is that QKI proteins regulate alternative splicing of genes involved in oligodendrocytes differentiation and CNS myelination.

Objective: To Identify QKI-regulated alternative splicing event in glial cells.

Methodology: To study novel differential exon usage regulated by QKI, RNA was harvested from QKI-expressing C6 glioblastoma cells in which QKI expression was knocked down (by sh-RNA). The RNA was sequenced by RNA-Seq and compared to RNA from control cells. Data was analyzed using TopHat and CuffLinks programs for differential gene expression and exon usage, respectively. In addition, growth rate and migration were characterized for control and knock down cells using scratch assay and Boyden chambers. Moreover, FLAG-QKI5 was overexpressed in C6 cells with either GFP-QKI6 or GFP-QKI7. FLAG-IP was performed and samples will be analyzed by Mass spectrometry to identify the nuclear components of the QKI complex. Once MS data is obtained, proteins involved in myelination that interacted with QKI5 will be further investigated.

Results: Knocking down the expression of QKI resulted in a decrease in growth rate by approximately 50%. QKI-deficient cells displayed reduced wound healing and migration compared to QKI-expressing control cells. The knock down cells were cuboidal, isolated and had a fewer cell-cell contact; whereas control cells were star-shaped, elongated and adherent. Data has been obtained for RNA-Seq project and is being analyzed for differential splicing events. Identified targets will be screened for QKI response elements. Then, the binding of QKI to the pre-mRNA will be assessed by CLIP-RT-PCR. The importance of the QRE for either splicing or gene expression will be determined by site-directed mutagenesis.

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599 Identifying Regulators of Alternative Splicing in *Schizosaccharomyces pombe*

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Alternative splicing is a common regulator of gene expression and allows the ability to generate multiple protein isoforms from the same gene. From a splicing perspective, the fission yeast Schizosaccharomyces pombe has a genome structure that in many ways closely resembles that seen in higher eukaryotes, including multi-intronic genes and degenerate splice sites. While several large RNA-seq experiments failed to detect instances of alternative splicing in S. pombe [1, 2], our lab has recently used a novel lariat-sequencing approach to identify nearly two dozen examples of exon-skipping [3]. Remarkably, an evolutionary analysis of these skipping events reveals deep evolutionary conservation of the gene structure for several of these splicing events, suggesting that understanding the mechanisms by which they are catalyzed in S. pombe will be informative about understanding mammalian alternative splicing. To understand the mechanism(s) by which these events are catalyzed in S. pombe, we are utilizing a high-throughput reverse genetic screening approach that was recently developed in our lab [4]. This approach uses quantitative RT-PCR to measure the cellular levels of each splice isoform of a gene in the background of thousands of mutant S. pombe strains. The mutant libraries being examined include both a collection of genome-wide gene deletion mutants as well as custom-generated libraries containing thousands of novel mutants within known splicing factors. By combining these libraries, we aim to characterize the complement of trans-acting factors which influence splice site decisions and regulate alternative splicing events. Given the conservation of these events over evolutionary space, we expect these results to provide important insights into broader mechanisms of alternative splicing in mammalian systems.

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600 Truncation of NTC related factor Prp45 delays co-transcriptional spliceosome assembly

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Splicing in *S. cerevisiae* proceeds co-transcriptionally on genes with longer ORFs [1]. Early in assembly, U1 snRNP binds the 5′splice site, forming the commitment complex 1, and is then joined by Msl5 (Branch point Binding Protein; BBP), which recognizes the branch site. Subsequently, BBP and its heterodimer partner Mud2 are displaced in favor of U2 snRNP and afterwards U4/U6.U5 tri-snRNP is included in the spliceosome. A complex of Prp19 associated factors (Nineteen complex; NTC) together with additional NTC related proteins is incorporated together with the tri-snRNP.

Previously, we reported that truncation of one of the NTC related factors, Prp45, weakens the association of the second step helicase Prp22 with spliceosomal complexes, affects the fidelity of 3′ splice site choice [2], and results in pre-mRNA accumulation of intron containing genes.

Here, we examined Mer1-dependent pre-mRNA and mRNA accumulation of *MER2* and other endogenous meiotic genes. *prp45*(1-169) cells accumulated ~three times higher levels of *MER2* pre-mRNA than WT cells when Mer1 was present, i.e., when the spliceosome was assembling on *MER2* pre-mRNA. We have used HA-tagged splicing factors Prp42, Msl5, Mud2, Msl1, Brr2, and Prp19 to monitor the presence of snRNP complexes along *ECM33* and *DBP2* genes. We found that *prp45*(1-169) differentially affected the co-transcriptional recruitment of BBP and the U2 component Msl1, indicating that Prp45 affects spliceosome assembly prior to NTC incorporation. Notably, we found earlier that *S. pombe* U2AF35, which forms part of early spliceosome and precedes NTC in the splicing cycle, interacts with Prp45 homolog SNW1 [3]. Our results suggest a new role for SNW proteins in early stages of spliceosome assembly.

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601 Analysis of co-regulated alternative exons using AVISPA's splicing codes

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Advancements in high-throughput sequencing have highlighted the genome-wide prevalence of alternative pre-mRNA splicing. However, fully understanding this complex layer of gene regulation involves identifying the underlying "splicing codes", or context specific operation of various *cis*- and *trans*-acting elements that control the splicing outcome. While machine learning algorithms proved successful in inferring such codes, their usage remained limited to pre-defined sets of exons by expert users. To address this limitation, we recently released AVISPA, a web tool that serves as a user-friendly front end to these splicing code models to facilitate regulatory analysis of any exon of interest.

Here we widen the scope of AVISPA's analysis from single exons of interest to large sets of exons defined by RASL-seq data from a Jurkat-derived cell line and primary human CD4+ T cells. This data set includes splicing quantification of over 3,000 events before and after antigen-induced stimulation in wild-type cells and cells treated with kinase inhibitors or depleted for various splicing factors previously identified in the lab to be important components of signal-induced alternative splicing in T cells. AVISPA was used to extract and analyze over 1,400 features for each event to give predictions for their relevance to splicing outcome. Comparative analysis of these features between subsets of the exons analyzed (e.g., CELF2 responsive exons compared to non-responsive exons) reveal unique feature enrichment profiles and give high-confidence predictions of novel splicing co-regulators. Specifically, within CELF2 regulated exons we identify combinations of regulatory features such as positionally biased CELF2, RBFOX, and hnRNP H binding sites and splice site strength. Currently, we are in the process of experimentally validating these predicted regulatory features. In all, our analysis highlights the power of AVISPA and splicing code modeling as a way to identify both shared and divergent features of sets of co-regulated alternative splicing events. This work has suggested future lines of investigation including additional experimental verification and building T cell condition- and splicing factor-specific regulatory code models for genome-wide predictions of T cell splicing.

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602 EJC components regulate alternative splicing and pervasive transcription but not NMD in *Cryptococcus neoformans*

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Cryptococcus neoformans is a capsular basidiomycete yeast responsible for opportunistic infections in patients presenting an immunity deficiency, mainly AIDS patients. A recent re-annotation of the *C. neoformans* genome revealed a spectacular complexity of its transcriptome compared to other fungi. A striking feature of its genome is its very high intron density. In fact, more than 99% of the genes contain introns and alternative splicing is very common. Moreover, most of the components of the RNA metabolism that are present in higher eukaryotes are found in *C. neoformans*, including factors that are not found in *S. cerevisiae*. This suggests a metazoan-like complexity of its RNA metabolism which could provide a mechanism for *C. neoformans* to adapt to different environments and be an efficient pathogen.

In order to get deeper insight into the RNA metabolism of *C. neoformans*, we identified the homologous proteins and constructed the corresponding mutant strains for the NMD factors Upf1, Upf2 and Upf3 and for the EJC components Magoh, eIF4A3, Y14 and UAP56. Noteworthy, we could detect an interaction between Magoh and Y14, suggesting the existence of an EJC-like complex in this yeast. We found that the depletion of the Upf proteins in *C. neoformans* does not affect viability, suggesting that the NMD pathway is not essential in this yeast. RNA-Seq analysis on an *upf1Δ* strain showed that the NMD pathway targets several endogenous PTC-bearing transcripts which arise from unproductive alternative splicing events. On the other hand, we found that the homologous of Magoh, eIF4A3, Y14 and UAP56 are all essential in *C. neoformans* although we demonstrated that none of them play a role on NMD in this yeast. Interestingly, the depletion of these factors has an unanticipated deep impact on the transcriptome of *C. neoformans*. Indeed, RNA-Seq analysis of a Magoh mutant showed the apparition of several new alternatively spliced mRNA isoforms, revealing that EJC components are necessary for accurate splicing in *C. neoformans*. Even more striking, we could detect the apparition of thousands of mostly antisense non-coding RNAs, suggesting an unexpected role for the EJC components in the regulation of pervasive transcription in *C. neoformans*.

603 High-Throughput Analysis of Alternative Splicing Regulatory Networks in Embryonic Stem Cells

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Alternative splicing (AS) is a crucial mechanism for post-transcriptional gene regulation and for expanding proteomic complexity. It is often controlled by the combined action of tissue-specific splicing regulators and changes in the concentrations and/or activities of ubiquitously expressed splicing factors. Given the increasingly extensive roles AS plays in embryonic stem cells (ESCs), understanding the molecular mechanisms underlying AS regulation is of critical importance. A major goal in this direction is to develop and apply high-throughput strategies to systematically discover factors that control specific AS events of interest. To this end, we have established an RNAi-based high-throughput screen for factors that regulate endogenous AS events. To further increase the throughput and sensitivity of the AS regulator screen, a multiplex RT-PCR barcode sequencing strategy was developed, which allows the parallel analysis of hundreds of AS events and thousands of knockdown conditions. Using these newly developed strategies, we have recently completed a high-throughput screen in both mouse ESCs (CGR8) and neuroblastoma cells (N2A) for factors that control ESC-differential AS. Analyses of the barcode sequencing data have revealed interesting new positive and negative regulators of ESC-differential AS events that will form the basis of exciting follow-up studies. The results from this study will provide critical new insights into the global regulatory networks of ESC-differential AS that contribute to fundamental aspects of ESC biology. Moreover, the high-throughput strategies developed here will transform our understanding of AS regulation and its integration with other layers of gene control under both physiological and pathological conditions.

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604 Inhibition of HIV replication by RNA *trans*-splicing technology

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More than 30 million people are infected with the human immunodeficiency virus (HIV) worldwide. Although existing anti-viral treatments are very effective in reducing plasma viral load and have led to a reduction in mortality, the virus cannot be eliminated as it integrates into the host cell genome and remains silent in long-lived memory T cells of the immune system. Thus, it is of great importance to develop novel therapies to eliminate the immune cells in which HIV remains latent. Research is ongoing into reactivating latent virus but this then has to be detected and the cell containing the virus eliminated. HIV uses alternative RNA splicing to process its genetic material into mRNA transcripts encoding viral proteins. Given its importance for the viral life cycle, HIV splicing is an attractive target for the development of novel anti-viral therapies. We are using a gene therapy technology called RNA *trans*-splicing to deliver the herpes simplex virus thymidine kinase-ganciclovir (HSVtk-GCV) cell suicide system into HIV-infected cells. An extensive *in silico* bioinformatics and RNA structural analysis approach has been undertaken to design ten HIV RNA *trans*-splicing constructs targeting a total of eight different HIV splice donor or acceptor sites in the HIV strain pNL4.3. The designed RNA *trans*-splicing constructs have been tested in an initial screen in 293T cells transfected with the HIV proviral clone pNL4.3. We have confirmed the occurrence of RNA *trans*-splicing in HIV-transfected cells by quantitative RT-PCR (qRT-PCR) with the successful detection of fusion RNA transcripts between HIV RNA and the HSV-tk RNA transcripts in six out of ten RNA *trans*-splicing constructs. RNA *trans*-splicing junctions have been confirmed with conventional PCR and by Sanger Sequencing. We are currently investigating the efficacy of HSVtk-induced cell death upon HIV RNA *trans*-splicing in the presence of ganciclovir. Production of spliced HIV RNA isoforms and proteins will be determined by qRT-PCR and western blotting, respectively. Having demonstrated the proof-of-concept of RNA *trans*-splicing against HIV in 293 T-cells, we will test RNA *trans*-splicing efficacy in HIV-infected T cells, and in cellular models of HIV latency.

605 Protein Arginine Methylation Affects pre-mRNA Splicing in *Saccharomyces cerevisiae*

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The posttranslational modification of arginine by a conserved family of enzymes, the protein arginine methyltransferases (PRMTs), has been linked to modulation of splicing both in budding yeast and in higher eukaryotes. Using the model organism *Saccharomyces cerevisiae*, we have established that loss of the major PRMT (called Hmt1) causes defects in splicing of specific transcripts instead of a global splicing defect in all transcripts. These Hmt1-mediated splicing defects are exacerbated at low temperatures. To determine the mechanism that underlies this phenotype, we used mass spectrometry to identify splicing factors that contain methylarginines and to map the location of these modified residues,. The identified methylarginine residues are then mutated to lysines, creating methylarginine-specific point mutants, These point mutants are then used to probe specific functional effects of Hmt1 on pre-mRNA splicing. As a proof of concept of this approach, we have determined that loss of arginine methylation on the SR- and hnRNP-like protein Npl3 is responsible for a loss of *in vivo* efficiency in splicing the *SUS1* and *SCS22* pre-mRNA transcripts. We are attempting to determine the molecular mechanism by which protein arginine methylation of Npl3 promotes transcript-specific pre-mRNA splicing.

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606 When splicing goes wrong – a mechanism for spermatid differentiation

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Sperm production involves many processes of fundamental importance including mitosis, meiosis and complex cellular differentiation. Each requires the tight regulation of gene expression. pre-mRNA splicing is an essential post-transcriptional process. Over 95% of multi-exon human genes undergo alterative splicing (AS) to create diverse arrays of mRNAs from a single gene. AS is a powerful and versatile regulatory mechanism, which enables cells to produce multiple protein isoforms of diverse and even antagonistic functions, thus increasing proteome diversity. Disturbance of splicing regulation is associated with many human diseases; however, key splicing factors that control tissue-specific alternative splicing remain largely undefined.

We have recently demonstrated that the RNA binding protein RBM5 is a splicing regulator in haploid male germ cells. Mice carrying a missense mutation (R263P) in the second RNA recognition motif (RRM) of RBM5 exhibited spermatid differentiation arrest, germ cell sloughing and apoptosis, which ultimately led to azoospermia (no sperm in the ejaculate) and male sterility. Within the adult mouse testis, RBM5 localized to somatic and germ cells including spermatogonia (stem cell), spermatocytes (germ cells during meiosis) and round spermatids (haploid). Through the use of RNA pull down coupled with microarrays, we identified 11 round spermatid-expressed mRNAs as putative RBM5 targets. Importantly, the R263P mutation affected pre-mRNA splicing and resulted in a shift in the isoform ratios, or the production of novel spliced transcripts, of most targets. Microarray analysis of isolated round spermatids suggested that altered splicing of RBM5 target pre-mRNAs affected expression of genes in several pathways, including those implicated in germ cell adhesion, spermatid head shaping, and acrosome and tail formation.

In summary, our findings demonstrate that RBM5 is critical splicing factor that regulates the splicing of specific subsets of pre-mRNAs in haploid male germ cells. RBM5-mediated splicing regulation is pivotal for spermatid differentiation and male fertility. Our results also suggest that the second RRM of RBM5 is critical for appropriate pre-mRNA splicing.

607 Functional study of the Ser/Arg-rich splicing factor SRSF5a during zebrafish embryonic development

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Nuclear pre-mRNA splicing is a key process regulating gene expression in eukaryotes. Splicing consists in the removal of introns and the joining of exons within a dynamic macromolecular complex called the spliceosome, which consist of five small nuclear ribonucleoproteins (snRNPs) and numerous non snRNPs proteins. Amongst these non snRNPs proteins, the SR proteins family constituted an important group of splicing factors that are involved in constitutive and alternative splicing. SR proteins are structurally related as they are characterized by one or two RNA-recognition motifs (RRMs) in N-ter and a C-terminal domain enriched in dipeptide Ser/Arg. Phylogenetic inference using the RRM domain allowed us to identify 13 encoding genes for SR proteins in the vertebrate model organism, *Danio rerio*. The Zebrafish is increasingly recognized as a powerful model for the study of vertebrate embryonic development in a physiological context. The roles of SR splicing factors during animal cell differentiation and development are largely unknown. The aim of the present research is to investigate SR proteins functions during zebrafish development by using molecular and genetic approaches. In this study, we investigated the role of the SR splicing factor SRSF5a. The expression profile was determined by *in situ* hybridization at 24, 48 and 72 hours post-fertilization and showed *SRSF5a* expression mainly in brain, retina and pharyngeal arches at these stages. Furthermore, *SRSF5a* knock-down by morpholinos microinjection strongly suggests an important role of this specific splicing factor during eyes and brain development. In order to gain insight into the molecular function of SRSF5a, we analysed control and morphant transcriptomes using high throughput RNA sequencing. Finally, we use a complementary approach to morpholinos and generate srsf5a mutant fishes using TALENs (Tal effector nucleases).

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608 The B52/SRp55 splicing factor modulates growth and cell competition

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Alternative splicing of mRNA precursors allows to create from a single gene, multiple mRNAs encoding protein isoforms of different and sometimes opposite functions. Almost all human genes produce alternatively spliced mRNAs, therefore alteration of alternative splicing is associated with numerous pathologies including cancers. SR proteins constitute a conserved family of RNA binding proteins involved in alternative splicing regulation. Several SR proteins are frequently overexpressed in human tumors, and at least one SR protein was shown to directly participate in cell transformation.

Here we describe a role of the SR protein B52 (SRp55) in cell growth in *Drosophila*. Overexpression of B52 in the bristle lineage leads to increased cell size and cell death. B52 overexpression strongly increases *myc* expression at a transcriptional level. By a genetic screen, we identified Brain tumor, a post-transcriptional repressor of Myc, as a suppressor of the phenotypes induced by B52 overexpression, revealing an antagonism between this SR protein and a tumor suppressor.

Whereas B52 upregulation increases Myc level and cell size, B52 depletion reduces cell size without apparent effect on *myc* expression. Therefore B52 level modulates growth through different pathways. By using clonal analyses in mosaic animals, we observed that depletion of B52 in epithelial cells of larval wing discs, triggers mutant cell elimination by a mechanism called ‘cell competition’. By RT-PCR screening, we identified alternative splicing events modulated by B52 in several genes involved in cell competition, including genes of the Hippo and insulin pathways. Our data suggest that B52 acts as a coordinator of a specific alternative splicing program modulating growth and cell polarity, two linked processes whose deregulation participates in tumorigenesis.

609 A conserved and cell-type specific program of regulated mRNA splicing supports postnatal liver development

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While the major regulatory programs controlling early specification and morphogenesis of liver are well studied, how the organ matures during the postnatal period is poorly understood. We performed large-scale high-resolution RNA-seq analysis of mouse livers across a postnatal developmental time course. We demonstrate a pervasive and a highly coordinated shift in liver transcriptome within the first four weeks after birth. Remarkably, the genes undergoing changes in expression, alternative splicing (AS) and alternative 3′-UTR usage not only show minimal overlap but also exhibit enrichment for unique functional categories. While many DNA-binding proteins follow the same overall pattern of expression as *all* genes, we report that a large number of RNA-binding proteins are down regulated postnatally.

Direct comparison of over one hundred developmentally regulated, and variable spliced regions between mouse and human livers show that nearly 50% are evolutionarily conserved in both timing and direction. When analyzed in freshly isolated hepatocytes and non-parenchymal cells, these variable regions show cell-type specific transitions such that subsets of events follow either similar or opposite patterns of splicing through development. A computational framework to correlate the changes in variable regions at specific developmental time points and various *cis* elements identified 117 RNA-binding proteins to be significantly associated with splicing alterations. Further, a *de novo* motif analysis showed a positional enrichment for many tissue-specific splicing factors around the variably spliced regions. Detailed temporal analysis of splicing and the associated regulatory factors revealed that a majority of AS changes are co-regulated, and follow either prenatal, postnatal or biphasic patterns of change. Together, our results identify a complex and a highly conserved program of mRNA processing associated with the development of the mammalian liver that supports its physiological growth and maturation.

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610 Capturing transcriptome-wide sites of spliceosome assembly and action using RIPiT-Seq

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To carry out the process of RNA splicing, the spliceosome must assemble *de novo* on each intron. While the dynamic nature of the spliceosome assembly is central to the high fidelity of intron removal, it also presents a formidable hurdle to investigating the nature of interactions between the spliceosome and its pre-mRNA substrate. An even greater challenge is to understand spliceosome assembly on the diverse landscape of the large collection of introns in the human genome. We have begun to tackle these issues by employing RNA immunoprecipitation in tandem followed by deep sequencing (RIPiT-Seq), a technique recently developed in our lab (1). Briefly, RIPiT purification of complex-specific RNA footprints can be achieved by tandem immunoprecipitation of a pair of proteins unique to the complex of interest. By pulling on complex-specific proteins characteristic of different spliceosome subcomplexes, we are able to follow spliceosome-RNA interactions throughout the assembly pathway across the entire transcriptome.

We specifically purified late-stage spliceosomes from cultured human cells (HEK293) via RIPiT by first targeting Magoh, the exon-junction complex (EJC) protein that bind pre-mRNAs within activated spliceosomes, followed by IP of either Prp19 or IBP160, proteins that stably associate with late-stage spliceosomes. The resulting protected RNA footprints differ from those occurring as a consequence of EJC deposition (2). By deep sequencing these footprints, we find that late-stage spliceosomal complexes remain associated with both ligated exons and excised intron lariats. As expected, we observe occupancy of these late-stage complexes at annotated splice sites, but now on an unprecedented transcriptome-wide scale. We are further characterizing these spliceosome-protected regions in a wide variety of contexts, such as long vs. short introns, constitutive vs. alternative exons, etc. We are also fine-tuning existing short read alignment algorithms to detect splicing intermediates and novel splice junctions that may unveil hitherto unknown sites of spliceosome activity. These analyses, along with ongoing similar profiling of early- and middle-stage spliceosomes, are poised to reveal many new secrets of this highly dynamic macromolecular machine.

1. Singh G ... Moore MJ. (2014) ***Methods***, 65(3):320-32.
2. Singh G ... Moore MJ. (2012) ***Cell,*** 151:750-764.

611 Splicing regulation of RBM4 in neuronal differentiation

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The RNA-binding motif 4 (RBM4) has multiple roles in mRNA metabolism including alternative splicing and translational control. RBM4 is also known to promote muscle and pancreas cell differentiation through regulating tissue specific alternative splicing. Using P19 cells as an in vitro neuronal differentiation model, we found that RBM4 was up-regulated in retinoic acid treated cells, which led us to investigate the role of RBM4 in neuron specific splicing. Numb displays multiple functions during differentiation, such as the control of asymmetric cell division, endocytosis, cell adhesion, cell migration, developmental processes, and cell fate determination. Mammalian Numb contains a phospho-tyrosine binding (PTB) and proline-rich region (PRR) domain, which are differentially affected by alternative splicing. In undifferentiated P19 cells, knockdown of RBM4 decreased exon-9 skipping, which is known for promoting neuronal differentiation and neurite growth. Moreover, stably RBM4 knockdown P19 cells had a reduced level of the exon-9 skipping isoform and accordingly exhibited delayed neuronal differentiation. We next demonstrated that RBM4 directly promoted exon-9 skipping by using the mini-gene reporter assay. Furthermore, we observed that exon-9 skipping was minimally reduced in e13.5 brain of Rbm4 knockout mice. We also found that knockdown of Rbm4 shortened the neurite length in cortical neurons. Taken together, our findings suggested that RBM4 may play an important role in neuronal differentiation and neurite growth via regulating alternative splicing of Numb.

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612 Concerted alterations in non-productive alternative splicing of core spliceosome components and splicing factors during smooth muscle cell phenotypic modulation

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Vascular smooth muscle cells interconvert between a contractile phenotype, typical of healthy blood vessels, and a more proliferative synthetic phenotype associated with vascular disease. We profiled transcriptome changes occurring during this phenotypic modulation. Hundreds of cassette exon events were regulated, with similar numbers upregulated in the contractile or synthetic phenotypes. Genes affected by alternative splicing were enriched for actin cytoskeletal functions while transcriptionally regulated genes were enriched for extracellular matrix, but significantly depleted for RNA binding proteins. In contrast to cassette exons, regulated intron retention events showed a skewed distribution, with the majority showing higher retention in the contractile phenotype most of which affected many splicing factor genes. Other splicing factor genes were also affected by non-productive alternative splicing events, such as poison cassette exon inclusion. In every case, the differentiated phenotype splicing pattern was predicted to be non-functional. Affected genes included snRNP components (e.g. Snrnp70, SnrnpA1, Sf3b1, Sf3b3) and splicing activators (Srsf1, Srsf2, Srsf6, Srsf7, Tra2β). Immunofluorescence analysis in rat aorta smooth muscle cells showed marked increases in expression during phenotypic modulation, while levels of RNA polymerase and other auxiliary splicing regulators (e.g. PTB and Mbnl) remained relatively constant. Thus the concerted non-productive splicing events appear to be associated with lower levels of the splicing machinery, rather than resulting from feedback responses to excessively high levels. Our data suggest that the alternative splicing programme in contractile smooth muscle cells is controlled not only by a range of auxiliary RNA binding proteins, but also by concerted down-regulation of the core splicing machinery. We are currently investigating the mechanism by which the concerted down-regulation of the core spliceosome components is established. Preliminary experiments point towards a change in chromatin marks within the alternatively spliced regions between the differentiated and proliferative phenotypes. We are now in the process of characterizing the state of the chromatin marks around those regulated regions by the use of HDAC inhibitors and ChIP.

613 Identification of Phosphorylation Sites in Spliceosome Components Reveals Roles in Removal of Suboptimal Introns in Fission Yeast

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Removal of introns from pre-mRNA by the spliceosome is a highly coordinated co-transcriptional process that is regulated by phosphorylation. Previous studies have shown roles for phosphorylation in both constitutive and alternative splicing; however, a systematic identification of phosphorylation events from specific kinases and how these modifications affect splicing genome-wide is currently lacking. To address these questions, we turned to *S. pombe*; this model system is attractive due to the presence of the SR protein kinase Dsk1 (Srpk1 human homolog), Clk/Sty kinase Lkh1 (Clk1 human homolog), Prp4 kinase, and almost 5,000 introns. We selectively inhibited each of these kinases using chemical analogue-specific mutations in the kinase active sites and identified broad splicing defects for Dsk1 and Prp4 using genome-wide splicing microarrays. Notably, the accumulated introns were longer than average, and enriched for splice sites that varied from consensus sequences. To tie these splicing defects to specific kinase substrates, we used a mutation in Dsk1 that enabled bulky ATP-ϒS analog-specific labeling *in vitro* and mapped substrates in yeast extract using LC-MS/MS. We identified 10 core-splicing factors as Dsk1 substrates, including the branch point binding protein (Bpb1) and SR and SR-like proteins. For each substrate, we designed alanine mutations at phosphorylation sites and analyzed genome-wide splicing using microarrays. Bpb1 was identified as having a broad splicing defect similar to inhibiting Dsk1. Although other Dsk1 substrate mutants did not have splicing defects on their own, combining the alanine mutants in Srp1 and Srp2, the two SR proteins in fission yeast, revealed a modest defect. Moreover, deletion of the non-essential Srp1 combined with the Bpb1 alanine mutant was lethal, and the Srp1 alanine mutant combined with the Bpb1 mutant was synthetic sick. We are currently expanding this analysis to test genetic crosses of all kinase substrate mutants. Taken together our data suggest that phosphorylation of Bpb1 by Dsk1 is required for suboptimal intron splicing and that other Dsk1 substrates have synergistic affects.

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614 Abstract Withdrawn

615 Transcriptome analysis of Drosophila sex-specific, neuronal alternative splicing and in vivo analysis of target genes

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Transcriptomic analysis by RNA Seq can be used to identify differences in alternative splicing (AS). To determine to what extent sex-specific AS is present in the neuronal system of flies, we analyzed the transcriptome of adult wild type female and male fly heads, using Next Generation Sequencing (NGS). The RNA-Seq data from three biological triplicates was evaluated using the AS Event Analysis (ASEA). For each AS event (ASE) the number of reads in the region of the event was collected. For each gene in the genome a pairwise comparison of all the gene´s transcripts was made to normalize to gene expression changes. A PSI (percent spliced in) value was calculated for each ASE by dividing the concentration of the long isoform´s amplicon by the sum of the concentrations of the long and short products.

Significant sex-specific expression changes were observed in 241 genes (FC $\geq$ 2, p value $\leq$ 0.05, reads $\geq$ 100). The wild type female to male comparison showed AS changes in 72 genes, corresponding to 162 ASE (PSIlogDiff $\geq$ 10%, p value $\leq$ 0.05, sum of reads $\geq$ 100). Several candidate genes were confirmed by qRT-PCR, showing a good quantitative correlation to the RNA-Seq data and a validation rate higher than 83%. For five of these candidate genes we generated transformant flies carrying a dual fluorescence minigene under control of an UAS promoter. These reporters are excellent tools to monitor AS and to identify trans-acting and cis-acting regulatory elements of the splicing process in cell culture and in vivo. Expression patterns of sex specifically spliced isoforms have been analyzed in third instar larval and adult female and male brains. Remarkably, different localization patterns are seen for the different isoforms. Although the minigenes are ubiquitously expressed under the control of an actin Gal4 driver, expression of the different isoforms is not observed in all cells of the fly brain. It will be intriguing to study the function of these tissue specific isoforms and to identify the regulatory mechanisms of these neuronal splicing processes in the future.

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616 Coupling between alternative polyadenylation and alternative splicing is limited to terminal introns

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Alternative polyadenylation (APA) has been implicated as an important regulator of gene expression. In some cases APA is known to couple with alternative splicing to influence last intron removal, however it is unknown whether APA events influence alternative splicing decisions at upstream exons. A genome-wide approach was used to examine the correlation between APA and upstream alternative splicing. CstF64 knockdown in HeLa cells coupled with Pas-Seq was used to trigger and identify APA events. APA genes were then evaluated for changes in alternative splicing using RNA-seq analyses of the same knockdown samples. Although a significant number of alternative splicing events were identified, no general correlation between APA and upstream alternative splicing events were observed. These results suggest that the coupling and diversification achieved between APA and alternative splicing in general, is fixed to defining the last exon. iClip-Seq experiments identified CstF64 binding to be predominantly in intronic regions. Interestingly, the genome-wide binding analysis also showed that CstF64 density is elevated upstream of skipped exons indicating a potential role for CstF64 in alternative splicing. We conclude that while the influence of APA on alternative splicing is generally limited to terminal introns, CstF64 binding to nascent pre-mRNA may contribute to modulation of splicing patterns.

617 Shaping of a Genetic Mutation Phenotype by Tissue Specific Alternative Splicing

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Trafficking of proteins in cilia is assisted by an adapter complex termed the BBSome. Mutations in the genes encoding the BBSome proteins result in Bardet-Biedl Syndrome, a genetic disorder characterized by retinitis pigmentosa, obesity, polydactyly, and renal dysfunction. A notable exception of this pattern of systemic disease associated with mutations in BBSome components is a recently discovered mutation in BBS8, which causes photoreceptor cell death (Retinitis pigmentosa) in the absence of any other symptoms. The mutation disrupts the 3′ splice site of BBS8 exon 2A and was predicted to block its splicing. However, the mechanism limiting the phenotype of this mutation to photoreceptors remained unexplained.

Using subretinal injection and electroporation of the minigene into developing mouse photoreceptors we demonstrate that the A to G mutation in the splice site of exon 2a activates nearby cryptic splice sites. In turn, the use of cryptic splice sites causes a frame shift that will disrupt the production of the BBS8 protein. We further show that exon 2a of BBS8 is included specifically in photoreceptors. Cell types other than photoreceptors do not splice exon 2a and consequently do not recognize the cryptic splice sites. As a result these cells are immune to the mutation and the disease phenotype is confined to photoreceptor cells.

Using BBS8 exon 2a as a model, we aim to characterize the mechanisms driving photoreceptor specific inclusion of alternative exons. To this end, we have utilized subretinal injection and electroporation of exon 2a mini-gene variants which include intron deletions and exon substitutions. We have identified two intronic regions that direct inclusion of BBS8 exon 2a in photoreceptors. Studies to identify the trans-acting factors that bind to these sequences are ongoing.

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618 Alternative Splicing in Neuronal Differentiation Identified by Novel Transcriptome Array Analysis

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Alternative mRNA splicing has potency to greatly diversify the repertoire of transcripts in multicellular organisms. Increasing evidences suggest that this expansive layer of gene regulation could play an important role particularly in the development and nervous system. However, the mechanisms still remain largely unknown. In this study, we aimed to reveal key splicing factors and regulatory networks of splicing in neuronal differentiation by analyzing transcriptome array data for neuronal cells differentiated from iPSCs.

Human iPSCs were differentiated into several types of neurons by 5 differentiating protocols. Human transcriptome arrays (HTA) were used for measuring whole exons and exon-exon junctions expression at two time-points of differentiation period for each protocol. In gene level expression analysis, neuronal signaling pathways which related to each type of differentiated neuron were up-regulated. Alternative splicing analysis identified approximately one hundred candidate alternative splicing events in each type of differentiation method. By RT-PCR, 216 out of 285 candidate alternative splicing events were verified. Common splicing evens (26 splicing events) in most of the protocols showed significant enrichment with function related to neuronal differentiation. We also found that splicing event types of alternative first exon and alternative last exon were frequently observed in differentiation into neuronal progenitor cells (NPC), whereas event of skipping exon was mainly observed in differentiation into neurons. Spliceosome related genes showed drastic expression changes during neuronal differentiation. Most of spliceosome factors were down-regulated in any type of differentiation protocol. On the other hand, up-regulated ones showed neuron type specific manner. CELF, ELAVL and NOVA family genes were up-regulated in GABAergic/glutamatergic neurons. HIPK family genes were up-regulated in dopaminergic neurons. Furthermore, transcription factors and 3′end processing related genes showed neuron type specific patterns of gene expression. Our findings suggest that differentiation stage-specific and lineage-specific regulatory mechanisms may exist for neuronal differentiation. This study gives a possibility that alternative splicing may be one of the important regulatory mechanisms of neuronal differentiation.

619 A Role for the Histone Variant H2A.Z and the SWR1 Nucleosome Remodeling Complex in Pre-mRNA Splicing in Fission Yeast

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Multiple lines of evidence have implicated chromatin factors in pre-mRNA splicing regulation. However, very little is known about roles of histone variants in this process. Here we describe a role for the histone variant H2A.Z in pre-mRNA splicing in fission yeast. Gene expression in Schizosaccharomyces pombe is highly reliant on splicing, in that ~60% of all genes contain at least one intron. Using Epistatic Mini-Array Profiles (EMAPs) to globally quantify genetic interactions between pairs of mutants, we have recently shown multiple connections between RNA processing and a large number of chromatin modification and remodeling factors in S. pombe. In particular, this high-throughput double mutant analysis revealed that many splicing factors had a significant enrichment of synthetic sick/lethal genetic interactions with components of the SWR1 complex, which deposits H2A.Z. To probe the functional basis of the genetic interactions between SWR1 complex components and the splicing machinery, we performed a splicing-sensitive microarray to screen for pre-mRNA accumulation in Δpht1 (H2A.Z) and Δswr1 (ATPase of the SWR1 complex) strains at 30°C and 16°C. Consistent with the EMAP results, we observed that both the Δpht1 and Δswr1 strains exhibit a splicing defect in an intron-specific manner at 30°C, which is greatly exacerbated at 16°C. The profiles of these splicing defects are complex, but generally correlate with genomic regions of H2A.Z localization from published ChIP-chip profiles. We have further characterized these splicing defects by qRT-PCR. One explanation for the splicing defect in the Δpht1 and Δswr1 strains is that H2A.Z facilitates the recruitment of the splicing machinery co-transcriptionally. To test this hypothesis we are performing chromatin immunoprecipitation-qPCR of early splicing factors in the absence of Pht1 or Swr1. To date, we see a decrease in U2AF association in the Δpht1 background, suggesting a role for H2A.Z in an early step in spliceosome assembly. Consistent with such a role, the SWR1 complex is the only chromatin remodeling complex represented in the EMAP to be enriched for negative genetic interactions with the U1 snRNP. Overall, our work indicates that efficient splicing is highly sensitive to mutations in both the splicing and chromatin machineries.

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620 Modulation of synaptic transmission, behavior, and alternative splicing in distinct neuron classes by a pair of RNA binding proteins

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Alternative splicing plays an important role in the function of the nervous system, but little is known about the extent to which alternative splicing is regulated between distinct neurons, the factors controlling such splicing decisions, or the neurophysiological roles played by networks of alternative isoforms. We use two-color fluorescent reporters in *C. elegans* to observe splicing at single-cell resolution and demonstrate that differential splicing between neurons is frequent and subject to highly specific regulation. One particularly interesting example of neuron subtype-specific alternative splicing is UNC-16/JIP3, in which exon 16 is skipped only in GABAergic motorneurons, but included in both GABAergic and cholinergic motorneurons. We utilize a forward genetic screen to identify two conserved RNA binding proteins, UNC-75/CELF and EXC-7/Hu/ELAV, which both bind to the downstream intron to mediate exon inclusion, and whose partially-overlapping expression is sufficient to explain the complex UNC-16 slicing pattern. RNA-Seq analysis reveals that UNC-75 and EXC-7 modulate extensive and overlapping networks of isoforms encoding neuronal proteins. We use the UNC-75 exon network to discover novel regulators of synaptic transmission, and to identify unique roles for isoforms of UNC-64/Syntaxin, a protein required for synaptic vesicle fusion. Our results indicate that combinatorial regulation of alternative splicing in distinct neurons represents a key mechanism to specialize metazoan nervous systems.

621 Genetic interaction mapping reveals a role for the SWI/SNF nucleosome remodeler in early steps of splicing in fission yeast

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Co-transcriptional RNA processing events are thought to be coordinated by connections between pre-mRNA splicing and chromatin remodeling. However, the precise nature of the crosstalk between nucleosome remodeling, RNA polymerase II transcription, and splicing remains unclear. Furthermore, the specific step(s) of spliceosome assembly and/or catalysis affected by chromatin factors is unknown.

We constructed a high-throughput epistasis mini-array profile (EMAP), assaying genetic interactions between 85 splicing factors and all genes represented in the non-essential fission yeast deletion library. Notably, splicing as a whole was significantly enriched for negative (synthetic sick) interactions with histone modifying and chromatin remodeling factors. Several U2 factors showed strong negative interactions with components of the nucleosome remodeling SWI/SNF complex. Subsequent splicing-specific microarray and gene-specific qRT-PCR analysis has showed that these sick double mutant strains have a broad splicing defect, suggesting that uncoupling splicing and SWI/SNF remodeling at a U2-dependent step leads to intron accumulation.

Further analysis has revealed the precise nature of the U2 splicing factor mutant alleles in our EMAP. In particular, we discovered that overexpression of the U2- specific SF3b factor Sap145 (Cus1 in *S. cerevisiae*) was synthetic sick with deletion of SWI/SNF. Interestingly, previous studies from the Ares lab in budding yeast have shown that overexpression of Cus1 can rescue temperature-sensitive defects in *S.c.* prp5 and the SF3a component prp11, suggesting that an excess of Cus1 may promote recruitment and stabilization of these U2-specific factors. Subsequent experiments showed that ectopic overexpression of Sap145 recapitulates the synthetic sick phenotype, as does overexpression of other SF3a/b subunits in a ΔSWI/SNF background. Notably, overexpression of Sap145 does not cause a growth defect in other chromatin-defective strain backgrounds, suggesting a special role for SWI/SNF nucleosome remodeling in promoting early steps of splicing.

SF3a/b must be destabilized from the U2 snRNP to allow spliceosome activation and catalysis, a reaction performed by the RNA-dependent ATPase Prp2. Our current evidence supports the hypothesis that wild-type Prp2 is required for viability in ΔSWI/SNF backgrounds. We propose that these two ATP-dependent functions must be coordinated to promote efficient splicing.

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622 DDD00944892 – A new splicing modifier

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Pre-mRNA splicing is an important step in gene expression. However in contrast to other steps involved in gene expression, including transcription and translation, very few well characterized chemical inhibitors are available with which to dissect or study the splicing process particularly *in vivo*. Therefore, the identification of specific and selective splicing inhibitors/modifiers would not only be extremely valuable for research purposes as biotools, but also potentially useful for therapeutic applications.

To date only a few natural compounds and their synthetic derivatives have been identified as general splicing inhibitors. In addition, several other natural compounds, derived either from extracts of plants, or microbes, have been reported either to inhibit splicing *in vitro,* or to change splicing of certain transcripts in cells.

Our aim is to identify new small molecules that modulate splicing *in vitro* and in cells. We conducted a high throughput screen of 71,504 small, drug-like chemical compounds by using a recently published high throughput *in vitro* splicing assay (1). Potential hits were verified by radioactive and RT-PCR based *in vitro* splicing assays and replicated between nuclear extracts from different human cell types. All compounds that tested positive for splicing inhibition *in vitro* were further tested in HeLa cells for their ability to inhibit splicing of endogenous genes in vivo. 10 new compounds were identified that modify splicing *in vitro* and inhibit pre-mRNA in cells. A further screen of ca. 100 derivatives of one of the new splicing inhibitors identified an additional 15 compounds that act as general splicing modulators.

We are currently investigating in detail the effect of one of these compounds, DDD00944892, on splicing and cell growth. We show that DDD00944892 alters splicing of a subset of pre-mRNAs in different cell lines and stalls splicing complex assembly at the pre-A complex.

(1) Samatov T et al., ChemBioChem Volume 13, Issue 5, pages 640–644, March 19, 2012

623 An Ultraconserved Element Controls Alternative Splicing of Differentially Localizing ARGLU1 mRNA

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Arginine and Glutamate Rich Protein 1 (ARGLU1) is a recently discovered protein that has been associated with the mediator complex as well as components of the splicing machinery. The protein is well conserved, and has highly conserved protein homologs as far back as fish and flies. We observed in HeLa cells that ARGLU1 contains two major splarglice isoforms that fractionate biochemically between the cytoplasmic fraction and the membrane/nuclear fraction. The isoform found exclusively in the membrane/nuclear fraction retains an intron, coding for an alternate C-terminus and generating an alternative 3′ UTR. Within this retained intron/alternative UTR is a long, extremely conserved stretch of nucleotides that fits the criteria for an Ultraconserved Element, in that it is 100% conserved for more than 200 bases between distinct taxa. Mutational analysis of a reporter vector strongly suggests that this UCE is necessary to repress splicing of the transcript. We have also further explored the role of ARGLU1 UCE in regulation of RNA localization.

Interestingly, analysis of RNA-Seq data from D. melanogaster indicates that the ARGLU1 homolog in Drosophila exhibits a similar phenomenon of intron retention alternative splicing, and is developmentally controlled. Conservation of the UCE in vertebrates and a similar phenomenon in insects indicates that post-transcriptional control of this gene is under strong selection, although the functional result of this alternative splicing is not known. Further analysis of the proteins derived from the two splice isoforms is necessary to understand the functional relevance of this regulation in both mammals and flies.

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624 Dissecting the in vivo functions of the neural splicing regulator nSR100/SRRM4

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Alternative splicing (AS) occurs at higher frequency in the nervous system than most other mammalian tissues. Which specific AS events shape and maintain neuronal functions is still widely unknown. We previously discovered the neural splicing regulator nSR100/SRRM4 and demonstrated that it controls a large program of AS events contributing to neuronal differentiation (Calarco et al. 2009, Cell; Raj et al. 2011, Mol Cell; see Raj et al. abstract). However, the extent to which nSR100 contributes to the development and function of the mammalian nervous system *in vivo* had not been previously explored.

To address this question we generated an nSR100 knockout mouse. The majority of mutant mice die shortly after birth with breathing difficulties and surviving individuals display tremor and circling behaviour. Using immunostaining we observe that innervation of the diaphragm is defective in mutant mice, likely explaining the high rate of perinatal mortality. These mice also display an abnormal distribution of cortical layers and dramatic midline crossing defects in the corpus callosum. Interestingly, disruption of cortical layering and failure to achieve midline crossing in the corpus callosum have been linked to neurological disorders in humans.

Next, we investigated the *in vivo* nSR100 AS regulatory program by performing RNA-Seq on brain tissues from mutant embryos. Depletion of nSR100 affects the regulation of all main classes of AS events, including cassette exons, alternative 5′/3′ splice sites and retained introns, many of which are found in genes associated with the aforementioned phenotypes. In addition, loss of nSR100 results in the mis-regulation of a large fraction of microexons, an emerging class of highly conserved <16 nucleotide alternative exons. RT-PCR confirmed that nSR100 promotes neural-specific inclusion of all tested microexons, including examples as short as 3- and 6-nucleotides. Intriguingly, many of these microexons are predicted to modulate protein interactions involving genes linked to phenotypes observed in the mutant mice.

In summary, our work highlights critical roles played by nSR100 during development of the peripheral and central nervous system, and further reveals a greatly expanded network of nSR100-dependent AS which includes highly conserved microexons likely to contribute important functions in the nervous system.

625 Actinomycin D modulates splicing decisions, CUG RNA and splicing factor levels across various DM1 in vivo models

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Myotonic Dystrophy 1 (DM1) is an inherited disease characterized by myotonia, or the inability to relax contracted muscles. On a molecular level, affected individuals carry large CTG expansions in the 3′ UTR of the DM protein kinase gene. When transcribed into RNA, this CTG expansion produces a CUG RNA that is capable of "sequestering" an important splicing factor, muscleblind-like 1 (MBNL1). Reduced levels of free MBNL1 in the nucleus result in mRNA mis-splicing of several critical genes, which lead to the symptoms observed in disease. While this mechanism is widely established, on-going research aims to explain differential splicing response between DM1-associated genes depending on differences in CUG RNA and MBNL1 levels. Previously, we identified pentamidine, a bisbenzamidine that reversed mis-splicing of several genes in DM1 tissue culture and mouse models by decreasing CUG RNA levels in a dose dependent manner. This prompted us to investigate other molecules that may inhibit transcription of CTG repeats. We performed a literature search to identify other molecules that may inhibit CUG transcription and identified actinomycin D: a potent transcription inhibitor and chemotherapy drug. Previous reports demonstrate that actinomycin D intercalates DNA at GpC sites and a crystal structure confirms its ability to bind CTG DNA. We demonstrate that actinomycin D decreases CUG transcript levels in a dose dependent manner in our DM1 cell model and does so at significantly lower concentrations (nanomolar) compared to its use as a general inhibitor of transcription. Furthermore, actinomycin D partially reversed splicing defects in a mouse model. In our cell culture model, we found that the ability of actinomycin D to exert splicing change is contingent on the differential sensitivities that DM1-associated pre-mRNAs possess to MBNL1 levels. We therefore investigated whether actinomycin D modulates MBNL1 protein levels, and found that it decreased them significantly in the nanomolar range. Collectively, our data indicate that actinomycin D is a powerful molecular tool when examining the interplay between CUG repeats and MBNL1 protein levels in splicing regulation of DM1-associated target transcripts.

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626 Functional Switch of a Human Protein through an Evolutionary G Tract "Invasion" into a 3′SS

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Alternative splicing greatly contributed to the expansion of transcriptomes during evolution. Particularly, mammals have evolved distinct alternative exons but the underlying molecular evolution mechanisms and their impact on the functional diversity of proteins are mostly unknown. Here we have identified G tracts that unusually "invaded" the 3′ splice sites (3′ SS) upstream of alternative exons of hundreds of human genes mainly through point mutations/insertions in marsupials, into a location where polypyrimidine tracts are expected for constitutive exons. The functions of G tract host genes cluster significantly for cellular growth and proliferation. One of them encodes the protein arginine methyl transferase 5 (PRMT5) that plays crucial roles in stem cell pluripotency, differentiation and animal development. We demonstrate by mutagenesis, depletion/add back and UV cross-linking analysis that the evolved G tracts are splicing silencers instead of enhancers often seen for other intronic G tracts. They act by recruiting mainly the hnRNP H1 protein to inhibit U2AF65 binding to the polypyrimidine tract, resulting in exon skipping and the creation of a shorter PRMT5 isoform (PRMT5S) in humans. Interestingly, the function of the PRMT5S isoform is completely switched to the inhibition of mitosis in RNA interference and rescue assays and promotion of dendritic cell differentiation upon ectopic expression, in opposition to that of the commonly known longer one (PRMT5L). Thus, the evolved 3′ SS G tract element causes exon skipping and the generation of two PRMT5 variants of opposite functions in cells, providing a unique molecular evolution mechanism for balanced control of cell mitosis and differentiation by the protein methylation pathway. The similarly evolved hundreds of other G tracts imply this as a common evolutionary mechanism facilitating the generation of diverse protein isoforms from a group of human genes in cell growth or proliferation.

627 Identify genes that are alternatively spliced by Sam68 in mTOR signaling pathway

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Sam68, the Src-associated substrate during mitosis of 68kDa, is an important member of the STAR RNA binding proteins. Previous studies have demonstrated that Sam68 is involved in alternative splicing of many genes. One of the recent publications in our lab demonstrated that ablation of Sam68 leads to increased energy expenditure and defective adipogenic differentiation in mice. We showed that the lack of Sam68 resulted in mTOR intron5 retention and the production of a short transcript. Although we have identified the *mTOR* gene to be alternatively spliced, other splicing events were also identified. Thus, the aim was to examine other Sam68 alternative splicing targets contributing to adipogenesis and cancer.

Using the candidate approach, we identified that S6K1, PRAS40, TSC1, Rheb, Akt and Deptor have many alternatively spliced isoforms. Besides, we demonstrate that alternative splicing of S6K1 and TSC1 are regulated by Sam68 in mouse pre-adipocytes, mouse white adipose tissue as well as mouse embryonic fibroblast. The short isoform of S6K1 regulated by Sam68 is called p31, according to its protein size (316aa). It has been shown that p31 is regulated by SRSF1 and has the oncogenic activity in human transformed cells. We also have identified that Sam68 regulates the production of p31 in the human breast cancer cell line. This splicing event is enhanced by growth factors both in mouse and human cell lines. Moreover, by examining the sequence, we have identified a Sam68 consensus binding sequence (SBS) in the intron 6 of S6K1 pre-mRNA. To investigate whether Sam68 associates with SBS, affinity pull-down assays and RNA immunoprecipitation assays were performed. We conclude that Sam68 directly binds to the SBS in S6K1 intron 6 to regulate its alternative splicing. Lastly, it has been shown that Sam68 deficiency leads to an adipogenesis defect. In our study, WT and Sam68 deficient preadipocytes were knocked down of p31 and induced differentiation for 4 days. We found that the adipogenesis defect in Sam68 deficient cells was rescued by knocking down of p31. In conclusion, we observe that increasing amount of p31 contributes to adipogenesis defect in Sam68 deficient preadipocytes.

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628 SRPK2 possesses distinct mechanisms for the phosphorylation of different SR proteins

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Serine arginine rich protein (SR protein) is an essential family of splicing factors that involves in the regulation of both constitutive and alternative pre-mRNA splicing. Different phosphorylation states of the C-terminal serine/arginine rich domain (RS domains) of the SR proteins govern their subcellular localization as well as their recruitment to the site of splicing and functions during spliceosome assembly. Extensive investigations have been done on how SRSF1, a prototypic SR protein that contains two RNA recognition motifs (RRMs), is regulated by SR protein kinase 1 (SRPK1). However, little information on the regulation of other SR proteins is available. Serine/arginine-rich splicing factor 3 (SRSF3), formerly known as SRp20, belongs to a subclass of SR proteins that contains only a single RRM followed by a C-terminal RS domain. The absence of the second RRM, which is important for the interaction between SRSF1 and SRPK1, suggests the mechanisms of binding and phosphorylation of SRSF3 by SRPKs are likely to be different.

In the present work, we aim to investigate the mechanisms of interaction as well as the phosphorylation of SRSF3 by SRPK2, another member of the SRPKs family which specificity and functions are distinct from SRPK1. Our results demonstrate that a conserved docking groove in the C-lobe of SRPK2, as well as residues between Arg106 to Ser128 of the RS domain of SRSF3 are essential for their interaction. Furthermore, unlike SRSF1, which only the N-terminal region of RS domain can be phosphorylated by the SRPKs, the entire RS domain of SRSF3 can be phosphorylated by SRPK2 at 12-14 sites and half of these sites are modified in a processive manner. These results suggest that SRPK2 adopts vastly different mechanisms in the phosphorylation of different SR proteins.

629 Molecular study of the MEC-8 splicing factor from *C. elegans*

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In multicellular organisms, proteomic diversity in each cell and tissue is provided initially by selective expression of gene subsets from the total genome which are further subjected to alternative splicing, such that a different pattern of exons can be retained or excluded in the final protein-coding mRNA. We are investigating the molecular details of the tissue-specific splicing factor protein MEC-8 from the worm *Caenorhabditis elegans*. The MEC-8 protein contains two distinct RNA recognition motifs (RRMs) connected by a linker, and is involved in alternative splicing of unc-52 and mec-2 genes in hypodermis and receptor neurons as well as numerous other pre-mRNA. MEC-8 interacts with several proteins based on previous biochemical and genetic data including EYA-1 (C49A1.4), F26B1.2, EXC-7 (F35H8.5), SUP-12 (T22B2.4) and ASD-2 (T21G5.5). Using NMR spectroscopy our aim is to structurally characterize the RNA binding as well as the interaction of MEC-8 with a partner proteins in the alternative splicing mechanism. We have therefore expressed and purified the individual RRM domains from MEC-8 as well as the full-length protein, and have initiated molecular characterization studies by using NMR spectroscopy. Early investigation into binding has including isothermal titration calorimetry and electrophoretic mobility shift assays. Our second goal is to use diverse biophysical, biochemical and in vivo techniques to look at the molecular details of RNA-binding by MEC-8 in order to help understand the important role this protein has in the muscular and neuronal development of the worm *Caenorhabditis elegans*.

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630 Deep-sequencing of RNA lariats from the fission yeast *Schizosaccharomyces pombe*

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Many eukaryotic genes require processing by the spliceosome to remove non-coding intronic regions, as intron lariats, which would otherwise preclude translation. In higher eukaryotes, splice site sequences that define introns are often information poor, both enhancing the spliceosome's ability to generate proteomic diversity via alternative splicing, but also potentially increasing the frequency of aberrant splice site selection. Although RNA-seq provides a powerful tool for monitoring genome-wide splicing patterns, read depth continues to limit its ability to robustly identify many splicing events, such as those producing unstable products. We recently demonstrated an alternative approach to monitor genome-wide splicing, which relies upon sequencing excised lariat introns. Lariat sequencing was previously employed in the unicellular yeast, *S. pombe*, whose introns display splice site degeneracy similar to mammals. Importantly, this approach discovered many previously unannotated splicing events in *S. pombe*, including examples of exon skipping – a class of alternative splicing once thought absent from *S. pombe*. Several of these skipping events are conserved evolutionarily, including with humans, but were unobserved in previous large-scale RNA-seq studies with 10-20 fold more depth. Importantly, the original lariat sequencing approach was optimized to recover long lariats, and identified only ~20% of the known *S. pombe* introns. Here, we improve upon the original approach, extending our coverage range to include ~80% of introns. Moreover, to better identify novel regulated splicing events, we independently sequenced lariats from *S. pombe* after exposure to several growth conditions, including heat shock and diauxic shift. For these conditions, we generated lariat sequencing and RNA-seq data from the same samples. Our current lariat sequencing datasets recover the majority of known introns, and identify many additional unannotated splicing and alternative splicing events. Importantly, because reverse transcription of lariat RNAs sometimes transcribes across the 2′-5′ linkage of the 5′ splice site and branch point adenosine, our approach directly identifies branch point usage along with the associated 5′ splice site. Our dataset contains hundreds of thousands such reads, allowing for genome-wide analysis of branch point selection and provides the highest resolution global pre-mRNA splicing study to date in an organism with degenerate splice site sequences.

631 Splicing regulation of BRCA1 exon 11

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BRCA1 is one of the major genes associated with familial breast cancer and mutations in this gene are responsible for about 40-45% of hereditary breast cancer. These include DNA changes that cause BRCA1 aberrant splicing by affecting regulatory sequences. Alternative splicing is a post-transcriptional process allowing the generation of multiple protein isoforms from the same gene. BRCA1 alternative splicing is altered in breast cancer therefore mutations in splicing regulatory elements along this gene sequence can contribute to the disease. Three BRCA1 isoforms are present in different proportions in breast cancer due to alternative splicing of exon 11. Mutations in exon 11 that affect splicing regulatory elements can alter the normal isoform ratio and cause breast cancer. An Unclassified variant (UV) c.693G>A was found in BRCA1 in a breast cancer patient with a family history of breast and ovarian cancer. This unclassified variant is situated in exon 11 in BRCA1 gene. Using a mini-gene approach as a splicing assay and when possible, blood samples from the patients, We show that the unclassified variant c.693 G>A has a strong effect on the splicing isoform ratios of BRCA1.Systematic site directed mutagenesis of the area surrounding the nucleotide position c.693 in exon 11 and mini-gene splicing assay suggests a splicing regulatory element exists in this region that is disrupted with the c.693 G>A sequence variant. Bioinformatic analysis and in vitro (Pull-Down) analysis have detected both enhancer and silencer factors as the regulatory proteins that bind this area regulating BRCA1 alternative splicing suggesting the presence of a CERES element. Correction strategies to revert aberrant splicing of exon 11 in the BRCA1 gene in the presence of the synonymous variant c.693 G>A using bifunctional oligonucleotides were undertaken.

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632 Unraveling the role of the MAPK pathway in alternative splicing regulation through Cdk12 phosphorylation

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Introduction: Alternative splicing is the process by which pairs of splice sites are differentially selected to generate multiple mRNA variants from a single precursor pre-mRNA. More than 95% of genes in human are thought to undergo splicing and its misregulation underlies many human diseases including cancer. Splicing is carried out by the spliceosome and involves a growing list of regulatory factors facilitating splice site recognition, one of which is the evolutionally conserved kinase Cdk12. How these factors are regulated by signal transduction pathways remains poorly understood. Interestingly, our laboratory identified Cdk12 as a candidate ERK1/2 substrate in a large-scale phosphoproteomic analysis.

Methods and Results: We first tested whether ERK1/2 can directly phosphorylate Cdk12 in vitro, using GST-tagged Cdk12 fragments produced in bacteria in presence of recombinant active ERK1 and [γ32P]ATP. We confirmed that ERK1 phosphorylates Cdk12 in vitro and identified specific phosphorylation sites by site-directed mutagenesis and MS analyses. We also showed that ERK1/2 physically interact with Cdk12. The functional impact of ERK1/2-mediated Cdk12 phosphorylation in vivo is currently being assessed.

Conclusion: We already validated the phosphorylation of Cdk12 by ERK1/2, consistent with a potential role of the MAPK pathway in alternative splicing. The functional impact of ERK1/2 signaling in alternative splicing process remains to be evaluated, as well as the repertoire of splicing factors targeted by these protein kinases.

633 Identification of minimal motifs required for circular RNA production

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Recent deep sequencing studies have surprisingly revealed thousands of circular RNAs generated from human protein-coding genes. These RNAs are produced when the splicing machinery fails to join the 3′ end of one exon to the 5′ end of the next and instead appears to "mis-splice" by, for example, joining the two ends of a single exon together. With the exception of the first and last exons of genes, every other exon in the genome has splicing signals at its two ends and theoretically can circularize. Yet, every exon does not circularize, and thus the splicing machinery must somehow determine whether a pre-mRNA yields a linear mRNA or a circular RNA. But how? To identify the minimal signals required for circular RNA production, we cloned the pre-mRNAs of several genes known to produce circular RNAs (e.g. ZKSCAN1, HIPK3, and EPHB4) into easily manipulatable expression vectors. By extensively mutagenizing these vectors and transfecting them into HeLa cells, we have found that most of the surrounding intronic sequences are dispensable for circular RNA production. In fact, miniature introns containing only the splice site sequences along with short inverted repeats (e.g. <36 nt for ZKSCAN1) are sufficient to allow the intervening exon(s) to efficiently circularize. This result is in stark contrast to previous studies that suggested inverted repeats of at least 400 nt in length are required for circle production. Interestingly, we find that increasing the stability of the hairpin between the repeats can sometimes strongly inhibit circular RNA biogenesis, suggesting that more than simple thermodynamics is at play. Further, an exon that circularizes when surrounded by its endogenous introns often does not circularize when placed between introns from a different gene (which do support circularization of that endogenous intervening exon). Circularization is thus tightly regulated and requires the intron repeats to collaborate with motifs in the exons. Our analysis identifying these crucial exonic sequences will be presented. In total, by determining the minimal elements sufficient for circular RNA biogenesis, we are generating detailed and generalizable models that explain how the splicing machinery distinguishes whether to produce a circular RNA or a linear mRNA.

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634 Exploring the complex post-transcriptional roles of the ESRPs in genome-wide regulatory programs of EMT

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The Epithelial to Mesenchymal Transition (EMT) is a process in which cobblestone-like epithelial cells lose apical-basolateral polarity, reorganize the cell-cell adhesions and acquire cell motility to become spindle, fibroblast-like mesenchymal cells. EMT, along with the reverse process MET, has multiple roles in embryonic development and organogenesis. Furthermore, EMT is implicated in cancer metastasis. Previous research focused on the transcriptional regulation associated with this process and many mesenchymal transcription factors, such as SNAIL1/2 and ZEB1, have been shown to promote EMT *in vitro*. However, more recent studies have begun to define an important role of post-transcriptional regulation during EMT. Our lab has identified Epithelial Specific Regulatory Proteins 1 and 2 (ESRP1 and ESRP2) that can regulate alternative splicing during EMT. Notably, ESPRs are among the most highly down-regulated RNA binding proteins (RBPs) during EMT.

To explore the function of ESRPs in the complex post-transcriptional regulatory network of EMT more comprehensively, we established an *in vitro* system for the rapid induction of EMT in H358 epithelial cells and performed strand-specific RNA-Seq to profile the post-transcriptional changes that occur during EMT. We also knocked down the ESRPs in the same cell line to define their role in regulating these changes. Using stringent cutoff, we identified hundreds of alternative splicing events associated with EMT, many of which are regulated by ESRPs. However for the majority of AS events, other RBPs are clearly playing a role. We identified about 50 known RBPs with at least 1.5 fold change at expression level during EMT, including MBNL1/3, PTBP3, MSI2. These data also reveal changes in alternative polyadenylation as well as the expression of several lincRNAs during EMT. We will further characterize the alternative polyadenylation program during EMT using PolyA-Site Sequencing (PAS-Seq) and again determine the contribution of ESRPs. To identify the direct targets of ESRPs, we will perform CLIP-Seq (cross-linking with immunoprecipitation followed by high-throughput sequencing) to identify the target transcripts and binding sites for ESPR1.

635 Dissecting protein-protein interactions that regulate the RNA-binding capacity of PSF

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PSF (SFPQ) is a ubiquitously expressed, essential nuclear protein that influences several genome maintenance and gene expression pathways including transcription, mRNA alternative splicing (AS) and polyadenylation. To perform this range of functions, PSF interacts with multiple protein cofactors and targets several types of DNA and RNA. Previous studies in our lab have revealed that PSF is essential for the signal-responsive splicing of the CD45 pre-mRNA in T cells. This splicing event is regulated by the serine/threonine kinase GSK3, which phosphorylates PSF T687 in resting, but not antigen-stimulated, T cells. PSF T687 phosphorylation controls the association of PSF with another nuclear protein, TRAP150, which subsequently prevents PSF from binding CD45 pre-mRNA.

To better comprehend the link between GSK3, TRAP150 and PSF, and the scope of the cellular consequence of this regulatory regime, we first mapped the minimal PSF/TRAP150 interaction interface. Intriguingly, our results showed that the PSF RNA recognition motifs (RRMs) are necessary and sufficient for binding TRAP150. Similar analysis of TRAP150 revealed that it binds PSF using an uncharacterized stretch of amino acids we have termed the central region (CR). Moreover, we have shown by UV-crosslinking that the TRAP150-CR is sufficient to disrupt the PSFRRMs/CD45 pre-mRNA interaction, suggesting that TRAP150 directly competes with some PSF RNA targets. We are currently testing whether TRAP150-CR also disrupts the binding of PSF to other RNA, DNA and protein partners. Furthermore, we are using next generation sequencing to define a set of mRNAs sensitive to GSK3 inhibition and/or TRAP150 and PSF knockdown. Finally, we have used limited proteolysis to compare PSF isolated from resting T cells to PSF from activated T cells and have found evidence that PSF undergoes a conformational change that depends on phosphorylation status. Altogether, our results suggest a mode of regulation in which phosphorylation of PSF by GSK3 causes the protein to assume an "open" conformation that promotes interaction with TRAP150 at the expense of RNA binding. This signal-induced conformational switching would mechanistically explain how PSF selectively engages with protein cofactors and nucleic acid targets to modulate its role in AS and other functions in T cells.

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636 Nuclear Export of Discarded Splicing Intermediates

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The precise removal of introns is essential for cell survival. To promote fidelity in nuclear pre-mRNA splicing, the spliceosome rejects and discards suboptimal splicing substrates that have engaged the spliceosome. Although intron-containing transcripts can be retained and degraded in the nucleus, a variety of spliceosome-discarded intermediates, such as mutated lariat intermediates, can be degraded in the cytoplasm. However, it is unknown why or how these suboptimal intermediates are exported for turnover. Here, we used RNA-FISH to provide direct evidence that mutated lariat intermediates are exported into cytoplasm. Moreover, disruption of DEAH-box ATPase Prp43p-mediated discard of suboptimal intermediates blocked export of mutated lariat intermediates. Further, we demonstrated that nucleoporins (Nup2p and Nup60p) and the general mRNA export receptor, Mex67p, play an essential role in exporting mutated lariat intermediates. Together, our findings establish the importance of the mRNA export pathway in transporting spliceosome-discarded intermediates for degradation. We are currently investigating why these intermediates are degraded in the cytoplasm instead of the nucleus.

637 Abstract Withdrawn

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638 Antisense Oligonucleotides for the Treatment of Batten Disease

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Juvenile neuronal ceroid lipofuscinosis (JNCL), or Batten disease, is an autosomal recessive disorder caused by mutations in the *CLN3* gene. Batten disease is a childhood neurodegenerative disease with a prevalence of approximately 1 in 25,000 births in the United States and Europe. Onset of the disease occurs at five-eight years of age with symptoms including progressive loss of motor function, seizures, vision loss, and loss of cognitive function, resulting in death before the age of 30. Currently, there are no treatments for the disease. The function of the CLN3 protein is not well understood, but it is implicated in membrane trafficking, phospholipid distribution, and response to oxidative stress. Most cases of Batten disease are caused by a deletion of exons 7 and 8 (*CLN3Δ78*), which causes a frameshift and a premature stop codon in exon 9. We have developed an antisense oligonucleotide that targets CLN3 splicing to restore the reading frame of the *CLN3Δ78* mRNA. This ASO efficiently restores CLN3 reading frame in patient cells in culture. Furthermore, mice with the *CLN3Δ78* mutation, which have motor deficits by two months of age, can be partially rescued by a single neonatal ICV injection of ASOs. Our results suggest that ASO-mediated reading-frame correction may be a viable therapeutic approach for Batten disease.

639 Antisense oligonucleotide-mediated splicing modulation for the treatment of Alzheimer's disease

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Antisense oligonucleotides (ASOs) are emerging as a promising drug platform for the treatment of neurological disease, having been proven safe, effective and long-lasting in the central nervous system. This development comes at a time when effective treatment for many central nervous system disorders with traditional small-molecule therapies has remained unsuccessful. Here, we investigated the therapeutic potential of ASOs for the treatment of Alzheimer's disease. We designed ASOs that target the splicing of two different gene transcripts that have been implicated in Alzheimer's disease. Altering the splicing of these transcripts is predicted to be therapeutic by decreasing the production of amyloid β-peptide, improving synaptic activity and/or reducing tau phosphorylation. We identified ASOs that successfully alter splicing of these gene transcripts in human and mouse cells in culture. These ASOs also effectively alter splicing and improve learning, memory and other behavioral abnormalities in a mouse model of Alzheimer's disease. We observed a reduction in the accumulation of Aβ plaques in some cases, suggesting that improved learning and memory in the mice is caused by amelioration of plaque formation. Together, our results validate new targets for Alzheimer's disease therapy and demonstrate the utility of ASOs for the treatment of the disease.

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640 A combinitorial microRNA therapeutc approach to suppressing non-small cell lung cancer

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Lung cancer is the leading cause of cancers deaths worldwide, and current therapies fail to treat this disease in the majority of cases. The RAS and p53 pathways are two of the most frequently genetically modified pathways in lung cancers. Alterations in both result in loss of responsiveness to current therapies leading to decreased patient survival. Because the microRNA, *mir-34* is a transcriptional target of p53, which is reduced in its expression in p53 null tumors, we hypothesized that targeting *Kras;p53* (*KP*) tumors with miR-34 would represent a powerful treatment option to suppress lung tumorigenesis. We characterized tumor progression in these mice following lung specific transgene activation observing mild hyperplasia 8 weeks post-transgene activation followed by carcinoma *in situ* and adenocarcinoma. Individual tumors were harvested and profiled for miRNA expression. p53 regulated miRNAs, including the miR-34 family, were down regulated, while oncogenic miRNAs, such as miR-155 and miR-21 were elevated. Epithelial cells from these tumors were harvested, cultured, and evaluated for miR-34 responsiveness. Transduction with miR-34 reduced proliferation and invasion. Based on these results we began two series of *in vivo* experiments. Firstly, miR-34 was evaluated as a tumor preventative agent. *KP* mice treated with miR-34 at the same time as activation of transgenes showed little evidence of tumorigenesis. The second series of *in vivo* experiments evaluated the ability of miR-34 to treat pre-formed tumors. While miR-34 was unable to reduce the preformed tumors in these animals it was able to prevent further growth. We have since advanced these findings using a combinatorial approach administering multiple candidate tumor-suppressive miRNAs in cell culture and *in vivo* using a systemic delivery vehicle already used in human patients. The results support the combinatorial use of miR-34 and *let-7*; half the dose of both resulted in a significantly enhanced effect relative to the full dose of either miR-34 or *let-7*. We show that the combination can increase survival of this aggressive model by over 40%. Preliminary results from these recent studies are encouraging a "first-in-human" Phase 1 clinical trial of miRNA therapeutics for lung cancer.

641 mRNA-ITGA4 transfection of mesenchymal stem cells to improve diapedesis for efficient cell delivery to the brain via a minimally invasive intravascular route

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Mesenchymal stem cell (MSC) transplantation has been explored as a new clinical approach to repair injured tissue, and an intra-arterial route has been proposed as a minimally invasive delivery method. With $\alpha_4\beta_1$ (VLA4) integrin likely involved in diapedesis, modulating expression of these molecules in the cell membrane is an attractive approach for enhancing parenchymal migration of intravascularly transplanted cells. The aim of our study was to overexpress the ITGA4 (α_4-subunit of VLA4 integrin) gene in MSC using mRNA-mediated transfection. Due to the superior safety, efficiency, and transient nature of the mRNA transfection, this method is well suited for this purpose. While there are reports of mRNA-induced gene expression, such large genes as ITGA4(3kb) have not yet been investigated.

Methods: A pSP72vector (P2191-Promega) with cloned ITGA4-gene cDNA was used as a template for mRNA production *in vitro* using the mMessage-mMachine®Kit (AM1344-Ambion) with a poly(A) tailing kit (AM1350-Ambion), or mMessage-mMachine®T7UltraKit (AM1345-Ambion) that included an anti-reverse-cap-analogue (ARCAcap). SSB-protein (S3917-Sigma) was used for mRNA stabilization. Transfection experiments were carried out in human MSC (PT2501-Lonza) and HEK293 cells. Lipofectamine®2000 (Invitrogen) was used as a transfection agent. RT-PCR was performed for mRNA-ITGA4 transfection efficacy. ITGA4-protein production was confirmed by immunocytochemistry.

Results: *In vitro* production of mRNA-ITGA4, as well as cellular delivery of this mRNA, was successful as confirmed by RT-PCR; however, no ITGA4-protein synthesis was detected. mRNA-ITGA4 stabilization by the SSB-protein resulted in ITGA4-protein production in HEK293 cells, but not in MSC. Further modification of mRNA-ITGA4, by introducing ARCAcap, resulted in detectable ITGA4-protein production in MSC. The ITGA4-protein distribution in MSC changed dynamically, with ITGA4-protein being gradually routed from the inner structures toward the cell membrane where it was detectable for up to 24h after transfection.

Conclusions: Cytoplasmic mRNA half-life and translation, rather than transfection efficiency, appear to be the major hurdles in exogenous mRNA-based protein production, and ARCAcap seems to be instrumental in overcoming that challenge. Ongoing studies will determine whether this short-lived ITGA4-protein presence in transfected MSC enables their migration from the vasculature to the parenchyma.

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642 In Silico design, in vitro characterization and in vivo delivery of multifunctional RNA-based nanoparticles

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Control over the concurrent delivery of different functionalities and their synchronized intracellular activation can significantly contribute to the fields of RNA and DNA biomedical nanotechnologies. We present several different, yet related methodologies depicting how one can computationally design, and experimentally assemble and deliver functionalized nucleic acid based nanoparticles that have single or multiple functionalities. We show, for example, how nanoscaffolds (nanorings) can be functionalized with multiple short interfering RNAs for combinatorial RNA interference, and also permit the simultaneous incorporation of assorted RNA aptamers, fluorescent dyes, proteins, as well as auto-recognizing RNA-DNA hybrids used to conditionally activate multiple split functionalities. These constructs were extensively characterized and visualized *in vitro*, in cell culture and *in vivo* by various experimental techniques. The results also revealed that the use of these functionalized RNA nanostructures promote high detection sensitivity in diseased cells and significant silencing efficiencies of targeted genes. Finally, we report a generalized methodology for the one-pot production of chemically modified functional RNA nanoparticles during *in vitro* transcription with T7 RNA polymerase. The efficiency of incorporation of 2′-fluoro-dNTP in the transcripts by the wild type T7 RNA polymerase dramatically increases in the presence of manganese ions, resulting in high-yield production of chemically modified RNA nanoparticles functionalized with siRNAs that are resistant to nucleases from human blood serum. Moreover, the unpurified transcription mixture can be used for functional *ex vivo* pilot experiments.

643 Formation of stress granules by anticancer drugs

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Stress granules (SGs) are cytoplasmic and dynamic bodies that serve as storage sites of untranslated mRNAs. SG formation is induced by various stresses including oxidative stress, heat shock, hypoxia and ionizing radiations. Since these stresses are known to inhibit translation initiation, SGs are thought to represent sites of repression of the translation of specific mRNAs. Moreover, SG formation allows the cell to survive by inhibiting apoptotic pathways via the sequestration of signaling molecules. In this study, we describe SG formation in cancer cells treated with anticancer drugs. We provide new details into the mechanism of SG induction by anticancer drugs and how SGs interfere with cancer cell death pathways through the sequestration and translation repression of specific mRNAs.

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644 Control of Stress Granules formation during caliciviruses infection

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Human norovirus (HuNV) is the major cause of gastroenteritis in the developed world, causing in 2012-13 over one million cases in the United Kingdom alone. Human norovirus (HuNV) is a member of the *Caliciviridae* family, with a (+) ssRNA genome containing a viral protein, VPg, linked to its 5′ end. Viruses recruit host ribosomes to translate viral mRNAs, gaining control of cellular translation factors and the signalling pathways regulating their activity. This ensures that viral proteins are produced, but also interfere with ongoing host protein synthesis thereby triggering and/or impairing innate responses of the infected host. To regulate host gene expression, viruses can also impact on the formation of RNA granules such as Stress Granules, where mRNAs are stalled, or P-bodies, where they are degraded. Previous studies on feline calicivirus (FCV) and murine norovirus 1 (MNV1), two prototype caliciviruses, demonstrated that the VPg directs translation by interacting with host translation factors. We now report an intricate relationship between caliciviruses and stress granules. We provide evidence that caliciviruses impair the formation of stress granules within infected cells, reflecting a survival strategy, while the infection foci impacts on the surrounding cells ability to form stress granules. Further evidence of global translational control during infection is given by polysomal profile analysis and by analysing the location of the viral RNA in intracellular compartments. Our findings support a model in which caliciviruses induce global translational control of the host during infection by modulating stress granules formation, to ensure survival within the host.

645 MicroRNA Regulation of Apolipoprotein B-100 mRNA Stability and Translational Control via 5′ and 3′ Untranslated Regions

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Hepatic apolipoprotein B-100 (apoB) synthesis and secretion appears to be regulated largely at the posttranscriptional and posttranslational levels. MicroRNAs are among posttranscriptional regulators of gene expression that bind to complementary sequences on target messenger RNA transcripts (mRNAs), usually resulting in translational repression or degradation. It is unknown whether specific miRNAs are involved in posttranscriptional regulation of apoB mRNA. We performed bioinformatic analysis, showing that two specific miRNAs with satisfactory E-values level (with lower levels indicating greater similarity between the input and its match) namely, miR-544 (E-value = 1.4) and miR-1202 (E-value=1.3) - have potential to interact with 3′ and 5′ UTR of apoB, respectively. We hypothesized that the interaction of these specific miRNAs (miR-544 and miR-1202) with the 3′ and 5′UTR of apoB mRNA leads to apoB mRNA translational repression and/or activation. Using a human hepatoma cell line model, HepG2, the effects of overexpressed miRNAs and inhibition of endogenous miRNAs on the expression of apoB mRNA and apoB protein synthesis were investigated. We further examined the effect of these miRNAs on apoB mRNA traffic into cytoplasmic P-bodies. Transfection of HepG2 cells with miR-544 led to a significant reduction in apoB mRNA expression and protein synthesis and induced an increase in the co-localization of apoB mRNA into P-bodies. The opposite effect was observed when anti-miR-544 was employed to inhibit the endogenous miR-544. In contrast to miR-544, miR-1202 overexpression induced an increase in apoB mRNA expression and protein synthesis. Similarly, the opposite effect was observed when using anti-miR-1202. Results from luciferase reporter assays indicated that the effects of miR-544 and miR-1202 may be mediated via interaction with the 3′ and 5′ UTR, respectively. Transfection of HepG2 cells with miR-5580, also predicted to target the 3′UTR (E-value = 0.79), did not appear to affect apoB mRNA expression. In summary, these data demonstrate that specific miRNAs are involved in the regulation of expression and translational control of apoB mRNA in hepatocytes. However, these miRNAs do not appear to mediate insulin regulation.

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646 Structural studies of the CCR4-NOT complex: CNOT1 recruitment and activation of the DDX6 ATPase

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The CCR4-NOT complex is a major deadenylation complex involved in cytoplasmic mRNA turnover. CCR4-NOT is also involved in translational repression, but the mechanisms are largely unknown. The CCR4-NOT complex is built around a large scaffolding protein, CNOT1. The central region of CNOT1 contains a MIF4G-like domain that interacts with the deadenylase CAF1, which in turn interacts with the deadenylase CCR4. MIF4G-like domains of other proteins have been shown to bind RNA-dependent ATPases of the DEAD-box family. Using pull-down assays from human cells and mass-spectrometry, we identified the DEAD-box protein DDX6 as an interacting partner of CNOT1 MIF4G. DDX6 (known as Dhh1 in yeast) is a translational inhibitor and mRNA decay factor.

To understand how DDX6 is recruited to CNOT1 and the implications of this interaction, we determined the structures of human DDX6 in complex with the CNOT1 MIF4G domain and of DDX6 in isolation. In the complex, DDX6 binds at the concave surface of CNOT1 MIF4G domain via conserved interactions. The interactions we observe rationalize why CNOT1 MIF4G does not bind related DEAD-box proteins, such as those of the eIF4A family. When bound to CNOT1, the conformation of DDX6 is similar to those previously reported for eIF4A-eIF4G and Dbp5-Gle1 (which represent activated ATPase states) but different from eIF4AIII-CWC22 (which represents an inhibited ATPase state). In *in vitro* assays, while isolated DDX6 had no significant RNA-dependent ATPase activity, addition of purified CNOT1 MIF4G stimulated ATP hydrolysis. The structure of DDX6 in isolation shows a closed conformation stabilized by intramolecular interactions, consistent with an inactive state of the ATPase. These findings show that CNOT1 activates DDX6 by modulating its conformation and might provide insights into the role of CCR4-NOT in post-transcriptional regulation in general.

647 Structural studies of the CCR4-NOT complex: the CNOT1-CNOT9 interaction and its tryptophan-binding pockets

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The CCR4-NOT complex has emerged as a central player in post-transcriptional regulation. The best studied function of CCR4-NOT relates to its action as a major deadenylase involved in shortening the poly(A) tail of cellular mRNAs, a key step in mRNA turnover. In addition, CCR4-NOT functions in mediating translational repression. In human cells, the CCR4-NOT complex is a key effector of miRNA-mediated translational repression, acting downstream of GW182/TNRC6 proteins.

CCR4-NOT contains a set evolutionary conserved proteins that are constitutive components of the complex in all species examined to date (yeast, humans, flies and trypanosoma). Among these are CNOT1 and CNOT9 (known as Caf40 in yeast). CNOT1 is a large multidomain protein that forms the binding platform for all other subunits of the complex. CNOT9 is an ARM-repeat protein, but its functions and the mechanism with which it is assembled within the complex are unclear.

We have determined the crystal structures of CNOT9/Caf40 in complex with the interacting region of CNOT1 from both yeast and humans. The structures reveal the remarkable evolutionary conservation of the interaction mechanism. Experiments performed in HEK293 cells indicate that this portion of CCR4-NOT interacts directly with GW182/TNRC6 proteins and that this interaction is dependent on GW motifs present in their silencing domain. By soaking tryptophan in CNOT1-CNOT9 crystals, we have identified two tryptophan-binding pockets in CNOT9 that we have validated using structure-based mutations in cell-based assays. These findings shows that CNOT9 has two pockets that synergistically bind tryptophan residues of the silencing domain of GW182/TNRC6 and provide insights into the repressive steps downstream of the GW182/TNRC6C proteins.

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648 Control of mRNA fate through dynamic regulation of DEAD-box helicases

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Cellular identity, function, and physiology are all determined by 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 mRNP is facilitated and controlled during gene expression. We hypothesize that members of the DEAD-box protein family, which have the ability to modulate RNA-RNA and RNA-protein interactions, are critical players in this control. DEAD-box proteins have been linked to virtually every aspect of RNA biology, but in many cases their cellular mechanism and particularly, their regulation are not fully defined. As a model for DEAD-box protein regulation, we have focused on Ded1, which has important roles in translation initiation and was recently show to be frequently mutated in medulloblastoma. We have previously shown that Gle1, a DEAD-box regulatory factor, inhibits Ded1 enzymatic activity during initiation. We have expanded these studies to show that the rate of translation is highly sensitive to Ded1 activity and that Gle1 modulates Ded1 to keep its activity at appropriate levels. Furthermore, Ded1 has been identified in proteomics studies as post-translationally modified, and we are conducting studies to examine this mode of regulation of Ded1 as well. This work will establish new paradigms in the control of RNPs during gene expression and contribute to a better understanding of the role of RNA biology in cancer.

649 Identification of inhibitory codon pairs that modulate translation in yeast

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Translation is modulated by the choice of synonymous codons, with a subset of 25 codons strongly selected in highly expressed genes in the yeast Saccharomyces cerevisiae. However, the mechanisms by which codons modulate translation remain elusive, since neither the identity nor properties of codons that confer poor translation are known. In a systematic analysis of 59 sense codons [1], we found that the arginine CGA codon is strongly inhibitory due to wobble decoding. Furthermore, CGA-CGA codon pairs have a synergistic inhibitory effect, implying that codon pairs modulate translation efficiency. Thus, it is likely that some combinations of non-identical codons also cause reduced expression.

To find inhibitory codon pairs, we generated libraries of superfolder GFP carrying insertions of three codons in the 5′ coding region [2], and subjected yeast with these integrated variants to fluorescence-activated cell sorting. The fluorescence score for each variant is calculated from deep sequencing of the variants in each bin; the sequence reads for each variant, reports on the distribution of cells with that variant in the bins, a method similar to that used to analyze protein function [3].

We identified inhibitory codon pairs based on the expectation that inhibitory pairs cause reduced GFP in most variants containing that pair relative to variants encoded with synonymous optimal codons. We have identified 12 strongly inhibitory codon pairs, including the known CGA-CGA codon pair, and have both experimental and computational evidence substantiating the inhibitory effects of these pairs. CGA codons are enriched in this set of inhibitory pairs.

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650 DDX3 Promotes Cancer Cell Metastasis Through Activating Rac1 Translation

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DDX3 is a DEAD-box RNA helicase involved in multiple steps of gene expression. DDX3 is upregulated in various cancers and cancer stem cells, and is considered as a biomarker of metastasis in squamous cell/ adenosquamous carcinomas. High frequency of oncogenic DDX3 mutations has been discovered in the Wnt type of medulloblastoma. To reveal roles of DDX3 in metastasis, we analyzed phenotypes caused by DDX3 depletion in HEK293, cervical cancer HeLa and neuroblastoma N2A cell lines. In these cell lines, DDX3 depletion alters cell adhesion properties and diminishes cell migration and invasion. Moreover, DDX3 overexpression N2A cell lines show increased migration ability. Metastasis assays further demonstrate that DDX3 is required for efficient metastasis of cancer cells. These results suggest that DDX3 may play an oncogenic role in controlling cancer cell adhesion, migration and invasion/metastasis via modulating cytoskeleton organization. To uncover potential targets of DDX3 involved in cytoskeleton remodeling, we performed pathway analysis of DDX3 targets that are regulated at the level of mRNA translation, and identified multiple pathways involved Rac1 functions. Our following experiments demonstrate that DDX3 may activate Rac1 translation by resolving its 5′UTR secondary structure. Moreover, we performed qRT-PCR array analysis of cell motility genes and found that transactivation of the Wnt/β-catenin target genes was down-regulated in DDX3 knockdown cells. Our results further showed that DDX3 depletion decreases β-catenin stability and attenuates Wnt/β-catenin signaling, and that Rac1 can rescue β-catenin expression, cell adhesion, invasion and metastasis of DDX3 knockdown cells. Rac1 is a major factor of the Wnt PCP pathway that controls cell movement and also regulates the stability of β-catenin in the canonical Wnt pathway. Thus, our study unveils a molecular mechanism by which DDX3 controls cancer metastasis via regulating Rac1 and β-catenin signaling of cancer cells and supports DDX3 as a culprit in Wnt type tumors.

651 Ribosome footprinting demonstrates an important role for translational control in the orchestration of skeletal muscle differentiation

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Gene expression is regulated at several different levels. In higher organisms regulation at the level of translation is probably studied and understood least. Translational control includes the use of upstream open reading frames (uORFs) and alternative open reading frames (aORFs). uORFs are short reading frames located in the 5′ untranslated region of an mRNA, which are able to reduce the translation efficiency of the primary open reading frame, or to completely prevent its translation. aORFs cover the normal coding region of an mRNA, and lead to the formation of other protein isoforms, generally differing in their localization or their biological function.

Ribosome footprinting is a genome wide high throughput sequencing technology monitoring translation at nucleotide resolution. We simplified the ribosome footprinting protocol and developed a novel data analysis pipeline to identify translation start sites, including aORFs and uORFs, as well as to quantify translation activity as an estimation of protein expression levels. We used our approach to investigate changes in translation during differentiation of C2C12 myoblasts cells, a model for skeletal muscle differentiation. We discovered many new, not annotated translation start sites, both for uORFs and aORFs. uORFs were enriched in non-AUG start codons, with the majority leading to stop codons before the known, annotated translation start site. We identified 315 genes that, during myogenic differentiation, switch to another translation start. These genes mainly cluster in two biological pathways, ribosome biogenesis and calcium signaling. The same pathways demonstrated a large discrepancy between total RNA and ribosome-associated RNA, as found by comparing RNA-seq and ribosome footprinting data. This highlights the importance of regulation at the level of translation and the role of uORFs in the control of protein expression during myogenesis. In conclusion, we show that the orchestration of skeletal muscle differentiation is multilayered and controlled at the level of transcription, RNA processing and translation.

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652 NMR-structure of PTBP1-RRM2 in complex with EMCV-IRES-domain F

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Internal ribosomal entry sites (IRESes) are large RNA structures present in the 5′ untranslated region (5′ UTR) of many viral and some cellular mRNAs (1). Unlike canonical translation initiation, IRES mediated translation initiation is 5′ cap independent but often requires cellular IRES trans-acting factors (ITAFs). The polypyrimidine tract binding protein 1 (PTBP1) with its four RNA recognition motifs (RRMs) is among the most frequently found ITAFs. The two N-terminal RRMs, RRM1 and RRM2, act independently whereas the two C-terminal RRMs interact with each other, thereby orienting their RNA-binding surfaces in opposite directions (2). Thus, PTBP1 might reorganize its RNA target and modulate the IRES activity by stabilization of its structure (3).

PTBP1 binding sites were mapped on the IRES of encephalomyocarditis virus (EMCV) by hydroxyl radical probing (4). It was found that two molecules of PTBP1 bind to the IRES of encephalomyocarditis virus (EMCV) in a distinct orientation: One PTBP1-molecule binds the IRES-domains H-L and the second one the domains D-F. The footprinting data suggested that RRM1 recognizes the gUCUUUu-pentaloop of the IRES-domain F and RRM2 binds its regular stem. We investigated the binding of RRM1, RRM2 and RRM12 by NMR titrations. Our NMR-data show that RRM12 bind the isolated domain F with RRM2 contacting the loop. We solved a preliminary structure of RRM2 bound to domain F: RRM2 binds the CU(U) U-motif present in the loop which is in agreement with its previously described binding motif, CU(N)Y(5). In addition, the loop-closing uracil is stacking to the sidechain of K271 and appears to be specifically recognized by hydrogen-bonding to the backbone amide-proton of T217. Hence, PTBP1-RRM2 binds an heptaloop.

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653 Embryonic stem cells control translation initiation to bypass mRNA upstream open-reading frames

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Embryonic and induced pluripotent stem cells require core transcription factors and exogenous growth factors to maintain pluripotency. MiRNAs and other RNA-binding proteins regulate pluripotency, but a role for general translational control is less defined. This is a particularly relevant question to stem cell biology since growth factors are known translational regulators. Here, we define how upstream open-reading frames (uORFs) function in embryonic stem cells (ESCs). uORFs are well-documented translation inhibitors, and we demonstrate that mammalian ESCs phosphorylate the translation initiation factor eIF2α to bypass uORFs in favor of downstream coding reading frames (CDs). We make these observations first with Nanog and c-Myc, two known pluripotency markers, and use ribosomal profiling to extend our findings to the genomic level. Furthermore, we demonstrate that uORF bypass shifts to uORF translation at an early step in differentiation. We also show that Nanog and c-Myc expression can be prolonged by chemically inducing eIF2α phosphorylation. In fact, mouse and human ESCs both upregulate phosphorylated eIF2α by activating the eIF2α kinase, protein kinase R (PKR), and downregulating the eIF2α phosphatase, constitutive repressor of eIF2α phosphorylation (CReP). Human ESCs and mouse ESCs require different growth factors, but we find that both ESC types maintain pluripotency by increasing uORF bypass.

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654 Rapid Kinetics of Iron Responsive Element (IRE) RNA/Iron Regulatory Protein1 and IRE-RNA/eIF4F Complexes Respond Differently to Metal Ions

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Metal ion binding was previously shown to destabilize IRE-RNA/IRP1 equilibria and enhanced IRE-RNA/eIF4F equilibria. In order to understand the relative importance of kinetics and stability, we now report rapid rates of protein/RNA complex assembly and dissociation for two IRE-RNAs with IRP1, and quantitatively different metal ion response kinetics that coincide with the different iron responses *in vivo*. k_{on}, for FRT IRE-RNA binding to IRP1 was eight times faster than ACO2 IRE-RNA. Mn^{2+} decreased k_{on} and increased k_{off} for IRP1 binding to both FRT and ACO2 IRE-RNA, with a larger effect for FRT IRE-RNA. In order to further understand IRE-mRNA regulation in terms of kinetics and stability, eIF4F kinetics with FRT IRE-RNA were determined. k_{on} for eIF4F binding to FRT IRE-RNA in the absence of metal ions was 5-times slower than the IRP1 binding to FRT IRE-RNA. Mn^{2+} increased the association rate for eIF4F binding to FRT IRE-RNA, so that at 50 μM Mn^{2+} eIF4F bound more than 3-times faster than IRP1. IRP1/IRE-RNA complex has a much shorter life-time than the eIF4F/IRE-RNA complex, which suggests that both rate of assembly and stability of the complexes are important, and that allows this regulatory system to respond rapidly to change in cellular iron.

655 Elongation Factor 1A binds the 3′ Translation Element of the Barley Yellow Dwarf Virus

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The Barley Yellow Dwarf Virus (BYDV) is especially devastating due to its global widespread distribution and its infection of staple cereal crops. Our research aims to understand mechanisms of BYDV mRNA translation to gain insight into combating the disease and its dire economic implications. Previous studies have shown that BYDV initiates translation in a way that is unique to most eukaryotic messages. Almost all eukaryotic mRNA consist of a 5′ cap and initiates translation at the 5′ untranslated region (UTR). BYDV mRNA, however, consists of a cap-independent translation element at the 3′ UTR (BTE). More interestingly, eukaryotic initiation factor 4F (eI4F) binds the 3′ end of BYDB mRNA, at the BTE, and translation occurs when the 3′ UTR interacts with the 5′ UTR. Pull-down experiments using the BTE RNA as bait have revealed not only eIF4F binding but also a small array of other proteins not present when a nonfunctional BTE (BTEBF) or the 5′ UTR is used as bait. One prominent protein that appears to bind BTE but not the control BTEBF or 5′ UTR RNA is an unknown 50 kDa protein. Using Mass Spectrometry, we identify this protein to be elongation factor 1A (EF1A). EF1A has previously been shown to bind the 3′ UTR of Tobacco Etch Virus (TEV) mRNA and may have a role in in regulation and replication of the viral genes.

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656 *Toxoplasma gondii* encodes two eIF4E isoforms that differ in stress response

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Toxoplasma gondii is an obligate intracellular parasite that infects warm blooded animals including up to a third of the human population. It can exist as one of two forms, the rapidly growing tachyzoites or the encysted bradyzoite that persists for the life of the host. In response to stressful conditions, such as those initiated from the immune response, tachyzoites differentiate into bradyzoites. These cellular stresses induce a general translational repression mediated through the phosphorylation of eIF2 and lead to a reprogramming of the transcriptome. Another pathway commonly used to mediate translational repression occurs through the regulation of the cap binding protein eukaryotic initiation factor 4E (eIF4E). This pathway remains unexplored in *Toxoplasma*. At least two eIF4E isoforms are encoded in the *Toxoplasma* genome. Tg4EI encodes a 50kDa protein and is transcriptionally downregulated in bradyzoites while Tg4EII encodes a 26kDa protein whose cognate transcript is maintained throughout both stages. Interestingly, Tg4EI and Tg4EII differ primarily in their N- and C- termini, suggesting the ability to differentially recognize regulatory binding partners. Reports have suggested that organisms expressing multiple eIF4E isoforms can respond to stress and may be involved in differential mRNA translation. The involvement of Tg4E isoforms in stress response will be discussed.

657 Analysis of the 5′ untranslated region of human *UPF1* mRNA indicates both cryptic promoter and internal ribosome entry site activity

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Apart from its role in nonsense-mediated mRNA decay, a mechanism that promotes rapid degradation of transcripts carrying premature translation termination codons, the human up-frameshift 1 (UPF1) DNA and RNA helicase protein plays a crucial role in telomere replication and homeostasis, and in cell cycle progression. Due to its relevance for several physiological roles, and to the fact that it is expressed during G2/M phase, in which overall protein synthesis is reduced, we hypothesized that its translation may occur *via* an internal ribosome entry site (IRES). IRESs can occur at the 5′ untranslated region (UTR) of transcripts and allow the direct recruitment of the ribosome to the vicinity of the main AUG, therefore bypassing the need of scanning the entire UTR.

To test this hypothesis, we cloned the human *UPF1* 5′UTR in the dicistronic vector p_Renilla_Firefly and transfected HeLa cells with either this construct or the control counterparts. We observed a 15- to 25-fold increase in relative luciferase activity of the *UPF1* 5′UTR-containing construct compared to the levels obtained from the empty counterpart, which suggests the presence of an IRES. However, these levels of luciferase activity could be due to the presence of a cryptic promoter. Hence, we transfected cells with promoterless plasmids and observed a 20-fold increase in relative luciferase activity levels. These data demonstrate that *UPF1* 5′UTR contains a cryptic promoter, whose activity may be masking IRES activity. To check the IRES activity alone, we have transfected cells with *in vitro* transcribed, capped and polyadenylated mRNAs and observed a 2-fold increase in protein levels. This is also observed in two other cell lines. Besides, *UPF1* IRES activity is maintained under conditions of global protein synthesis inhibition. Deletional analysis of *UPF1* 5′UTR revealed that the first 50 nucleotides at the 5′ end of this region are essential for both cryptic promoter and IRES activity. These results evidence, for the first time, the existence of both a cryptic promoter and an IRES element within *UPF1* 5′UTR and provide new insights on the regulation of UPF1 expression in human cells.

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658 Hypoxia induces autophagy through translational up-regulation of lysosomal proteins in human colon cancer cells

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Colorectal cancer (CRC) is one of the most common cancers in humans. Every year, more than 1 million patients are diagnosed with CRC in the world. The incidence of CRC has been rising steadily in the last 20 years. Although the genetic process of carcinogenesis has been well documented, most patients with metastatic CRC still die within five years due to treatment failure. Thus, a better understanding of the molecular mechanisms underlying CRC progression may facilitate the development of new anti-cancer therapies. Hypoxia occurs in a wide variety of physiological and pathological conditions, including tumorigenesis. Tumor cells have to adapt to hypoxia by altering their gene expression and protein synthesis. Here, we show that hypoxia inhibits translation through activation of PERK and inactivation of mTOR in HCT116 cells. Prolonged hypoxia (1% O2, 16 h) dramatically inhibits general translation in HCT116 cells, yet selected mRNAs remain efficiently translated under such a condition. Using microarray analysis of polysome-associated mRNAs, we identified a large number of hypoxia-regulated genes at the translational level. Efficiently translated mRNAs during hypoxia were validated by sucrose gradient fractionation and quantitative real-time RT-PCR. Pathway enrichment analysis showed that many of the up-regulated genes are involved in lysosome, glycan and lipid metabolism, antigen presentation, cell adhesion, and remodeling of the extracellular matrix and cytoskeleton. The majority of down-regulated genes are involved in apoptosis, ubiquitin-mediated proteolysis, and oxidative phosphorylation. Further investigation revealed that hypoxia induces lysosomal autophagy and mitochondrial dysfunction through translational regulation in HCT116 cells. Moreover, the abundance of several translation factors involved in the mTOR and PERK signaling pathways is regulated by hypoxia. Our studies highlight the importance of translational regulation for tumor cell adaptation to hypoxia.

659 Determining the Role of Pea Enation Mosaic Virus (PEMV) mRNA Untranslated Region (UTR) in Translation Initiation

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5′ m^7GpppN cap and the 3′ poly adenosine (A) tail in two opposite ends of eukaryotic mRNAs are two key elements for their translation initiation machinery recruiting and mRNA circularizing. Unlike host mRNAs, many viruses lack those elements and yet they are translated efficiently. The most striking feature is the complex structures within their untranslated regions (UTR) that allow them to bypass some cellular translation control steps. In PEMV 3′ UTR, a cap independent translation element (3′ PTE) and a tRNA-like Structure (3′ TSS) were found to be essential for translation initiation. However, the detailed molecular mechanisms of PEMV 3′UTR-mediated translation initiation are poorly known. To understand these, we used fluorescence quenching and anisotropy techniques, gel shift binding assay and toe-printing experiment to investigate initiation factors (eIFs) and ribosome binding events. We found that initiation factors, eIF4E (K_d=~7.2nM) and eIF4F (K_d=~8.4nM) had high binding affinity with 3′ PTE. These binding affinities were as high as those for eIF4E and eIF4F binding with the cap analogue m^7GTP and much higher than those for m^7GpppG. We also demonstrated that eIF4A, eIF4B and Poly (A) tail binding protein (PABP) slightly increased the binding affinity of eIF4F with the 3′ PTE RNA (K_d<5nM). These binding affinities suggest the PTE element can sequester eIFs from the host cell. The mechanism of 3′TSS and 3′ PTE working together to recruit host ribosome remains unclear and is being investigated with gel shift binding assay, sucrose gradient sedimentation assay and Toe-printing assay.

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660 Global translational control and regulation of eIF4E activity during norovirus infection

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Human norovirus (HuNV) is the major cause of gastroenteritis in the developed world, in 2012-13 GII.4 strains were responsible for over one million cases in in the United Kingdom alone. Human norovirus (HuNV) is a member of the *Caliciviridae* family, with a (+) ssRNA genome containing a viral protein, VPg, linked to its 5′ end. As obligate intracellular parasites, viruses depend on the translation machinery of the host. Viruses recruit host ribosomes to translate viral mRNAs, gaining control of cellular translation factors and the host signalling pathways that regulate their activity. This not only ensures that viral proteins will be produced, but also interfere with ongoing host protein synthesis thereby triggering and/or impairing innate responses of the infected host. Previous studies on feline calicivirus (FCV) and murine norovirus 1 (MNV1), two prototype caliciviruses, demonstrated the VPg acts to direct translation by hijacking the host protein synthesis machinery.

We now report that MNV1 infection modulates the sequestration of eIF4E via the mTOR pathway, and that eIF4E phosphorylation is important for MNV1 replication. While Akt, mTOR and p70S6K are activated during MNV1 infection, the activity of the mTOR downstream target, 4E-BP1, regulating eIF4E availability, is impaired. Our results also show that the ERK1/2 and p38 MAPK pathways, that are responsible for the phosphorylation of eIF4E, are activated during MNV1 infection. These changes are important as the inhibition of mTOR or MAPK pathways impairs viral replication. Moreover, using polysomal profile analysis, we show that phosphorylated eIF4E relocates to the polysomes during infection and this is critical to the translation of a subset of mRNAs. Further evidence of global translational control will be provided by showing the dynamic regulation of stress granules formation during calicivirus infection.

Our findings support a model in which caliciviruses induce global translational control of the host during infection by modulating the mTOR and MAPK cell signalling pathways, and control stress granules formation, to ensure survival within the host.

661 Identification of an IRES element in the human mTOR transcript: its structural and functional features

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Mammalian target of rapamycin (mTOR) is a conserved serine/threonine kinase that integrates signals from the cellular nutrient- and energy-status, acting namely on the protein synthesis machinery. Deregulation of mTOR signaling is implicated in major diseases, such as cancer, mainly due to its role in regulating protein synthesis. The main mTOR targets are proteins responsible for ribosome recruitment to the mRNA, thus, a specific inhibitor of mTOR, for example rapamycin, leads to global inhibition of translation. Major advances are emerging regarding the regulators and effects of mTOR signaling pathway, however, regulation of mTOR gene expression, is not well known. Knowing that in stress conditions such as hypoxia, overall protein synthesis is reduced, but synthesis of mTOR protein is not inhibited, we hypothesized that mTOR 5′UTR harbors an IRES allowing cap-independent synthesis of mTOR protein in stress conditions. By using dicistronic reporter plasmids we have tested and confirmed this hypothesis. In addition, we have shown that IRES-dependent translation of mTOR is stimulated by hypoxia with associated eIF2α phosphorylation, in a manner that is independent of HIF1α induction per se. The anti- and pro-apoptotic outcomes of the unfolded protein response induced by endoplasmic reticulum stress also stimulates mTOR IRES activity, with a more pronounced effect in the pro-apoptotic phase with associated eIF2α phosphorylation. Furthermore, we have demonstrated that mTOR IRES activity is potentiated by mTORC1 inactivation, suggesting a feedback loop in order to maintain mTOR expression. Our data point out a novel regulatory mechanism of mTOR gene expression that integrates the protein profile rearrangement triggered by global translational inhibitory conditions.

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662 Regulation of mRNAs stability and translation upon heat shock in *Trypanosoma brucei*

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The life cycle of the human pathogen Trypanosoma brucei includes commuting between the homoeothermic mammal host and a poikilothermic invertebrate vector. Parasite adaptation to the temperature differences between the hosts (~37°-40°C and ~25°-35°C) demands tight control of heat-shock genes. However, gene expression in Kinetoplastids relies almost entirely on the post-transcriptional mechanisms. Thus, mRNA stability and translation efficiency are main controllers of heat-shock proteins levels.

We recently showed that ZC3H11, a zinc finger protein that binds to AU-rich elements, is required for the procyclic (insect) form heat-shock response. The level of ZC3H11 increases over 10-fold after heat shock, mostly because of increased protein stability and higher translation efficiency. Polysome profiling showed that under normal culture conditions bulk of ZC3H11 mRNA co-migrate with small ribosomal subunit suggesting either a block of start codon scanning or impaired large subunit binding. However, upon heat shock ZC3H11 mRNA moves to heavy polysomes and its 3′UTR is responsible for this effect. At the moment we are elucidating the exact mechanism of such translational control.

We have now conducted a transcriptome-wide study and have detected over hundred mRNAs that shift to polysomes upon heat shock of the procyclic form. As expected, that list of mRNAs was enriched for chaperones and co-factors required for protein refolding. Also there were few protein phosphatases and kinases, proteins involved in signaling and some RNA-binding proteins, including ZC3H11. These effectors of different pathways might be potential regulators of heat-shock response.

Interestingly, around 130 mRNAs (including ZC3H11 mRNA) that move to polysomes upon heat shock have long poly(AU) repeats (~20-30 nucleotides) within their 3′UTR. We found that these elements increase reporter mRNA abundance indicating their possible involvement in mRNA stabilization.

Currently we are testing the relevance of our top candidates for heat-shock response and investigating the mechanism for translational control and mRNA stability upon heat shock in details.

663 Defining the neuronal function of the polyadenosine RNA-binding protein ZC3H14

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RNA-binding proteins are tasked with the major responsibility of regulating the co- and posttranscriptional events of gene expression. Impairments to this process can result in a wide variety of human diseases including intellectual disability (mental retardation). Recently, our group identified inactivating mutations in the ZC3H14 gene, which encodes an evolutionarily conserved, RNA-binding protein, Zinc finger Cys_3His domain-containing protein number 14 (ZC3H14), that lead to non-syndromic intellectual disability. ZC3H14 is an RNA-binding protein that binds with high affinity to polyadenosine RNA. Although ZC3H14 is ubiquitously expressed, ZC3H14 mutant patients only display brain-specific phenotypes. ZC3H14 is expressed highly in hippocampal neurons compared to other regions of the brain, suggesting that these neurons may be more susceptible to loss of ZC3H14. While the steady-state localization of ZC3H14 is primarily nuclear, our recent studies reveal that a population of ZC3H14 is present in the axons of primary hippocampal neurons. We also found that ZC3H14 associates with 80S ribosomes. Taking advantage of the Drosophila model system, we performed a series of suppression screens with flies mutant for the ZC3H14 Drosophila orthologue, dNab2. These studies reveal functional interactions with two translation regulators FMRP and Ataxin-2, both of which have been linked to diseases of neuronal dysfunction. Taken together, these findings raise the possibility that ZC3H14 could play a role in modulating translation. We are exploiting a ZC3H14 knockout mouse to assess the requirement for ZC3H14 in translation and to define the spectrum of ZC3H14-interacting RNA regulatory factors. Ultimately, the information gained from the proposed research could provide insight into the molecular role of ZC3H14 and how ZC3H14 affects proper higher order brain function.

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664 Translational control of the human erythropoietin *via* an upstream open reading frame in cardiac tissue

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Cellular stress activates an integrated stress response, which includes rapid changes in global and gene-specific translation. Translational regulation of specific transcripts mostly occurs at translation initiation and is mediated *via* different *cis*-acting elements present in the mRNA 5′ untranslated region (5′UTR); these elements include upstream open reading frames (uORFs). uORFs modulate translation of the main ORF by decreasing the number and/or efficiency of scanning ribosomes to reinitiate at the start codon of the main ORF. However, in response to abnormal stimuli, they mediate translational derepression of stress-responsive proteins.

Erythropoietin (EPO) is the main hormone that regulates erythropoiesis. Beyond its well-known hematopoietic action, EPO has diverse cellular effects in non-hematopoietic tissues, including cardioprotection. Indeed, numerous experimental data in animal models of ischemia and acute myocardial infarct support the cardioprotective effects of EPO, which seem to be mediated by a local increase of EPO expression in response to the tissue injury. The 5′ leader sequence of the human EPO mRNA has one uORF with 14 codons that is conserved among different species, indicating its potential regulatory role. In the present work, we aimed to test whether EPO expression is translationally regulated in response to ischemia in cardiac tissue. Reporter constructs containing the normal or mutant EPO 5′ leader sequence fused to the Firefly luciferase cistron were tested in H9C2 (rat heart/myocardium myoblasts) and C2C12 (mouse muscle myoblasts) cell lines. Luciferase activity was measured by luminometry assays and the corresponding mRNA levels quantified by real-time RT-PCR. Results have revealed that the EPO uORF represses translation of the main ORF at about 60-70%, in both cell lines, and synthesis of EPO protein mainly occurs by reinitiation after uORF translation. In addition, our results show that specifically in H9C2 cells, the uORF-mediated translational repression is not affected by the presence of the EPO 3′-enhancer, while in C2C12 cells, the EPO 3′-enhancer induces a 4-fold increase in EPO expression. Nevertheless, in H9C2 and C2C12 cells under chemical ischemia, EPO uORF-mediated translation repression seems to be released. These findings show that cardioprotection effects of EPO might be regulated at the translational level.

665 HuD accelerates cap-dependent translation in a distinct way from PABP mediated translation stimulation

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Hu proteins are RNA-binding proteins that bind to adenosine-uridine (AU)-rich elements (AREs). Hu proteins affect almost every posttranscriptional aspect of RNA metabolism, including the control of mRNA translation. HuD is one member of the neuronal Hu family of proteins and promotes neuronal differentiation. We have previously shown that HuD enhances cap-dependent translation through a direct interaction with eIF4A and poly(A). We have also shown that the stimulatory effect of HuD on neurite outgrowth depends on the interaction of HuD with eIF4A- and poly(A). However, the underlying molecular mechanism(s) and interactions are poorly understood. In particular, little is known about the relationship between HuD and PABP on mRNA. The strategy of HuD to interact with eIF4As has some similarity to that of the PAIP-1, which binds eIF4A and stimulates cap-dependent translation. However, HuD contributes to cap dependent translation in a distinct way and not by stabilizing the interaction between eIF4G and PABP because HuD dose not bind to PABP directly.

Here, we depleted PABP from the translation extracts with or without HuD by PAIP-2 mediated affinity chromatography to directly evaluate the role of PABP for HuD-mediated translation stimulation. We observe that this leads to decreased levels of translation from cap - poly(A) mRNA but in the presence of HuD suggesting that HuD stimulates cap dependent translation independently of PABP. To address this hypothesis, we are currently performing in vitro translation and mRNA pull-down assays using HeLa cell extracts that are depleted of PABP. The status of these experiments will be discussed.

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666 Translational control of specific mRNAs is important for cellular survival and the anti-inflammatory feedback during macrophage activation

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Control of mRNA translation has emerged as critical mechanism by which gene expression is regulated. We analyzed the regulation of mRNA translation during the course of macrophage activation, which is characterized by a rapid and transient induction of pro-inflammatory factors followed by the delayed induction of anti-inflammatory cytokines. We tested the contribution of translational control by recording polysome profiles from RAW264.7 macrophages that were stimulated with lipopolysaccharide (LPS). At the global level, we found that polysomes increase in the early phase of activation, followed by a strong decrease in the late phase. Loss of polysomes in the late phase correlates with phosphorylation of eukaryotic translation initiation factor 2, a central regulator of translation initiation.

To identify individual mRNAs whose translation is specifically regulated during macrophage activation, mRNAs were isolated from different parts of the polysome profile and quantified by microarray analysis. Early after stimulation, strong activation at the level of translation was observed for inhibitors of the NFkB signaling pathway, Nfkbid (IkB-delta), Nfkbiz (IkB-zeta), Ier3 and Nr4a1. Posttranscriptional inhibitors of cytokine expression such as Zc3h12a (Regnase-1) and Zfp36 (TTP) were also among the translationally activated mRNAs. In resting cells, these mRNAs had a significantly lower polysome association than the average mRNA with a similar open reading frame length, suggesting that translational de-repression is the primary mechanism responsible for the regulation of these proteins. Genome-wide transcriptome analysis by RNA-Seq revealed a very high correlation between the expression profiles of Ier3 and TNF, and we could show that Ier3 is important for macrophage survival. Interestingly, many of the NFkB inhibitors were still de-repressed late after activation, indicating the importance of ongoing feedback inhibition for resolution of inflammation. In addition, translational activation in the late phase was observed for Nos2 (iNos) and Nox1, enzymes that mediate the respiratory burst. Taken together, our analysis reveals that translational control during macrophage activation is important for cellular survival, effector functions, as well as for feedback inhibition that promotes the resolution of inflammation. We are currently in the process of identifying cis-acting elements that control translation in macrophages, and will present our latest results at the meeting.

667 Human *Argonaute 1* 5′ untranslated region can mediate cap-independent translation initiation *via* an internal ribosome entry site

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Argonaute proteins (AGOs) are essential effectors in RNA-mediated gene silencing pathways. There are eight AGO-like proteins in human cells, grouped in two families: the eIF2C/AGO subfamily and the PIWI subfamily. The eIF2C1 gene encodes AGO1, a member of the former subfamily, that is ubiquitously expressed at low to medium levels and it is highly conserved during evolution reflecting its important physiological roles. Moreover, recent studies concluded that AGO1 protein is overexpressed in colorectal cancer, relative to adjacent non-cancer tissue, without a concomitant increase in mRNA levels. These pieces of evidence lead us to suspect that high AGO1 protein levels may be due to internal ribosome entry site (IRES)-mediated translation. IRESs are structures that can mediate cap-independent translation initiation by directly recruiting ribosomes to the AUG vicinity, thus skipping the scanning of the whole 5′ untranslated region (UTR), in response to stress. To confirm this hypothesis, we transiently transfected colorectal cancer HCT116 and cervical cancer HeLa cells with an AGO1 5′UTR-containing dicistronic vector, and luciferase activity was measured by luminometry assays. Results have shown a 2-fold increase in relative luciferase activity in both cell lines, when compared to the cells transfected with the empty counterpart ($P<0.05$). Transfection of the corresponding promoterless plasmids ruled out the hypothesis of this fold to be due to the existence of a cryptic promoter. In addition, RT-PCR analyses of the dicistronic mRNAs confirmed that no cryptic splicing occurs. Besides, the knock-down of the eIF4E subunit induced a significant 2- to 4-fold increase in IRES activity. Taken together, these data suggest the presence of an IRES in the AGO1 5′UTR whose biological relevance is still under thorough investigation.

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668 Sequence Specific Modulation of G-Quadruplex Folding

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G-quadruplexes (G4) are stable non canonical structures that can be adopted by guanine rich nucleic acids. Many important biological roles have been attributed to DNA and RNA G4. Indeed, RNA G4 motifs present in the UTR of mRNA have been shown to regulate translation. Hence, many researches have focused on targeting G4 with chemical compounds that specifically bind those structures and prevent or enhance their folding. Although these compounds are able to discriminate between G4 and other nucleic acid structures, they are not able to recognize one particular G4 sequence. Since there are more than 300 000 potential G4 sequences in the human genome, off-target effects remain a major issue. The aim of this project was to target specific G4 with short antisense oligonucleotides (ASO). The specificity is obtained using Watson-Crick base pairing. To ensure that there would not be any off-target effects, we focused on G4 harboring a particular topology: a long loop 2 (L2). 2′O-methylated and LNA ASO were used for their high affinity and great stability in human cells. Using in line probing, we showed that the ASO could modulate the folding of G4 positively or negatively, depending on where the ASO is binding. This modulation was obtained on artificial G4 sequences and on sequences present in the 5′-UTR of human genes. By inserting them in the 5′-UTR of a luciferase reporter, we also showed that L2 G4 can decrease translation in human cells. We showed that this translation inhibition can be increased or decreased by co-transfecting ASO with the luciferase gene. Among the targeted genes was a histone variant: h2ay. This gene has two isoforms, one of which contains a G4 in its 5′-UTR. The ratio of the isoforms has been shown to change when the cells enter senescence and during some cancer cells differentiation. By targeting the G4 present in the isoform 1, we were able either to increase or decrease its level of translation. This is the first report of targeting of a specific G4 and it also paves the way for a new kind of therapeutic tool to modulate specific gene expression.

669 Elimination of translational repressor ELAVL2 accompanies acquisition of developmental competence of mouse oocytes

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Full developmental competence appears in mouse antral follicle oocytes at the end of the growth phase. In the mouse, this event is marked by the transition from the so-called non-surrounded nucleolus (NSN) chromatin configuration into the transcriptionally quiescent surrounded nucleolus (SN) configuration, which is named after a prominent perinucleolar condensed chromatin ring. However, the SN chromatin configuration alone is not sufficient for determining the developmental competence of the SN oocyte. There are additional nuclear and cytoplasmic factors involved while a little is known about the changes occurring in the cytoplasm during the NSN/SN transition. Here, we report functional analysis of maternal ELAVL2, an AU-rich element binding protein. *Elavl2* gene encodes an oocyte-specific protein isoform (denoted ELAVL2^{O}), which acts as a translational repressor. ELAVL2^{O} is abundant in fully-grown NSN oocytes, is ablated during the NSN/SN transition, and remains low during the oocyte-to-embryo transition (OET). ELAVL2^{O} overexpression during meiotic maturation causes errors in chromosome segregation, indicating the significance of naturally reduced ELAVL2^{O} levels in SN oocytes. On the other hand, *Elavl2* expression is important for proper oocyte development, since its prematurely reduced expression in growing oocytes results in lower yields of fully-grown and meiotically matured oocytes. Moreover, *Elavl2* knockdown showed stimulating effects on translation in fully-grown oocytes. We propose that ELAVL2 has an ambivalent role in oocytes: it functions as a pleiotropic translational repressor in efficient production of fully-grown oocytes while its disposal during the NSN/SN transition contributes to the acquisition of full developmental competence.

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670 An oxygen-regulated switch in the cap-dependent translation machinery is required for the adaptation to hypoxia and the tumor microenvironment

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The initial step of protein synthesis consists of the eukaryotic translation initiation factor 4F (eIF4F) binding to the 7-methylguanosine (m^7-GpppG) 5′ cap of mRNAs. Low oxygen tension (hypoxia) represses cap-mediated translation by sequestering eIF4E, the cap-binding component of eIF4F, through mammalian target of rapamycin (mTOR)-dependent mechanisms. This raises a fundamental question in cellular biology as to how proteins are synthesized as part of the robust transcriptional response to hypoxia and eIF4E inhibition. Here, we uncover an oxygen-regulated translation initiation complex that mediates selective cap-dependent protein synthesis. Hypoxia stimulates the formation of a complex that includes the oxygen-regulated hypoxia-inducible factor 2α (HIF-2α), the RNA binding protein RBM4, and the cap-binding eIF4E2, an eIF4E homologue. PAR-CLIP analysis identified an RNA hypoxia response element (rHRE) that recruits this complex to a wide array mRNAs, including the epidermal growth factor receptor (EGFR). Once assembled at the rHRE, HIF-2α/RBM4/eIF4E2 captures the 5′cap and targets mRNAs to polysomes for active translation thereby evading hypoxia-induced repression of protein synthesis. Furthermore, a variety of cancer cell lines require this pathway to adapt to the hypoxic microenvironment en route to tumor formation. These findings demonstrate that cap-dependent translation is not ‘on’ or ‘off’ based on eIF4E activity, but that it can utilize functional translation initiation factor homologs to adapt to environmental stress for selective protein synthesis.

671 Fail-safe Mechanism of GCN4 Translation Control – uORF2 Promotes Reinitiation by Analogous Mechanism to uORF1 and thus Secures its Key Role in GCN4 expression

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One of extensively studied mechanisms of gene-specific translational regulation is reinitiation. It takes place on mRNAs where main ORF is preceded by upstream ORF (uORF). Even though uORFs generally down-regulate main ORF expression, specific uORFs exist that allow high level of downstream ORF expression. The key is their ability to retain 40S subunits on mRNA upon termination of their translation to resume scanning for the next AUG. Here we took advantage of the exemplary model system of reinitiation, the mRNA of yeast transcriptional activator GCN4 containing four short uORFs, and show that contrary to previous reports, not only the first but the first two of its uORFs allow efficient reinitiation. Strikingly, we demonstrate that they utilize a similar molecular mechanism relying on several cis-acting 5′ reinitiation-promoting elements, one of which they share, and the interaction with the a/TIF32 subunit of eIF3. Since a similar mechanism operates also on YAP1 uORF, our findings strongly suggest that basic principles of reinitiation are conserved. Furthermore, presence of two consecutive reinitiation-permissive uORFs followed by two reinitiation-non-permissive uORFs suggests that tightness of GCN4 translational control is ensured by a fail-safe mechanism that effectively prevents or triggers GCN4 expression under nutrient replete or deplete conditions, respectively.

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672 A blood pact: significance and implications of eIF4E in hematological diseases

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The initiation factor 4E (eIF4E) acts as a rate-limiting component of the eIF4F complex that assembles at the 5′ cap structure present in eukaryotic mRNAs: it binds to the m(7)G-containing cap and participates in recruitment of mRNA to the ribosome allowing translation initiation to take place. Beside its role as cap-binding protein, eIF4E also functions in other RNA processing events such as mRNA nucleocytoplasmic transport and its stabilization against decay in the cytosolic compartment. The activation of EIF4E is regarded as a key event in oncogenic transformation; its overexpression has been observed in many cancers and reported to play a significant role in the development and progression of hematological malignancies, at least in animal models. In the last decade, the advent of next-generation sequencing has greatly enhanced our understanding of the pathogenesis of lymphoid malignancies. Taking advantage of whole-genome and transcriptome sequencing techniques, we aimed to identify genetic modifications and changes in sequence that affect cellular pathways in T-cell acute lymphoblastic leukemia (T-ALL). Results of our quest for cap-translation inhibitors affecting the druggable 4EBP/eIF4E axis downstream of mTOR will be presented. In addition to providing novel insights into the leukemogenesis process, our studies have also identified potential new markers for diagnosis and/or therapeutic intervention, although the complexity of lymphoid malignancies demands future sequencing efforts to acquire a comprehensive understanding of its genetic basis.

673 Hypoxia-Activated HnRNP L Induces Mir-574-3p Dissociation from Polysomes and Promotes Oncogenesis

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MicroRNAs (miRNAs), heterogeneous nuclear ribonucleoproteins (hnRNPs), and protein-directed RNA switches are interconnected, posttranscriptional gene regulators that control a spectrum of cellular processes — including hypoxic responses, pluripotency induction, and leukemic oncogenesis. HnRNP L is a multifunctional protein that binds CA-rich elements (CARE) in pre-mRNAs and mRNAs to mediate alternative splicing and RNA switching, respectively. We have previously reported that hypoxia induces phosphorylation and cytoplasmic relocalization of hnRNP L in myeloid cells. Phospho-hnRNP L binds to the CARE in the 3′UTR of vascular endothelial growth factor-A (*VEGFA*) mRNA, blocks the binding of the GAIT (Gamma-interferon Activated Inhibitor of Translation) complex and miR-297-RISC (RNA-induced silencing complex), and thus redirects the *VEGFA* RNA switch to increase VEGFA translation. Here, we report a novel regulatory role of hnRNP L for inactivation of miRNA functions. We show that Src kinase-phosphorylated hnRNP L sponges CA-rich miR-574-3p, dissociates it from both Argonaute 2 (Ago2) and translating polysomes, thereby reversing its canonical RISC-dependent tumor suppressive activity. Mutagenesis-based structure-function studies reveal that hnRNP L binds the 3′-terminal CA-rich region of miR-574-3p but not the 5′-terminal seed sequence. Under hypoxic conditions, overexpression of miR-574-3p decoys cytoplasmic hnRNP L, preventing the *VEGFA* RNA switch and subsequent translational activation. Furthermore, we show that hnRNP L antagonizes miR-574-3p decoy activity and promotes tumorigenesis capacity of monocytic leukemia cells *in vivo*. Altogether, our results indicate that the interplay between hnRNP L and miR-574-3p, dependent on their stoichiometric ratio, regulates their activities under physiological, pathological, and therapeutic conditions. These studies reveal an alternative regulatory mechanism of defective miRNA function that leads to oncogenic gene upregulation, and provide a miRNA-based hnRNP-targeted therapeutic approach to cancer.

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674 DDX3 modulates neurite outgrowth via Rac1-mediated signaling pathway DDX3 modulates neurite outgrowth via Rac1-mediated signaling pathway

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The DEAD-box RNA helicase DDX3 is reported as a component of cytoplasmic RNA granules in neurons. To explore the role of DDX3 in neuron process, we used siRNA to deplete DDX3 in N2A neuroblastoma cells and rat primary cortical neurons. Knockdown of DDX3 significantly reduced the neurite length and growth cone ares in cortical neurons. It also decreased neurite outgrowth in N2A cells under differentiation condition. Moreover, we observed that depletion of DDX3 reduced Rac1 expression in cortical neurons and N2A cells. Over-expression of constitutive active but not wild type Rac1 was able to rescue the neurite outgrowth inhibition in DDX3-depleted N2A cells. Further analysis revealed that DDX3 was required for Rac1 translation as well as activation. We then identified that DDX3 activated both Rac1 and PKA translation through their 5′UTR in helicase-dependent manner. Our following experiments suggested that Forskolin was able to activate Rac1 to induce neurite outgrowth in N2A cells and cortical neurons in PKA-dependent manner. Knockdown of DDX3 diminished Forskolin-induced Rac1 activation and neurite outgrowth indicating that DDX3 was required for PKA-dependent Rac1 activation. Our immunostaining and local translation assays indicated that DDX3 might activate local translation of Rac1 and PKA in neurites through their 5′UTR sequences. Furthermore, bioinformatic analysis revealed that DDX3-activated translational targets shared sequence and structure signatures at their 5′UTRs. Pathway analysis of the mRNA targets and polysome profile confirmed that DDX3 regulated multiple factors involved in Rac1 pathways. In conclusion, our results suggested that DDX3 might be able to regulate a mRNA regulon (operon) composed functionally coherent genes involved in Rac1 signalling pathway. We speculated that this translational control of mRNA regulon might provide cells a way to control signalling strength in spatial and temporal axes.

675 Triticum Mosaic Virus 5′ leader acts as a bona fide internal ribosome entry site for translation

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Many RNA viruses rely on an internal ribosome entry site (IRES) element to maximize viral-encoded proteins. IRES elements recruit, by definition, ribosomes in a cap- and 5′ end-independent manner. Few plant viruses have been reported to rely on an IRES for their translation. In contrast to most animal viruses, plant IRESes rely on very short sequences, have no defined sequence requirement, are devoid of stable secondary structure and show maximal translational activity with an open 5′ end, suggesting potential organism-specific regulation of translation. Here, we investigated the potential IRES activity of the leader of the uncapped Triticum mosaic virus (TriMV, Potyviridae) RNA. The TriMV 5′ leader is able to drive cap-independent translation under limiting cap-binding protein, eIF4E. We found that optimal IRES activity requires the entire 739 nt leader, which by far exceeds the typical length of plant viral leaders. When compared to previously reported plant IRESes in the context of a bicistronic construct for the translation of a downstream gene, the TriMV IRES activity was clearly 5′ end-independent and retained maximal efficiency when ribosome entry from the 5′ end was blocked by a highly stable secondary structure. Its activity was 100 fold higher than prototypical plant viral IRESes. Sequence and structural analysis of the TriMV 5′ UTR revealed characteristical features of picornavirus IRESes, including stable structures, a polypyrimidine tract and multiple AUGs. Our preliminary results reveal that the TriMV 5′UTR appears to be the first bona fide plant viral IRES element.

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676 The RNA affinity landscape of the core eukaryotic translation factor eIF4G

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The initiation of eukaryotic translation requires the activity of more than a dozen initiation factors (eIFs) to recruit ribosomes to mRNA and begin the process of scanning and peptide synthesis. A key player in this process is eIF4G, a large scaffold protein that links the cap binding activity of eIF4E to the poly-A binding activity of PABP and the helicase activity of eIF4A, resulting in a circularized mRNA competent for ribosome recruitment. Recent studies have shown that eIF4G has several RNA-binding domains that can directly engage mRNA, and may play a role in recruiting eIF4G to a message, or in circularizing mRNA. Thus the RNA sequence specificity of eIF4G may contribute to the wide range of translation initiation rates observed for different mRNAs. To test this hypothesis, we are using RNA Bind and Seq , a newly developed method that can comprehensively determine the intrinsic RNA sequence affinity of a protein *in vitro* (1). We have applied this method to *S cerevisiae* eIF4G, and preliminary analysis suggests that yeast eIF4G1 has a preference for poly-U homopolymers, particularly in unstructured contexts. Experiments are now underway to confirm these preferences and determine their effects on translation.

(1) Lambert N, Robertson A, Jangi M, McGeary S, Sharp PA, Burge CB. Quantitative Analysis of Protein-RNA Binding Reveals Novel Regulatory Motifs and Impact of RNA Structure (submitted)

677 Determining the mechanism of action of the tumor suppressor snoRNA U50

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Small nucleolar RNAs (snoRNAs) are highly conserved non-coding RNAs with well-characterized molecular functions directing post-transcriptional modification of ribosomal RNA (rRNA). Unexpectedly, snoRNA U50 was recently identified as the tumor suppressor residing on chromosome 6 q14-q22, a genetic deletion frequently observed in a variety of cancers including breast and prostate cancer. U50 is a mammalian C/D Box snoRNA that directs 2′-O-methylation of two positions on the ribosome, C2848 and G2863. However, it is unclear how loss of snoRNA U50 affects cell proliferation and oncogenic transformation and whether these effects are downstream of defects in rRNA modification or represent uncharacterized functions of U50 in regulating cell growth. Here we show that multiple cancer cell lines have low U50 expression compared to non-transformed control cells. We further show that knockdown of U50 in non-cancer cells leads to increased proliferation, whereas overexpression of U50 in multiple prostate cancer cell lines reduces cell proliferation >3-fold. These in vitro results substantiate the identification of U50 as a bona fide tumor suppressor. Remarkably, decreased cell proliferation was detected only 8 hours after U50 overexpression, long before significant changes in U50-directed rRNA modification were observed. Furthermore, overexpression of point mutants in U50 incapable of directing rRNA target modifications still reduced cell proliferation when overexpressed, suggesting a novel function of snoRNA U50 that affects cell growth. To provide insight into key U50-regulated transcriptional nodes that potentiate altered cell proliferation, we performed RNA-Seq analysis 8, 12 and 16 hours after U50 overexpression. Gene set enrichment analysis revealed that target genes of the E2F1 transcription factor, which can function as either an oncogene or a tumor suppressor depending on context, were significantly upregulated. Work is ongoing to elucidate the mechanism by which U50 impacts E2F1 levels and/or activity and to determine the contribution of E2F1 misregulation to the carcinogenic effects of deleting U50. Together, these results reveal a novel function for snoRNA U50 in regulating cell proliferation by a mechanism that is distinct from the well-known role of U50 in directing rRNA modification.

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678 Analysis of RNA interactome of human RNA methylase ABH8

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ABH8 is a member of family of proteins homologous to bacterial Fe2+/2-oxoglutarate dependent dioxygenase AlkB. While AlkB is a DNA repair enzyme, its mammalian homologues (ABH1-8 and FTO) evolved variety of functions. The main known function of ABH8 is final methylation step during modification of uridines present in anticodon of specific tRNAs. Methyltransferase activity of ABH8 is conferred to the methyltransferase (MT) domain. However ABH8 also possesses RNA recognition motif (RRM) and an AlkB domain. Function of MT domain is well characterized, whereas the ile AlkB domain and RRM may contribute to previously uncharacterized RNA binding and thus putative novel functions.

In order to search for novel ABH8 substrates we prepared a human cell line expressing 3xFLAG tagged ABH8 and performed crosslinking and immunoprecipitation coupled to next generation sequencing (CLIP-seq). With high confidence our data shows that apart from known tRNA substrates ABH8 binds previously unknown tRNA substrates and also exonic regions in mRNAs. Whether these novel RNA targets harbour tRNA-like structures, other motifs or are methylated by ABH8 remains matter of further research. Importantly, since ABH8 was identified as one of the factors contributing to pathogenesis of cancer, better characterization of its functions and RNA interactome might help to understand the role of ABH8 during cancer progression.

Altogether, our data indicate that ABH8 protein may, apart from known tRNAs also bind range of mRNA substrates. The functional significance of this binding is the subject of our ongoing studies.

679 The identity of the discriminator base has an impact on CCA-addition

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CCA-adding enzymes synthesize and maintain the C-C-A sequence at the tRNA 3′-end, generating the attachment site for amino acids. While tRNAs are the most prominent substrates for this polymerase, CCA-addition on non-tRNA transcripts is described as well. To identify general features for substrate requirement, a pool of randomized transcripts was incubated with the human CCA-adding enzyme. Most of the RNAs accepted for CCA-addition carry an acceptor stem–like terminal structure, consistent with tRNA as the main substrate group for this enzyme. Most surprising, however, is the observation that the identity of the 3′-terminal nucleotide has a great impact on the efficiency of CCA incorporation. In tRNA, this position is described as discriminator base, an important identity element for correct aminoacylation by aminoacyl-tRNA synthetases. Mutational analysis of the impact of the discriminator identity on CCA-addition revealed that purine bases (with a preference for adenosine) are strongly favoured over pyrimidines. Furthermore, depending on the tRNA context, a cytosine discriminator can cause a dramatic number of misincorporations during CCA-addition. The data correlate with the frequencies of adenosine and cytosine residues at the discriminator position observed *in vivo*. Originally identified as prominent identity element for tRNA recognition by cognate aminoacyl-tRNA synthetases, the discriminator base represents a likewise important substrate recognition element for efficient and accurate CCA-addition by CCA-adding enzymes.

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680 Role of wobble uridine tRNA modifications in translational frame maintenance

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Maintaining the correct reading frame during protein synthesis is crucial as an alternation leads to incorporation of incorrect amino acids and truncated proteins. In order to maintain the correct reading frame, the translational machinery has evolved features to improve translational accuracy. Here we perform an *in vivo* assay addressing the role of tRNA wobble uridine (U34) modifications in sustaining the correct reading frame in *Saccharomyces cerevisiae*. We utilized a dual luciferase frameshifting assay [1] to investigate the effects of specific tRNA wobble uridine modifications, including 5-carbamoylmethyl (ncm^5), 5-methoxycarbonylmethyl (mcm^5), and 2-thio (s^2) groups, on the translational frame maintenance in yeast. Our preliminary results show that tRNA wobble uridine modifications play a vital role to improve the translational frame maintenance.

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681 An improvement of tRNAscan-SE: revised analysis of tRNA genes in the human genome more than doubles the complete set

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tRNAscan-SE [1] has been widely used for whole-genome tRNA gene prediction for more than fifteen years. The program was optimized for speed and sensitivity to detect domain-specific tRNA genes in genomes; for example, a eukaryotic-specific tRNA covariance model was employed to annotate mammalian genomes. The initial analysis of the human genome yielded slightly more than 600 tRNA and tRNA-like genes. More recently, nuclear mitochondrial sequences (NumtS), apparently derived from random insertions of fragmented mitochondrial genomes, have been identified in many eukaryotic nuclear genomes. Within the human nuclear genome, over 700 NumtS regions have been mapped [2]. To better detect these and other possible mitochondrial tRNAs not yet identified in the nuclear genomes, we integrated the latest version of Infernal (v1.1) [3] into tRNAscan-SE and employed mt-tRNA specific covariance models [4], along with traditional eukaryotic tRNA models. In the human genome alone, we identified 715 likely mt-tRNAs, 112 of which are located outside of the previously noted NumtS regions. These nuclear-encoded mt-tRNAs now outnumber the estimate of cytosolic tRNA genes.

To provide access to the more complete collections of human and all other species' tRNA gene sets, we are expanding the Genome tRNA Database [5] to include classifications of the newly detected nuclear mt-tRNAs, distinct from cytosolic tRNAs. We are also working with model species consortia to establish a new tRNA naming convention that will be more informative and stable between assembly updates. New tRNA gene names will be included in the Genome tRNA Database along with the legacy gene identifiers previously produced by tRNAscan-SE. These and other new enhancements of tRNAscan-SE and the Genome tRNA Database will provide researchers more accurate detection and more comprehensive annotation for over a million tRNA genes found in existing genomes.

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2. Simone, D., et al. BMC Genomics, 2011. 12:517.
3. Nawrocki, E.P. and S.R. Eddy. Bioinformatics, 2013. 29:2933-5.
4. Juhling, F., et al. Nucleic Acids Res, 2012. 40:2833-45.
5. Chan, P.P. and T.M. Lowe. Nucleic Acids Res, 2009. 37:D93-7.

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682 Alternative pre-mRNA splicing patterns are refractory to disruptions in ongoing Sm-snRNP supply in *Drosophila* larvae

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Sm-class small nuclear ribonucleoproteins (snRNPs) are core components of the spliceosome, which catalyzes pre-mRNA splicing. Previous studies in human cells suggest that changes in snRNP levels affect alternative pre-mRNA splicing (AS) patterns. To address whether or not such changes contribute to the normal regulation of AS, we sought to test the contribution of snRNP levels to AS patterns in a genetically tractable model system. Illumina RNA sequencing was performed on wild-type and snRNP biogenesis mutant *Drosophila* larvae using ribosomal RNA subtracted (total) RNA as well as on poly(A)-enriched RNA. Mutants studied here include *Phax (phosphorylated adapter of RNA export), Smn (survival motor neuron) and Ars2 (arsenite resistance 2)*. Phax and Ars2 are involved in the nuclear export of snRNAs, whereas SMN participates in the cytoplasmic assembly of snRNPs before they are imported into the nucleus to function in pre-mRNA splicing. Sm-class snRNPs levels were disrupted to various degrees in these mutants, as measured by snRNA levels. All of the mutants exhibited developmental delays relative to wild-type larvae, the timing of which could be determined through comparison of overall gene expression profiles with modENCODE developmental datasets. The gene expression profiles of the mutants clustered with late second to early third instar modENCODE data, whereas the wild-type had progressed to a later third instar stage. Despite containing decreased snRNA levels, none of the mutants displayed major changes in overall AS patterns. In fact, the biggest changes in AS were seen in the *Ars2* mutant, which showed the smallest decreases in overall snRNA levels. Some small changes in AS in a subset of pre-mRNAs were common among the different mutants. Ongoing work is aimed at determining the possible impact of these changes on developmental progression and the different phenotypic outcomes of the mutants.

683 Determinants of tRNA function and rapid tRNA decay in yeast

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tRNAs are under strong evolutionary pressure to ensure that the genetic code is correctly and efficiently interpreted for protein synthesis. However, it is only partly understood how tRNA sequence variation influences its structure, modification, and stability, affects translation fidelity, impacts the activity of isodecoders, and leads to human diseases. We report here the first high throughput quantitative analysis of a tRNA, using the model tRNA, $SUP4_{oc}$ of *Saccharomyces cerevisiae*. Using a GFP reporter (Dean, K. M. and Grayhack, E. J. (2012) RNA **18**:2335-2344) and a library of yeast strains carrying $SUP4_{oc}$ variants, we quantified the function of ~26,000 variants with one to three or more mutations.

We find that $SUP4_{oc}$ tRNA is highly tolerant to mutation, and exhibits a number of unexpected interactions between residues. We report examples of tertiary interactions that can slip by a base, loss of invariant tertiary interactions that can be accommodated by other interactions, long distance stabilization of mutations by other non-interacting mutations, and evidence for an alternative conformation centered around the hinge region of the tRNA, which is subject to conformational changes during ribosome passage. These data, although derived from a single tRNA species, lay the foundation for understanding other variations in tRNA sequence, such as those implicated in mitochondrial disease and the isodecoders of metazoans.

In addition, we used this approach to comprehensively define $SUP4_{oc}$ variants subject to the rapid tRNA decay (RTD) pathway, which targets specific tRNAs with a weakened acceptor/T-stem, or lacking certain stabilizing body modifications, resulting in 5′-3′ exonucleolytic degradation. We compared the function of a library of variants in an RTD mutant strain and a wild type strain, and find that RTD is much more prevalent than anticipated. RTD substrates have mutations throughout the tRNA, implying that RTD monitors the integrity of the entire tRNA molecule, and may involve additional cellular factors. Since this assay system measures the net contribution of all steps of tRNA biogenesis and translation, except fidelity, the approach of comparing $SUP4_{oc}$ variant function in wild type and mutant strains should be widely applicable to many problems in tRNA biology.

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684 Characterization of nematode-specific tRNAs (nev-tRNAs) that can decode an alternative genetic code

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Transfer RNAs (tRNAs) are one of the classical non-coding RNAs and play a crucial role in protein biosynthesis as the links between the codons and the amino acids. Although high translational fidelity is achieved by accurate tRNA aminoacylation and essential for protein and cell integrity, we have previously found unexpected nematode tRNAs (nev-tRNAs) that possess Gly (CCC) or Ile (UAU) anticodon but can be charged with leucine (1). An *in vitro* translation analysis also showed that nev-tRNAs is incorporated into eukaryotic ribosomes and participate in protein biosynthesis. However, its biological significance is still unclear.

Here, to examine whether nev-tRNAs are used in translation in *Caenorhabditis elegans*, we analyzed their maturation and subcellular localization. We first tried to determine the nucleotide sequence of 3′ CCA end which is one of the essential post-transcriptional tRNA modifications. As a result, the nucleotide sequence CCA was found at the 3′ end of both nev-tRNA^{Gly} (CCC) and nev-tRNA^{Ile} (UAU) by RT-PCR analysis followed by nucleotide sequencing. We next performed subcellualar fractionation studies using by differential centrifugation. The expression of nuclear markers; U6 small nuclear RNA and U3 small nucleolar RNA were enriched in nuclear fraction, whereas the majority of both nev-tRNAs were enriched in post-nuclear (cytoplasmic) fraction like cytoplasmic marker; tRNA^{iMet}. Common tRNA^{Gly} (UCC) and tRNA^{Ile} (UAU) were also detected in the cytoplasmic fraction. These findings suggest that nev-tRNAs are expressed, matured and exported to cytoplasm, and might compete with common tRNAs. Now, we are constructing an over-expression system of nev-tRNAs for a multi-omics approach. Possible functions of this RNA molecule will be discussed.

1. Hamashima, K. et al., (2012) *Nucleic Acids Res*, **40**, 3653-3662.

685 Conditional Mouse Models in the Study of the Regulation of RNA Polymerase I Transcription

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The eukaryotic ribosome is a 4-MDa complex assembled in the nucleolus, the largest subnuclear organelle, from four ribosomal RNAs (rRNAs) and ~82 ribosomal proteins (r-proteins). Several hundred other proteins and hundreds of small RNAs are also needed to assemble the ribosome. In growing cells, the rRNAs account for 35% to 60% of all gene transcription. As the manufacture of ribosomes is so energetically demanding and closely linked to cell growth, it is important to understand the mitogenic regulation of rRNA transcription.

Two important RNA Polymerase I (RPI) transcription factors have both been previously shown to be regulated by mitogenic stimulation, Upstream Binding Factor (UBF) and Transcription Initiating Factor 1A (TIF1A). UBF has been identified as a key architectural RPI transcription factor that most probably replaces histone chromatin on the active rRNA genes. It has also been shown to be the probable factor that regulates RPI elongation rates. TIF1A has been shown to be an essential initiation factor, interacting with RPI and SL1 forming the pre-initiation complex. Numerous growth pathway-regulated phosphorylation sites have been shown to be important for function of these factors *in vitro,* through ERK/MAPK, JNK, mTOR and CDK regulated pathways.

As a starting point to determine which posttranslational modifications of UBF and TIF1A regulate ribosome biogenesis we have studied the effects of deletion of the mouse UBF and TIF1A genes. Inactivation of the UBF gene arrests mouse development at the 8-cell morula stage and conditional inactivation of the UBF gene in cell culture leads to a rapid depletion of UBF protein, a shutdown of rRNA gene transcription and the arrest of cell proliferation. Concomitantly we observe major changes in rRNA gene and nucleolar structure. Contrary to previously published data, the TIF1A null embryos appear to arrest development earlier than E9.5 and the conditional cell lines do not easily excise the gene, leading to a partial knockout. Rescue experiments are being used to evaluate the *in vivo* requirements for UBF splice variants and UBF, and eventually TIF-IA, phosphorylation site mutants. Preliminary data confirm previous observations that suggest only UBF variant 1, not UBF variant 2, is essential.

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686 An archaeal RNA binding protein, FAU-1, is a novel ribonuclease involved in the processing of rRNAs

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Ribosomal RNAs are important non-coding RNAs in all organisms. It is reported that precursor rRNAs (pre-rRNAs) are processed to mature rRNAs by various ribonucleases (RNases). RNase E is one of the well-known endoribonucleases involved in the processing of rRNAs in *Escherichia coli*. However, in Archaea, enzymes responsible for the processing of rRNAs have not been clearly understood yet. Previously, we identified an RNA-binding protein called FAU-1 that consists of 472 amino acid residues in the hyperthermophilic archaeon *Pyrococcus furiosus*. We showed that the N-terminal half of the FAU-1 had a degree of similarity (25%) with RNase E from *E. coli* (Kanai *et al*., 2003). In the current research, we have detected an endoribonuclease activity of the FAU-1 protein.

Production of the recombinant FAU-1 protein with a His_6 tag sequence was induced in *E. coli*. We then purified FAU-1 to near homogeneity by His-affinity column chromatography, followed by RESOURCE-Q ion exchange column chromatography. Using the purified protein, it was tested whether FAU-1 was able to process *P. furiosus* pre-5S rRNA, one of the known RNase E substrates. As a result, accumulation of FAU-1-dependent cleavage product was observed *in vitro* by Northern blot. The cleavage site specificity of FAU-1 was also examined using a short RNA probe (73 nt) partially representing the *P. furiosus* pre-5S rRNA. We found that FAU-1 preferentially cleaved the UA-rich sequences of the RNA probe. These results suggest that FAU-1 could provide a pathway to process at least the pre-5S rRNA in archaea. Next, to investigate the role of FAU-1 *in vivo*, we constructed a deletion mutant of the *fau-1* gene in *Thermococcus kodakarensis*, which is a closely related species to *P. furiosus*. Consequently, the *fau-1* mutant was not lethal but a growth defect was observed. Currently, we are investigating the relationship between the growth defect and amounts of rRNAs in *Δfau-1* strains. The results will be discussed.

687 Precise mapping of mitochondrial and genomic tRNA-derived fragments (tRFs) in the development of *Triops cancriformis* (Tadpole shrimp)

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MicroRNAs are 18-24 nucleotide (nt) non-coding RNAs that are deeply involved in development, especially in morphogenesis. However, the detailed biological information about 25-45 nt long small RNAs remains unclear. In order to characterize 25-45 nt long small RNAs, we performed deep sequencing of small RNAs in each six developmental stage (egg, 1st-4th instar larvae, and adult) of *Triops cancriformis* (Tadpole shrimp), whose morphology dramatically changes during development. As a result, the length distribution of small RNA reads showed that 32 nt long small RNAs were specifically detected in adult stage. By the nucleotide sequence comparison between the 32 nt long reads and genomic DNA, it was found that these small RNAs were derived from genomic $tRNA^{Gly}$(GCC). To reveal overall features of these tRNA-derived fragments (tRFs), all small RNA sequences were compared with mitochondrial and genomic tRNA sequences. Consequently, we found that small RNAs were derived from mitochondrial and genomic tRNAs corresponding to at least 16 and 39 anticodons, respectively. Interestingly, the main regions of these tRFs in each host tRNA were different depending on their anticodon. Mitochondrial tRF^{Ser}(GCU) were abundantly produced from the 5′ half regions, while tRF^{Val}(UAC) were mainly produced from the 3′ end regions of the host tRNAs. In the case of genomic tRFs, highly abundant tRFs, tRF^{Gly}(GCC), tRF^{Gly}(CCC), tRF^{Glu}(CUC) and tRF^{Lys}(CUU), were derived from the 5′ half regions of the host tRNAs. Further analysis of tRF read counts in individual stages suggested that the expression of mitochondrial and genomic tRFs were altered in six developmental stages. For example, mitochondrial tRF^{Ser}(GCU) and genomic tRF^{Gly}(GCC) were highly detected in late larval and adult stages, respectively. Moreover, expression of genomic tRF^{Gly}(GCC) and tRF^{Lys}(CUU) were confirmed at adult stage of *T. cancriformis* by northern blot analysis. Taken together, our results suggest that mitochondrial and genomic tRFs are not random degradation products, but may have important role(s) in *T. cancriformis* development.

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688 Computational analysis of ribosomal RNA gene organization and their evolutionary divergence in Archaea

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Ribosomal RNAs (rRNAs) are the major components of ribosomes and their sequences are widely used for understanding evolutionary relationships in three domains of life, Bacteria, Archaea and Eukaryota. Since Archaea is isolated and identified from extreme environments such as hyperthermia and anoxia, which imitate the primordial Earth environment, it is considered an ideal model organism to understand the evolution and the origin of life. To date, over 150 archaeal complete genomes have been registered in NCBI database, however, our knowledge of the rRNA gene evolution is still limited. Here, we analyzed the patterns, localization, and organization of rRNA gene clusters in complete genomes to understand their evolutionary changes in Archaea.

First, we collected positional information on 779 rRNA genes from 148 complete archaeal genomes and analyzed the distance between adjacent rRNA genes. The distribution of the distances is bimodal with group I (around 10~10^3 bases, peak at 300 bases) and group II (around 10^4~5 x 10^6 bases, peak at 3 x 10^6 bases), where group I is considered to reflect the features of rRNA gene clusters. Although we found similar patterns of distributions in two major phyla of Archaea, Euryarchaeota and Crenarchaeota, the gene organization in clusters is very different. In Crenarchaeota, rRNA gene cluster mainly consist of 16S-23S, whereas in Euryarchaeota, they mainly consist of 16S-23S-5S. 5S rRNA genes are also found in Crenarchaeota but independently exist from other rRNA genes. Significantly, in the particular species in early diverged Euryarchaeota, Crenarchaeota-like (16S-23S clusters and 5S) and Euryarchaeota-like (16S-23S-5S clusters) patterns are coexisted. Moreover, the data also suggests that in late diverged Euryarchaeota, after the acquisition of a 16S-23S-5S rRNA gene cluster, the 16S-23S-5S cluster was duplicated in the genome, and/or 5S rRNA, which is located in the last position of the cluster, was subsequently duplicated. In this conference, I will discuss our molecular evolutionary analysis, focusing on the rRNA gene duplication and translocation, and propose a prototype of rRNA gene cluster.

689 Phenotypes and translational deficiencies of tRNA-i6A37 anticodon loop modification in yeast and human patient cells

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Isopentenylation of N6 of A37, immediately adjacent to the anticodon, is an ancient modification mediated by conserved tRNA isopentenyltransferases (IPTase); MiaA in *E. coli*, Mod5 in *S. cerevisiae*, Tit1 in *S. pombe* and TRIT1 in humans. We developed a facile detection assay and found i6A37 on different subsets of tRNAs, both cytosolic and mitochondrial, in different yeast and other species (*1,2*). Experiments in *S. pombe* using a reporter enzyme activity-sensitive codon swap assay showed that i6A37 increases a substrate tRNA's specific activity for decoding nearly 4 fold. Consistent with a role in promoting translational efficiency (more so than fidelity), the *S. pombe* mRNAs that are most highly enriched in the cognate codons are also most abundant, those for ribosomal proteins, translation factors and enzymes of energy metabolism (*3*). However, the cognate codon enrichment profile of human mRNAs is more complex. We found for *S. pombe* and human cells that while cytosolic tRNA substrates are efficiently modified with i6A37, the mitochondrial substrate-tRNAs are typically modified at only 25-50%. The phenotypes of *tit1-deletion S. pombe* can be rescued by human TRIT1, and some of the phenotypes can be rescued by over-expression of cytosolic cognate tRNAs. A pediatric patient presenting to the clinic with severe combined mitochondrial respiratory chain defects was subjected to whole-exome sequencing which identified a homozygous p.Arg323Gln mutation in *TRIT1*. Examination of pre-existing Mod5-tRNA cocrystal structures revealed that the homologous residue's side chain makes RNA backbone contacts with the anticodon stem of the substrate tRNA, somewhat removed from the active center. We show that the mutant enzyme is deficient for IPTase activity in vitro and deficient for rescue of *S. pombe tit1-deletion* phenotypes. The patient's cells are severely deficient in i^6A37 in cytosolic and mitochondrial tRNAs. Complete complementation of the i^6A37 deficiency of these tRNAs was achieved by transduction of patient fibroblasts with wild-type but not mutant TRIT1.

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2. T. N. Lamichhane, S. Mattijssen, R. J. Maraia, *Mol Cell Biol* **33**, 4900 (2013).
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690 Identification of Novel Determinants for the Rapid tRNA Decay Pathway in Yeast

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tRNA function is crucial to all cells and defects in tRNA function are associated with several neurological defects and mitochondrial diseases. In Saccharomyces cerevisiae, the quality of mature tRNA is monitored by the rapid tRNA decay (RTD) pathway in which the 5′-3′ exonucleases Rat1 and Xrn1 degrade certain hypomodified or destabilized tRNAs. The RTD pathway is suppressed by deletion of the gene encoding Met22 whose substrate has been shown to inhibit Rat1 and Xrn1 in vitro. Previous work on the RTD pathway has shown that the predicted stability of the combined acceptor and T-stem was a strong indicator for whether variants of the tRNASer family were susceptible to RTD mediated degradation (Whipple et al. Genes Dev 2011). However, the acceptor and T-stem stability does not accurately define RTD substrates in some other tRNA gene families.

Our lab generated a comprehensive library of variants of the ochre suppressing tRNA SUP4oc in both WT and met22Δ backgrounds to more thoroughly define determinants of the RTD pathway and to assess the effects of tRNA sequence variation on function (See Guy et al. Abstract). tRNA function of variants in both libraries was quantified by expression of GFPoc, which was analyzed by fluorescence activated cell sorting and deep-sequencing. This analysis yielded many RTD candidates in unexpected regions including the D-stem, anticodon stem, and anticodon loop. Most interesting were those with mutations in the anticodon stem-loop, which is remote from the acceptor stem and not predicted to have a large effect on 5′ accessibility.

These RTD candidates were reconstructed and analyzed by flow cytometry and poison primer extension to quantify tRNA levels in both met22Δ and WT backgrounds. Our results indicate that tRNA levels of novel RTD candidates are in fact decreased in WT backgrounds suggesting their degradation by the RTD pathway. These findings indicate that the RTD pathway may monitor the tRNA more holistically than previously thought and may be aided by some other yet undetermined component in its degradation of tRNA substrates.

691 Empirical fitness landscapes of the U3 snoRNA

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We present an experimental approach for high-throughput mapping of fitness effects of mutations in yeast cells. We use synthetic biology techniques to generate a library of random mutants of a gene of interest, and associate each mutant with a unique "barcode sequence". We then pool all the mutants together, and use next-generation sequencing to track the growth rate of each mutant, by comparing the frequencies of their barcodes in a competitive growth experiment.

We apply this method to the Saccharomyces cerevisiae U3 (snR17) gene. U3 is an essential gene encoding a small nucleolar box C/D snoRNA responsible for the cleavage of the primary rRNA transcript and for the formation of the "central pseudo-knot" in 18S rRNA. We synthesised library of random mutants of U3, transformed these mutants into a U3::PGAL-U3 strain, and measured the fitness of each mutant in conditions where the genomic copy of U3 was expressed (in Galactose), or repressed (in Glucose). Fitness measurements were highly reproducible between biological replicates and were confirmed by growth-curve analysis. The identity of U3 mutants significantly affected fitness on Glucose, but not on Galactose. On Glucose, certain mutations in U3 appear to be beneficial, but most were harmful. We will present a mathematical model that allows determining the fitness contributions of individual positions in U3, and of epistatic interactions between sites, based on the data collected.

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692 Structure and function effects of *E. coli* $tRNA^{Arg4}_{UCU}$ anticodon domain modifications

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Post-transcriptional modifications to nucleosides within the anticodon-stemloop (ASL) domain of transfer RNAs play a key role in translational regulation and gene expression, promoting accurate codon recognition and affecting tRNA stability and conformational dynamics. The *Escherichia coli* $tRNA^{Arg4}_{UCU}$, which decodes the rare codon AGA, contains four endogenous modifications within its ASL: 5-methylaminomethyluridine (mnm^5U_{34}), N^6-threonylcarbamoyladenosine (t^6A_{37}) and pseudouridine (Ψ_{40}), as well as a relatively uncommon 2-thiocytidine (s^2C_{32}). The structural and functional contributions of these modifications were assessed by a variety of biophysical and structural techniques for unmodified ASL^{Arg4}_{UCU}, singly modified ASL^{Arg4}_{UCU}-s^2C_{32} and triply-modified ASL^{Arg4}_{UCU}-mnm^5U_{34};t^6A_{37};Ψ_{40}. Codon-specific ribosome binding assays show that only the triply-modified ASL construct obtains an efficient codon-anticodon binding interaction. The single modification s^2C_{32}, although observed in the $tRNA^{Arg1,2}_{ICG}$ isoacceptors to inhibit wobble decoding, does not negate dual codon recognition of AGA and AGG in $tRNA^{Arg4}_{UCU}$; instead, biophysical studies indicate a possible role in the modulation of thermal stability of certain secondary structure elements. Structurally, NMR spectroscopy experiments reveal that the mnm^5U_{34};t^6A_{37};Ψ40 triple modification promotes adoption of the rigid loop conformation essential to codon-anticodon interaction, despite lacking the spectral characteristics of the canonical U-turn motif. These results suggest that the endogenous anticodon-stem loop modifications observed in *Escherichia coli* $tRNA^{Arg4}_{UCU}$ direct conformational dynamics toward a pre-structuring of the anticodon and are required to facilitate accurate and efficient codon recognition on the ribosome.

693 Identification of discrete classes of small nucleolar RNA featuring different ends and RNA binding protein dependency

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Small nucleolar RNAs (snoRNAs) are among the first discovered and most extensively studied group of small non-coding RNA. However, most previous studies have focused on a small subset of snoRNA that guide the methylation (C/D snoRNA) or pseudouridylation (H/ACA snoRNA) of ribosomal RNA (rRNA). In this study, we annotated the expression pattern of all box C/D snoRNA in both normal and cancer cell lines independent of their functions. The results indicate that box C/D snoRNA are expressed as two distinct forms differing in terms of their ends with respect to boxes C and D and the number of base pairing between their 3′ and 5′ termini. Both long and short forms of C/D snoRNAs are overexpressed in cancer cell lines but their overall end distribution is conserved across both normal and cancer cells lines. Surprisingly, the long forms are much more dependent than the short forms on the expression of the core snoRNP protein NOP58, which is thought to be essential for C/D snoRNA production. In contrast, a subset of short forms are dependent on the expression of the splicing factor RBFOX2. Further analysis of the potential secondary structure of both forms indicates that the k-turn motif required for the binding of NOP58 is less stable in the short form and thus is less likely to mature into a canonical snoRNP. Together the data suggest that C/D snoRNA are divided into at least two groups of RNA with distinct maturation and functional preference.

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694 Archaeal Elp3 Catalyzes tRNA Wobble Uridine Modification at C5 via a Radical Mechanism

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Approximately a quarter of cytoplasmic tRNAs in eukaryotic organisms have the wobble uridine (U34) modified at C5, which is critical for efficient and accurate protein translation. Genetic studies indicated that the eukaryotic Elongator complex carries out the central step of the modification, but the mechanism of the reaction is unknown. Here we show that a single protein, the archaeal homolog of the third subunit of the eukaryotic Elongator complex, is able to catalyze the same reaction. Our studies revealed that archaeal Elp3 generates a radical in the methyl group of the bound acetyl-CoA, which reacts with C5 of U34 in tRNA to form a C-C bond. Hydrolysis of the covalent CoA in tRNA completes the modification reaction. The mechanism of action by Elp3 represents an unprecedented application of acetyl-CoA for chemical transformations in living cells via a radical reaction of the methyl group of acetyl-CoA.

695 The exosome and its cofactors contribute to multiple steps of 18S rRNA maturation in *Arabidopsis thaliana*

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Maturation of 18S rRNA from the polycistronic rRNA precursor involves a number of conserved endonucleolytic cleavages. Recent studies in yeasts and human have now revealed that exoribonucleolytic degradation by RRP6, a cofactor of the nuclear exosome, can also contribute to processing or degradation of 18S precursors. Here, we investigate the impact of the exosome and its cofactors on 18S rRNA maturation in *Arabidopsis thaliana.*

We show that plants deficient in the core exosome, the exoribonuclease RRP44, or the nucleolar RNA helicase AtMTR4 accumulate different forms of both 3′- and 5′- extended 18S rRNA precursors. Downregulation of the core exosome or RRP44 is associated with accumulation of a 2.5 kb 18S precursor spanning from P to A3 processing sites. In *rrp41* mutants, these P-A3 precursors are frequently polyadenylated with long poly(A) tails up to 200 nucleotides. By contrast, loss of the RNA helicase AtMTR4 results in decreased levels of P-A3 and to increased levels of 5′ shortened P'-A3 fragments, which have no or only short oligo(A) tails. Preliminary results indicate that more than one poly(A) polymerase is involved in the addition of poly(A) tails to rRNA precursors prior to their degradation by the exosome. Moreover, loss of the exoribonuclease RRP6L2 is specifically associated with the accumulation of precursors that have mature 5′ ends but carry a 3′ extension of 20 nucleotides (18S-A2), indicating that 18S precursors undergo exoribonucleolytic trimming by RRP6L2. Interestingly, the 18S-A2 fragments that accumulate in *rrp6l2* mutants often carry short oligo(U) tails and can be detected in the cytoplasm.

Taken together, our data suggest that in addition to conserved endonucleolytic cleavages, 3′-5′ exoribonucleolytic degradation by the exosome and its cofactors contribute to different steps of 18S maturation in plants.

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696 Mitochondria Outer Membrane Proteins Are Required For The Proper Function And Localization Of tRNA Splicing Endonucleases

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tRNAs function to bring amino acids to ribosomes during protein synthesis. In yeast, splicing of pre-tRNAs is essential for the production of 10 families of tRNAs. Intron removal is catalyzed by the heterotetrameric tRNA splicing endonuclease (SEN) complex, which is located on the cytoplasmic surface of mitochondria. Sen2 and Sen34 are the catalytic subunits of the SEN complex, whereas Sen15 and Sen54 are thought to serve a structural role. However, how and why SEN subunits assemble on the surface of mitochondria is unknown. We previously showed that when SEN subunits are relocalized in the nucleus, tRNA splicing, nuclear export, and aminoacylation are normal, but cells are inviable. Thus, there is an unknown requirement for the SEN complex to reside on mitochondria surface. Our recent genome-wide screen to search for all yeast gene products involved in tRNA biology identified two mitochondrial proteins, Tom70 and Sam37. Deletion of *TOM70* and *SAM37* cause pre-tRNA splicing defects and the accumulation of end-matured, intron-containing tRNAs. Tom70 is a component of the translocase of the outer mitochondrial membrane (TOM) complex and Sam37 stabilizes the sorting and assembly machinery (SAM) complex which functions in inserting beta-barrel proteins into the mitochondria outer membrane. In *tom70Δ* cells, a portion of the Sen15 and Sen54 pools are relocated from the mitochondria surface to the cytoplasm, but Sen2 remains associated with mitochondria. In *sam37Δ* cells, Sen15 fails to maintain proper mitochondrial localization, but Sen54 is appropriately distributed to mitochondria. Thus, via direct or indirect interactions, Tom70 and Sam37 are required for the proper localization, assembly, and function of the SEN subunits on mitochondria. Our results show that appropriate assembly of the SEN complex proteins on mitochondria is necessary for efficient pre-tRNA splicing. These data provide the first information for how the SEN complex locates to and assembles on the mitochondrial surface.

697 Elimination of a persistent endosymbiont virus in Leishmania by RNAi

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Leishmania parasites are a genus of early-diverging single-celled eukaryotes that cause the disease leishmaniasis. Select strains of Leishmania (Viannia) parasites are known to harbor a double-stranded RNA virus of the Totiviridae family known as Leishmania RNA virus 1 (LRV1), the presence of which increases the severity of disease caused by the parasite in mammals. These viruses infect their parasite hosts persistently, and there is currently no reproducible published method to generate parasites lacking LRV1 from a line that contains the virus. Parasites of the Viannia subgenus have an active RNAi pathway, which we here exploit to generate LRV1-negative lines from strains originally containing the virus. Leishmania braziliensis and L. guyanensis parasites engineered to knock down LRV1 are virus-negative by immunostaining with an anti-capsid antibody and by qPCR. Consistent with naturally-occurring and spontaneously-arising LRV1-negative parasites, knockdown parasites result in the release of fewer cytokines during in vitro macrophage infections than do their LRV1-containing counterparts. This provides us with a method to generate LRV1-negative lines from any strain of LRV1-containing Leishmania with an active RNAi pathway for use in studying the effects of LRV1 on parasite biology or virulence. It also raises questions as to the interaction between LRV1 and the parasite RNAi pathway under natural (not genetically engineered) conditions. How the Leishmania RNAi pathway interacts with LRV1 under natural conditions, and whether parasite RNAi is likely to be antiviral, will be discussed.

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698 Harmine Inhibits HIV-1 Gene Expression Independent of Its Effects on DYRK1a Function

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Previous studies have demonstrated that HIV-1 RNA processing can be altered to suppress virus replication by modulating the activity/abundance of the SR or hnRNP proteins of the host cell. We were interested in assessing whether similar repression could also be achieved by altering the activity of host kinases known to regulate host cell RNA splicing such as DYRK1a. Genome wide association studies have recently identified variations in DYRK1a as being linked with altered HIV-1 replication. Consequently, we examined the effect of DYRK1a depletion or inhibition with harmine on HIV-1 RNA processing and gene expression in our experimental system. We observed that harmine was a potent inhibitor of HIV-1 gene expression, reducing levels of all viral proteins (Gag, Env, Tat) evaluated. Consistent with this response, harmine also reduced accumulation of all classes of HIV-1 RNAs and blocked export of viral genomic RNA to the cytoplasm. Subsequent tests confirmed anti-HIV-1 activity in the context of HIV-1 replication in PBMCs raising the prospect that this approach could offer an alternative to existing treatments for this infection. However, parallel experiments evaluating the effect of DYRK1a depletion revealed that reduction of DYRK1a levels by >90% had little to no effect on HIV-1 gene expression nor did it alter the ability of harmine to repress viral protein synthesis. Although harmine is also an inhibitor of monoamine oxidase, another inhibitor of this enzyme (moclobemide) failed to elicit a similar suppression of HIV-1 gene expression. Together, the data suggest that the action of harmine on HIV-1 replication is mediated by an unknown host factor and current efforts are directed at defining the host factor(s) mediating its effect.

699 Widespread Alternative Transcription Start Site Usage Expands the Human Cytomegalovirus Proteome

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Human cytomegalovirus (HCMV) is one of the largest viruses, with a double stranded DNA genome over 220 kilobases in size. The HCMV genome was originally predicted to encode approximately 150 open reading frames. However recent genome-wide transcriptomic and proteomic studies have revealed that HCMV possesses a much greater coding capacity than previously appreciated. Massively parallel sequencing studies have demonstrated that HCMV encodes at least 750 unique peptide-coding regions derived from an extensively spliced transcriptome. Based on this data we hypothesized that the viral genome extensively uses alternative transcription start sites (TSSs) to expand the HCMV proteome. However, the dense coding capacity of the viral genome prevents the accurate determination of HCMV transcript structure using standard RNA-seq approaches, as multiple overlapping transcripts are transcribed from both strands of the viral genome. CAPSEQ, a next-generation sequencing approach that precisely defines TSSs with nucleotide resolution, has been been used to define TSSs on a genome wide scale. CAPSEQ identifies TSSs without a priori knowledge and focuses sequencing reads on the 5′ end of transcripts, allowing distinct TSSs to be defined in transcriptionally complex sequences such as the HCMV genome. In this study we used CAPSEQ to identify HCMV TSS usage during distinct temporal stages of the virus lytic cycle. This approach faithfully identified previously annotated HCMV TSSs, and identified several hundred additional TSSs. Clustering of TSSs allowed for the definition of HCMV promoters and an evaluation of promoter architecture. Most viral promoters were of the narrow archetype, suggesting that most HCMV promoters respond to specific regulatory cues. Bioinformatic analysis of HCMV TSSs identified new motifs in the proximal promoters of HCMV genes that may underlie the temporal regulation of viral gene expression. By comparing the HCMV CAPSEQ data to previous ribosome profiling data, we identified novel promoters controlling the expression of recently identified novel coding regions of HCMV, suggesting that an unappreciated degree of transcriptional diversity underlies the extremely dense coding capacity of the HCMV genome. In sum our data suggest that alternative promoter usage significantly expands the HCMV proteome, allowing for the regulated expression of internal and antisense coding regions.

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700 Interplays between RNA binding proteins determine viral infection outcomes

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RNA binding proteins play important roles in antiviral innate immunity. Three RNA binding proteins, namely RIG-I, MDA5 and PACT, cooperate with each other to detect viral RNA species in host cells. Activation of these RNA binding proteins triggers the production of type I interferons which potently attenuate viral replication.

Interestingly, viruses also encode RNA binding proteins with various functions. In this study, we identified that two viral RNA binding proteins, Us11 from HSV-1 (herpes simplex virus type-1) and ORF-4a from MERS (Middle East respiratory syndrome) coronavirus, antagonize the induction of interferons by interacting with cellular RNA binding protein PACT.

Both Us11 and ORF-4a bind with PACT tightly but not with RIG-I and this interaction prevents PACT from activating RIG-I. Us11 deficient HSV-1 virus induces IFN-β more potently than Us11 expressing virus, highlighting the importance of Us11 in immune evasion. More importantly, this difference was not observed in PACT knockout cells, suggesting that the function of Us11 is mediated through PACT. MERS-CoV ORF-4a protein inhibits PACT-induced activation of RIG-I and MDA5, but not the activity of downstream effectors, including RIG-I, MDA5, MAVS, TBK1 and IRF3.

Taken together, our findings suggest a novel function of viral and cellular RNA binding proteins. Viruses employ viral RNA binding proteins to circumvent innate antiviral responses by binding to cellular RNA binding protein PACT and perturbing its function. Recent findings from us and others further suggest that additional viral RNA binding proteins, including Ebola virus VP35 and NS1 from influenza A virus, function in a similar way to suppress innate immunity. Thus, PACT targeting represents a novel viral strategy to counteract host defense.

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701 Identification of RNA partners of viral proteins in infected cells

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RNA viruses exhibit small-sized genomes encoding few proteins, but still establish complex networks of protein-protein and RNA-protein interactions within a cell to achieve efficient replication and spreading. Deciphering these interactions is essential to reach a comprehensive understanding of the viral infection process. To study RNA-protein complexes directly in infected cells, we developed a new approach based on recombinant viruses expressing tagged viral proteins that were purified together with their specific RNA partners. High-throughput sequencing was then used to identify these RNA molecules. As a proof of principle, this method was applied to measles virus nucleoprotein (MV-N). It revealed that in addition to full-length genomes, MV-N specifically interacted with a unique population of 5′ copy-back defective interfering RNA genomes that we characterized. Such RNA molecules were able to induce strong activation of interferon-stimulated response element promoter preferentially *via* the cytoplasmic pattern recognition receptor RIG-I protein, demonstrating their biological functionality. Thus, this method provides a new platform to explore biologically active RNA-protein networks that viruses establish within infected cells.

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702 Quantitative assessment of influenza's cap-snatching repertoire by RNA sequencing

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Influenza cleaves host mRNAs ~10-13 nucleotides downstream of their 5′ ends and uses the resulting capped fragment to prime viral mRNA synthesis. Previous studies have suggested that influenza preferentially targets a subset of mRNAs as substrates, but these studies rely on low-throughput cloning and sequencing techniques. To quantitatively assess the targets of influenza cap-snatching, we developed two high-throughput methods that specifically sequence the 5′ ends of influenza viral mRNAs. We found clear evidence for the prime-and-realign hypothesis of viral transcription initiation and that the extent of realignment was strongly influenced by the viral template sequence. In addition, we developed a bioinformatic method to identify the targeted host transcripts despite the limited information content in the capped fragments. This method revealed that a subset of U snRNAs were by far the most abundant cap-snatching substrates. This study underscores the diversity of influenza's cap-snatching repertoire, showing that noncoding RNAs as well as mRNAs are used to make viral RNAs.

703 Conserved features of an RNA promoter for RNA Polymerase II determined from high-throughput sequencing of a population of hepatitis *delta* virus

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The hepatitis *delta* virus (HDV) is an RNA pathogen that uses human RNA polymerase II (RNAP II) for its replication. Previous studies identified the right terminal domain of genomic HDV RNA as an RNAP II promoter. In order to explore the features of this region, we developed a pipeline to filter, align and analyze sequence conservation and covariation of this region from data obtained by high-throughput 454 sequencing of an HDV population actively replicating in human cells. We generated 473,139 sequences representing 2,351 new HDV variants and demonstrated that this region of HDV accumulates as a population of different sequences. Despite sequence heterogeneity, our analyses revealed the conservation of the rod-like conformation of this region and identified conserved nucleotides at the tip of the rod-like structure, near the proposed transcription initiation site. These features, which are also conserved in sequences from HDV variants isolated from various hosts, are likely important to act as RNA promoter for RNAP II during HDV replication.

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704 Selection of an Antiviral RNA Aptamer against Ebola Virus Glycoprotein

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The filoviruses, Ebola (EBOV) and Marburg (MARV), cause periodic hemorrhagic fever, and high rates of mortality and transmissibility in human population are associated with these viruses. No anti-viral therapies are currently available against these viruses. Recent studies suggest that reduction in virus load during the infection has a significant impact on deleterious outcomes. Thus, a transient reduction of virus load may be quite effective at decreasing mortality associated with filovirus infection. My PhD work involves developing an RNA Aptamer against the EBOV glycoprotein GP2 ectodomain. Aptamer binding to EBOV GP2 is predicted to prevent virus fusion with cellular membranes thereby inhibiting virus entry into permissive cells. This antiviral therapy could be an effective stop gap measure against the sporadic outbreaks of this virus. An alternative use of the aptamer is as a sensor to quantify the EBOV virus in biological samples.

705 Real time analysis of HIV-1 transcription with single polymerase sensitivity

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Transcription of HIV-1 is subjected to stochastic variations that have an important impact on viral progression, leading either to acute infection or to transcriptional latency. To precisely measure HIV transcription in living cells, we have developed a system based on improved MS2-repeats that allow single polymerase visualization. We found that HIV-1 is transcribed in a discontinuous burst like fashion. The intensity, duration and frequency of transcriptional activation events of an HIV-1 reporter gene were determined with a high temporal resolution. Transcription is achieved by polymerase trains composed by a high number of very closely spaced polymerases. The exact initiation rate, spacing and 3′ processing time of individual polymerases within a train were also measured.

By analyzing mutant HIV-1 reporters with decreased TBP-binding, we show that the stable assembly of the Pre Initiation Complex (PIC) on the promoter can regulate the activation events of the gene. Tat levels could also be determinant by stabilizing the PIC or inducing the release of promoter proximal paused Pol II.

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706 The Lsm1-7-Pat1 complex promotes viral RNA translation and replication by differential mechanisms

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The Lsm1-7-Pat1 complex binds to the 3′end of cellular mRNAs and promotes 3′end protection and 5′-3′ decay. Interestingly, this complex also specifically binds to *cis*-acting regulatory sequences of viral positive-strand-RNA genomes, and promotes their translation and subsequent recruitment from translation to replication. Yet, how Lsm1-7-Pat1 complexes regulate these two processes remains elusive. Here, we show that Lsm1-7-Pat1 complexes act differentially in these processes. By using a collection of well-characterized *lsm1* mutant alleles and a system that allows the replication of Brome mosaic virus (BMV) in yeast we show that the Lsm1-7-Pat1 complex integrity is essential for both, translation and recruitment. However, the intrinsic RNA-binding ability of the complex is only required for translation. Consistent with an RNA binding-independent function of the Lsm1-7-Pat1 complex on BMV RNA recruitment, we show that the BMV 1a protein, the sole viral protein required for recruitment, interacts with this complex in an RNA-independent manner. Together, these results support a model wherein Lsm1-7-Pat1 complexes act by binding consecutively to the BMV RNA regulatory sequences and the 1a protein to promote viral RNA translation and recruitment.

707 Investigating Nuclear Envelope Budding of Lytic Viral Transcripts During Kaposi's Sarcoma-Associated Herpesvirus Infection

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A recent publication on a post-synaptic response in *Drosophila* muscles revealed an alternative nuclear mRNA export pathway, in which ribonucleoproteins (RNPs) containing post-synaptic transcripts and a signaling peptide bud across the nuclear envelope (NE) in a similar fashion to herpesvirus egress (1). On this similarity, Speese et al. (1) speculate that the herpesviruses hijack the host nuclear RNP export pathway to release virions. Likewise, we hypothesize that herpesviruses also utilize the NE-budding export for selective transport of viral transcripts to escape the host shutoff effect. Thus we expect to observe NE buds containing viral transcripts for their export, independent of nuclear pore complexes.

Our experiments into alternative mRNA export include microscopic and biochemical approaches with Kaposi's sarcoma-associated herpesvirus (KSHV)-infected cells. With fluorescence *in situ* hybridization (FISH) and co-immunofluorescence (IF), we observe that foci of viral mRNAs (K8.1) localize to the nuclear periphery of lytic wt KSHV-infected cells. To explore the mechanism, we knocked down Torsin, an AAA+ ATPase mediator of both herpesvirus egress and NE-budding RNP export, expecting that NE buds containing viral mRNAs will become more concentrated or stuck at the NE (2). Knockdown of TorA protein causes isoform compensation from TorB and did not reveal a difference in viral transcript localization. Future directions include multiple Torsin isoform knockdown, inhibition of classic mRNA export pathways, and examination of more viral transcripts at different lytic stages.

References:

1. Speese, S. D., Ashley, J., Jokhi, V., Nunnari, J., Barria, R., Li, Y., Ataman, B., Koon, A., Chang, Y.-T., Li, Q., Moore, M. J., and Budnik, V. (2012) *Cell* 149, 832-846.
2. Jokhi, V., Ashley, J., Nunnari, J., Noma, A., Ito, N., Wakabayashi-Ito, N., Moore, M. J., and Budnik, V. (2013) *Cell Reports* 3, 988-995.

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708 Global intersection of long non-coding RNA (lncRNA) genes with processed and unprocessed pseudogenes in the human genome

Michael Milligan, Leonard Lipovich

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In the more than one decade since the completion of the Human Genome Project, a key revelation in post-genomic biology has been the prevalence of non-protein-coding functional elements in the human genome. Highlighted by the ENCODE and FANTOM consortia, these elements include tens of thousands of pseudogenes, as well as comparably numerous long non-coding RNA (lncRNA) genes. Pseudogene transcription, still poorly understood, is a field of great importance for human disease and specifically oncology research, due to the high sequence similarity between pseudogenes and their parental genes, generating the potential for sequence-specific regulation. Recent case studies have established essential functional roles of both pseudogenes and lncRNAs in development and disease in metazoan systems, and some have even highlighted functional impacts of lncRNA transcription at pseudogene loci on the regulation of the pseudogenes' parental genes. To compute the complete regulatory space of integrated pseudogene-lncRNA regulation, we developed and implemented an algorithm, using the PERL programming language, to identify all pseudogenes that are overlapped by lncRNA transcription in both sense and antisense orientations. As inputs to our algorithm, we imported three public repositories of pseudogenes: Gencode v17 (processed and unprocessed; n=8110); UCSC Genome Database (processed only; n=13742) and Yale (processed and unprocessed; n=17876), plus two public lncRNA catalogs: Broad Institute (n=21630) and Gencode v17 (n=10716). All datasets were retrieved from the UCSC Genome Database and the UCSC Table Browser. The intersection hence comprised six pseudogene-lncRNA genomewide overlaps. We identified 2,047 loci containing genomic-span (exon and/or intron) overlaps between Yale pseudogenes and Gencode lncRNAs, of which 201 had exon overlaps with public mRNA sequences, providing direct evidence of transcription. A similar analysis of the other five data intersections yielded a total of 5709 additional overlaps, of which 1103 had mRNA-supported exons. This project has generated an mRNA-supported list of all pseudogene-lncRNA overlaps in the human genome to serve as a foundation for future manual curation, parental-gene ontology analysis, and post-transcriptional RNA processing assessments. Specifically, we observed piRNA clusters at many of our pseudogene-lncRNA sense-antisense overlaps, suggesting that these overlaps may function as hotspots for functional small RNA biogenesis in epigenetic and post-transcriptional silencing.

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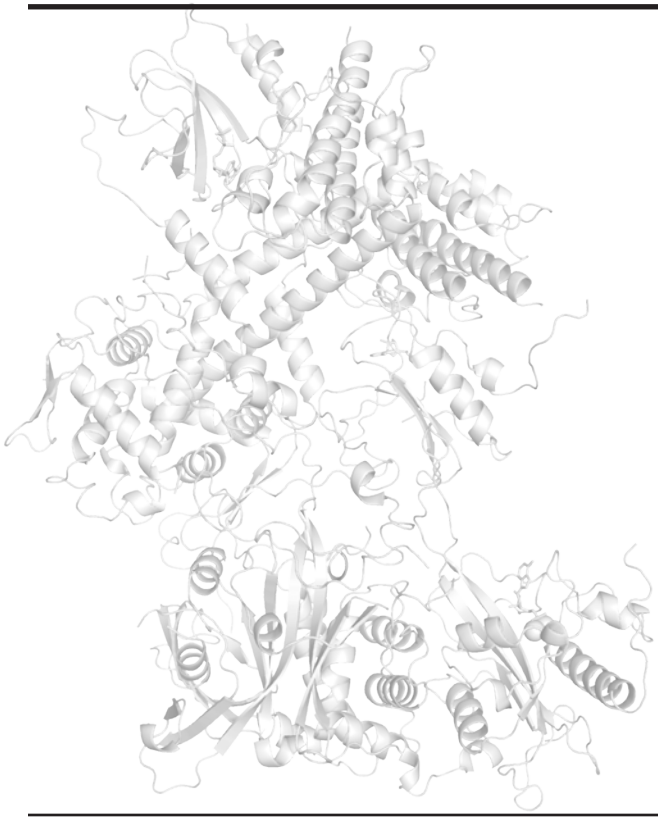

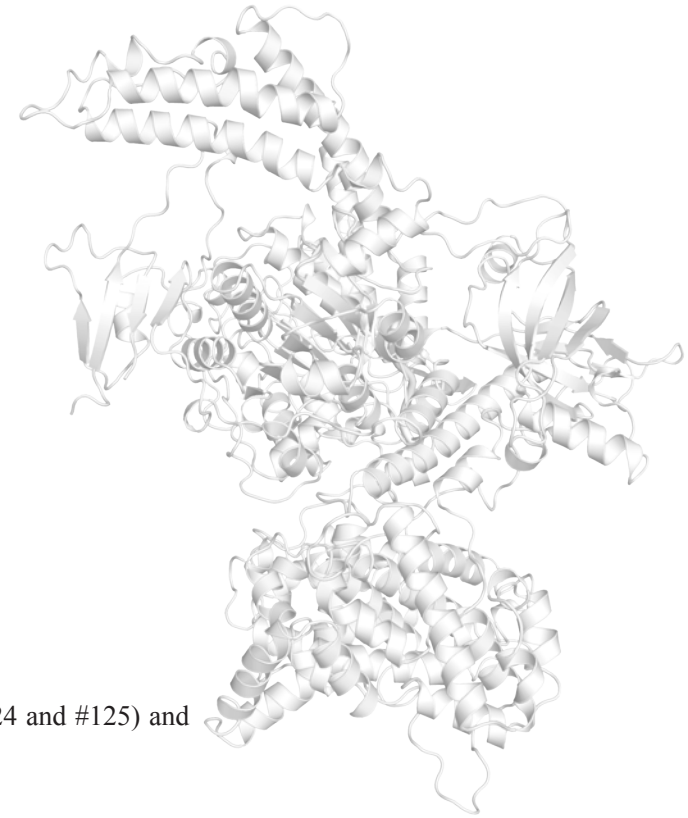

Structures of a yeast Pan2-Pan3 core complex (see abstracts #124 and #125) and the spliceosomal helicase Acquarius (see abstract #11).

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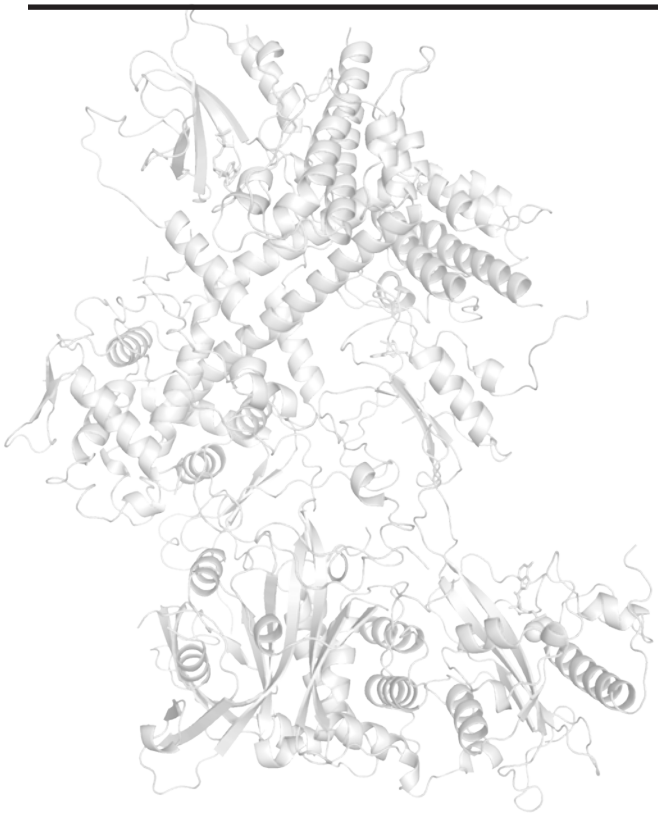

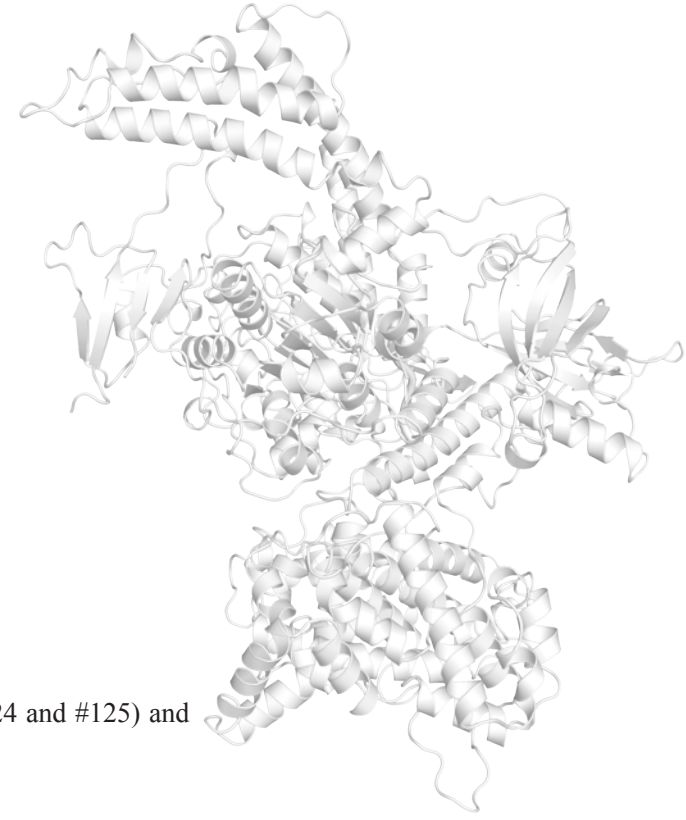

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KEYWORD INDEX

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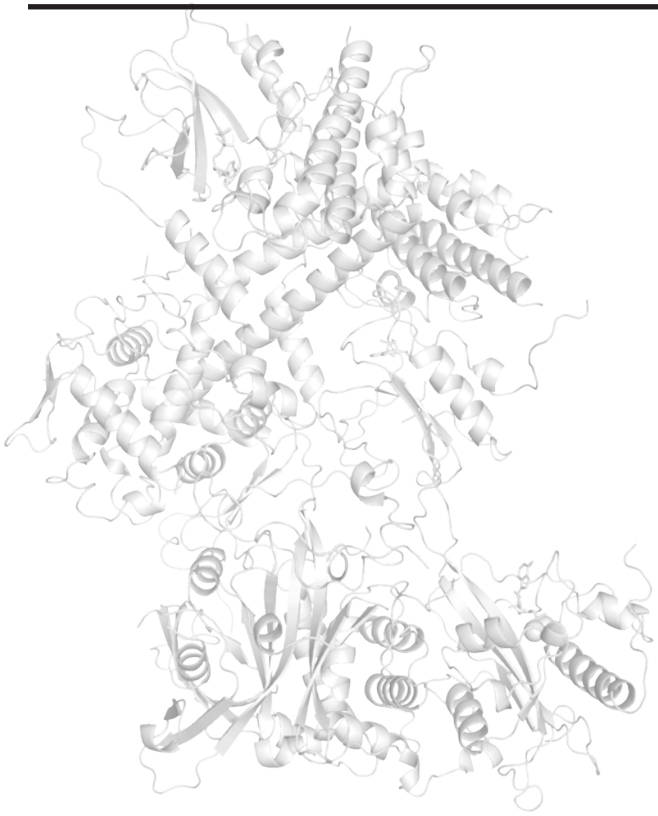

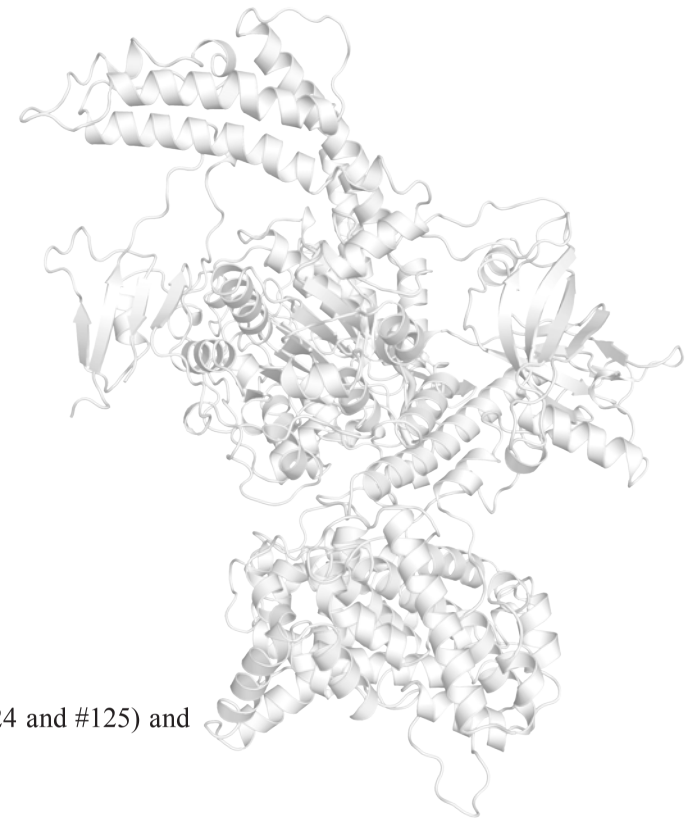

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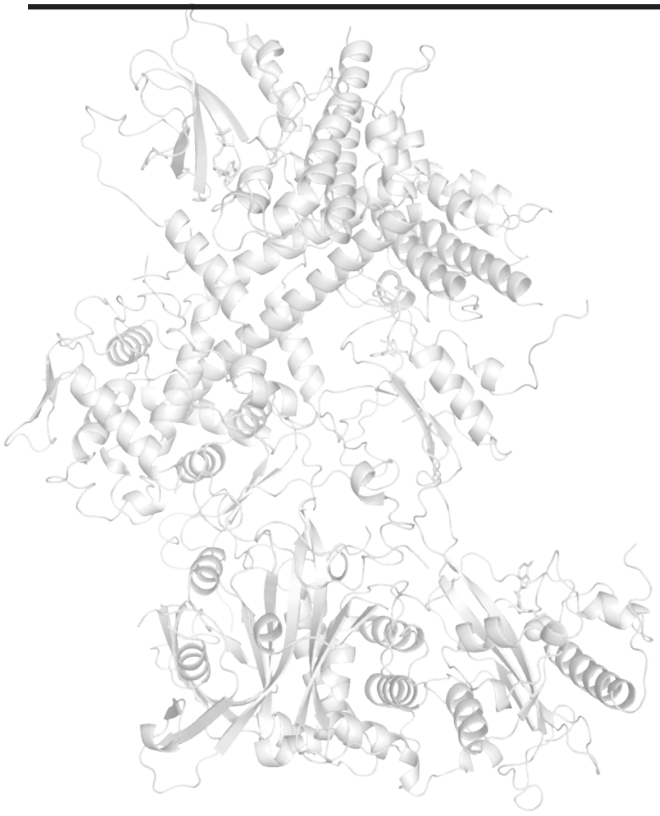

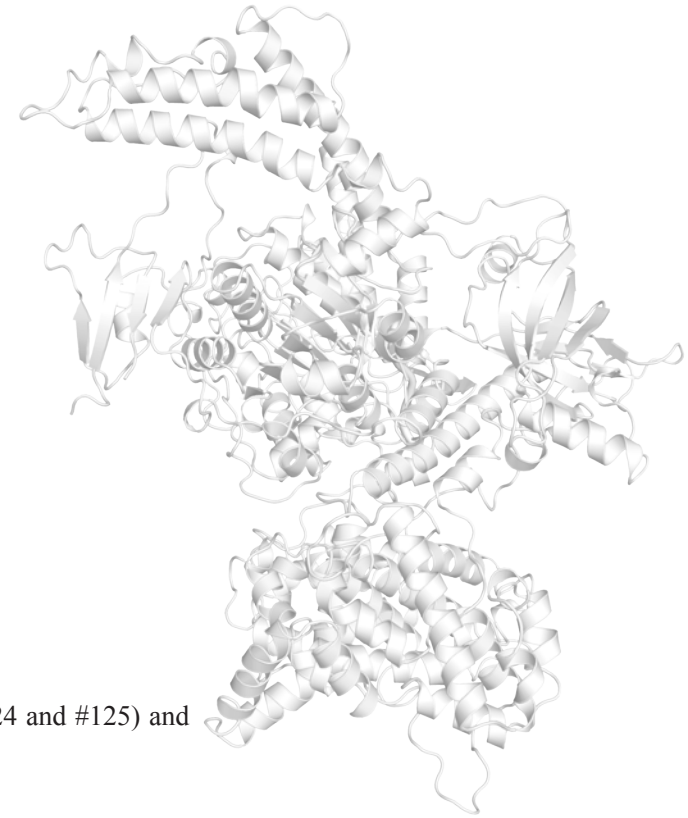

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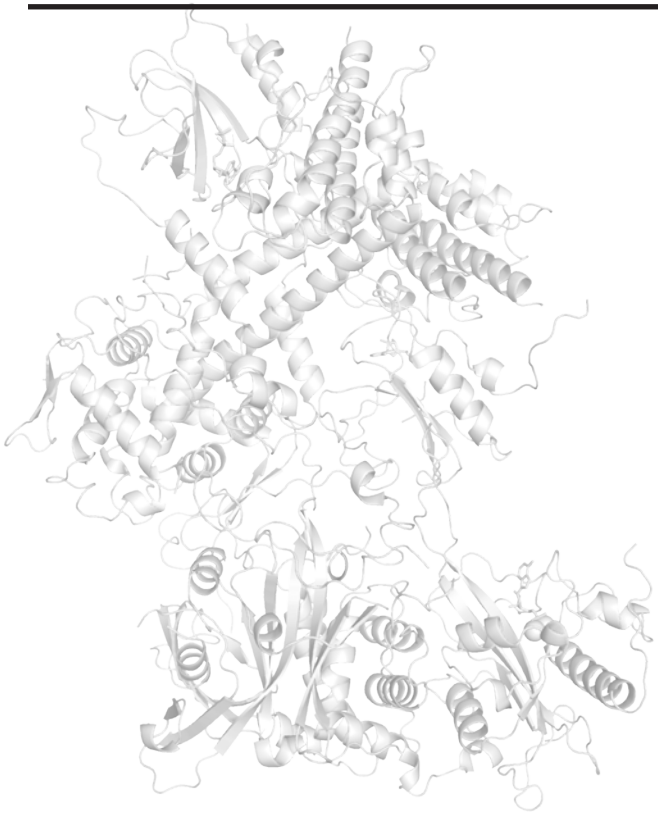

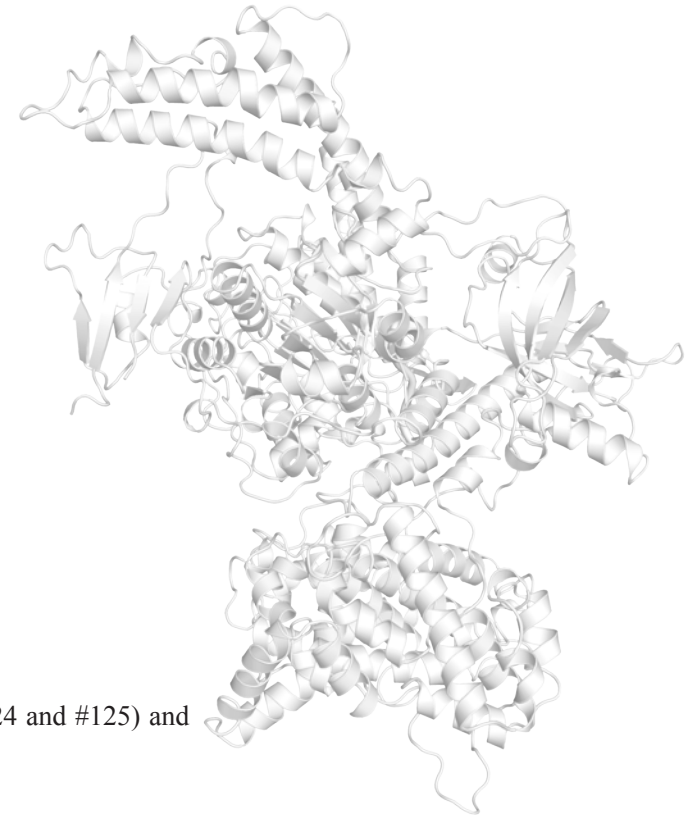

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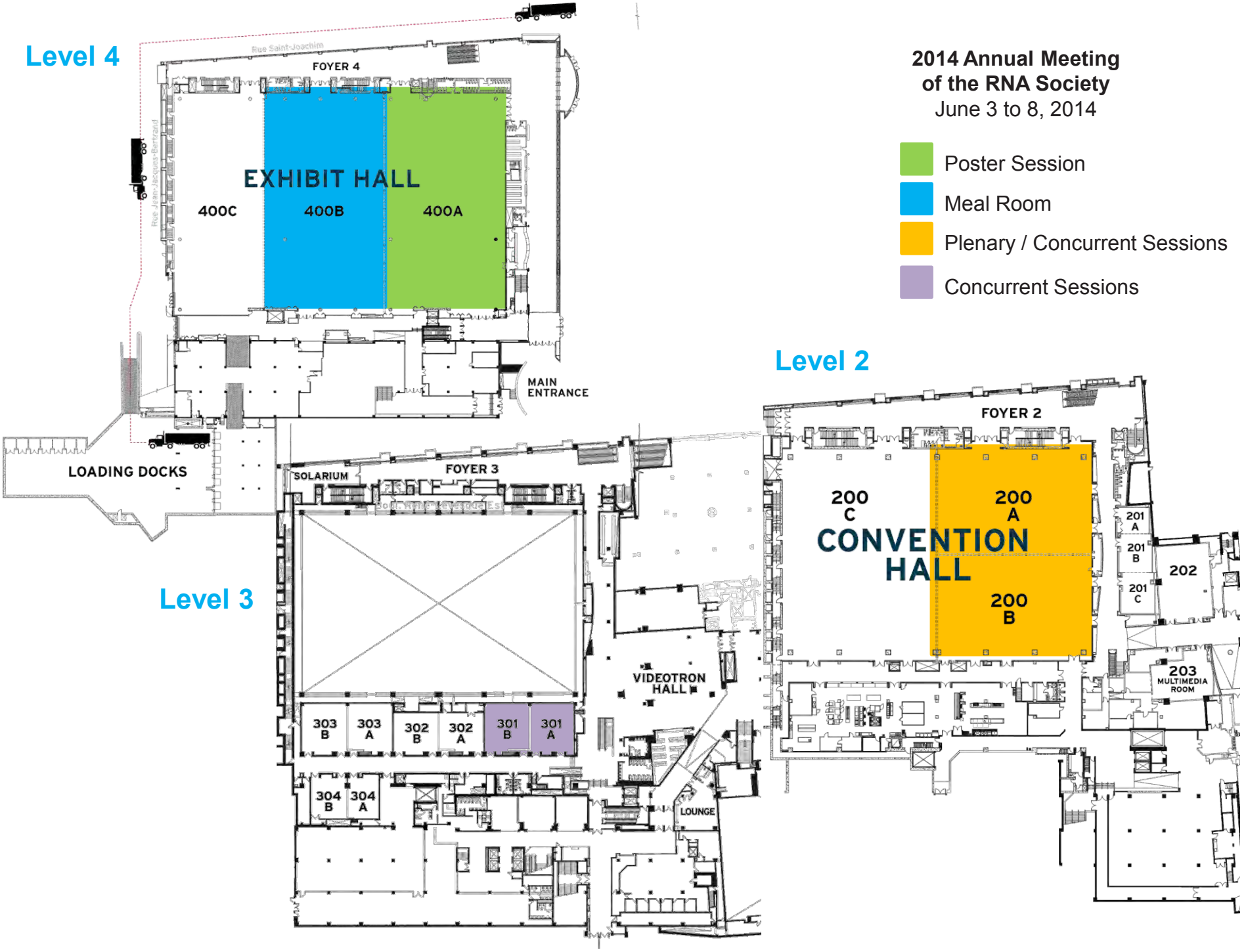
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LOUNGE
2014 Annual Meeting of the RNA Society
June 3 to 8, 2014
Poster Session
Meal Room
Plenary / Concurrent Sessions
Concurrent Sessions

TUESDAY JUNE 3

14:00 – 20:00	Registration	Foyer 4
17:30 – 20:00	Welcoming reception and dinner party	Foyer 4
20:00 – 20:10	Welcoming remarks	200AB
20:10 – 21:10	Keynote address: Phillip Zamore, UMass Medical School	
21:10 – 22:10	Keynote address: Robert Schneider, NYU School of Medicine	

WEDNESDAY JUNE 4

07:30 – 20:00	Registration continues	Foyer 4
08:30 – 10:30	Plenary session 1: RNA-Protein Interactions (1-15) [Kathy Collins]	200AB
11:00 – 12:30	Plenary session 1 (continued)	200AB
12:30 – 14:00	Lunch	400B
14:00 – 16:45	Plenary session 2: Splicing Regulation (16-25) [Ben Blencowe]	200AB
17:15 – 18:45	Workshop 1: RNA Chemistry (26-31) [Scott Strobel]	200A
	Workshop 2: RNA Regulation in Protozoa (32-37) [Barbara Papadopoulou]	301AB
	Workshop 3: RNA Programmable Genome Editing (38-42) [Emmanuelle Charpentier]	200B
18:45 – 20:30	Dinner	400B
18:45 – 20:30	Meetings Committee dinner/meeting	202
19:30 – 20:30	Junior Scientists Social	Solarium
20:30 – 23:00	Poster Session 1: (20:30 – 21:45 even numbers; 21:45 – 23:00 odd numbers)	400A

THURSDAY JUNE 5

08:00 – 19:00	Registration continues	Foyer 4
08:30 – 10:15	Plenary session 3: Emerging and High-Throughput Techniques (43-54) [Gene Yeo]	200AB
10:45 – 11:45	Plenary session 3 (continued)	200AB
11:45 – 13:15	Lunch	400B
11.45 – 13:15	Mentor/Mentee luncheon	400B
13:15 – 15:30	Concurrent session 1: RNA Silencing (55-63) [Yukihide Tomari]	200A
	Concurrent session 2: Ribosome Mechanisms and Assembly (64-72) [Katrin Karbstein]	200B
16:00 – 17:00	Keynote address: Anne Ephrussi, EMBL	200AB
17:30 – 19:00	Concurrent session 3: Ribozymes and Riboswitches (73-78) [Ron Breaker]	200A
	Concurrent session 4: RNA Transport and Localization (79-84) [Anita Corbett]	200B
	Concurrent session 5: 3' End Processing (85-90) [Elmar Wahle]	301AB
19:00 –	Dinner and evening in Quebec City on own – tours and other activities will be offered	

FRIDAY JUNE 6

08:00 – 18:30	Registration continues	Foyer 4
08:30 – 10:30	Plenary session 4: RNA in Disease (92-105) [Claudia Bagni]	200AB
11:00 – 12:30	Plenary session 4 (continued)	200AB
12:30 – 14:00	Lunch	400B
14:00 – 16:15	Plenary session 5: Non-coding and Regulatory RNAs (106-114) [Jørgen Kjems]	200AB
16:45 – 19:00	Plenary session 6: RNA Technologies and Therapeutic RNAs (115-123) [Matt Disney]	200AB
19:00 – 20:30	Dinner	400B
19:00 – 20:30	Board of Directors dinner/meeting	202
20:30 – 23:00	Poster session 2: (20:30 – 21:45 odd numbers; 21:45 – 23:00 even numbers)	400A

SATURDAY JUNE 7

08:00 – 18:30	Registration continues	Foyer 4
08:30 – 10:00	Concurrent session 6: RNA Decay (124-129) [Jeff Coller]	200A
	Concurrent session 7: RNA Interconnections (130-135) [Karla Neugebauer]	200B
10:30 – 12:00	Workshop 4: Bioinformatics of RNA Interactions (136-141, 141a–141d) [Eric Westhof, Janusz Bujnicki, and François Major]	200A
	Workshop 5: RNA Editing in Cellular Function (142-148) [Marie Öhman]	200B
	Career Development Workshop [Michael Matrone]	301AB
12:00 – 13:30	Lunch	400B
13:30 – 15:00	Concurrent session 8: Translational Control (149-154) [Wendy Gilbert]	200A
	Concurrent session 9: Splicing Mechanisms (155-160) [Jonathan Staley]	200B
15:30 – 18:15	Plenary session 7: RNA Architecture: Structure, Folding and Modification (161-172) [Michael Sattler]	200AB
18:30 –	Apero/Banquet/Awards/Dance	Hilton Hotel, First floor foyer and ballroom

SUNDAY JUNE 8

Conference concludes

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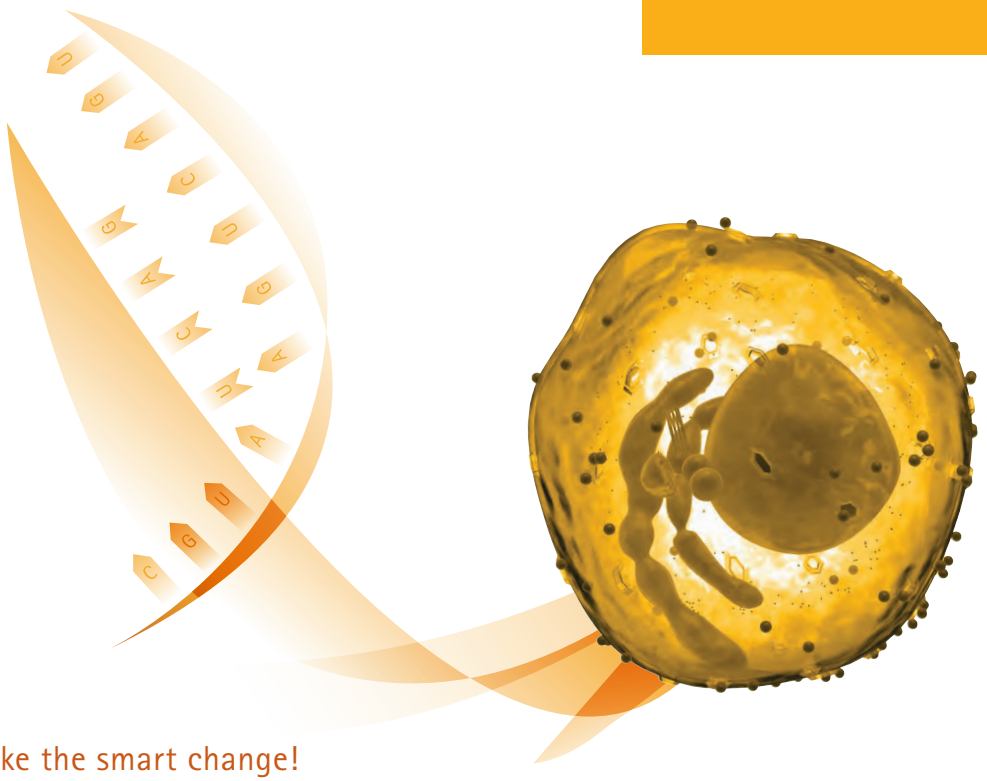

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