

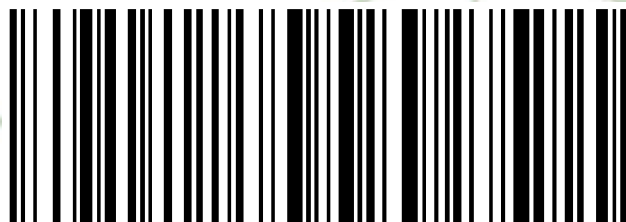
# RNA 2017



The 22<sup>nd</sup> Annual Meeting of the RNA Society



**May 30 – June 3, 2017**  
**Prague Congress Center**  
**Prague, Czech Republic**



# Cell Research



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Published in partnership with the Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS), *Cell Research* is a premium international life science journal with a broad scope in basic molecular and cell biology. The journal publishes original research results that are of unusual significance or broad conceptual or technical advances in all areas of life sciences, as well as authoritative reviews and sharply focused research highlights.

\*2015 Journal Citation Reports®, Science Edition (Thomson Reuters, 2016)



**SPRINGER NATURE**

# RNA 2017

THE 22<sup>ND</sup> ANNUAL MEETING OF THE RNA SOCIETY

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## PROGRAM & ABSTRACTS

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May 30 to June 3, 2017

Prague Congress Center

Prague, Czech Republic

**Andrea Barta**, *Medical University of Vienna, Vienna*

**Rachel Green**, *Johns Hopkins University, Baltimore*

**Christopher Lima**, *Sloan-Kettering Institute, New York*

**Ron Micura**, *Leopold Franzens University, Innsbruck*

**Petr Svoboda**, *Institute of Molecular Genetics ASCR, Prague*

**Yukihide Tomari**, *University of Tokyo IMCB, Tokyo*

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## GENERAL INFORMATION

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



**NO UNAUTHORIZED PHOTOGRAPHY IN SESSIONS:** To encourage sharing of unpublished data at the RNA Society Meeting, taking of photographs and/or videos during scientific sessions (oral or poster), or of posters outside of session hours, is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

**USE OF SOCIAL MEDIA:** The official hash tag of the 22<sup>nd</sup> Annual Meeting of the RNA Society is #RNA2017. The organizers encourage attendees to tweet about the amazing science they experience at the meeting, so that those who could not come to Prague can join in from afar. However, please respect these few simple rules when using the #RNA2017 hash tag or talking about the meeting on Twitter and other social media:

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

**Front Cover Image:** The front page art shows a timeless structure of a double helix dsRNA model emerging from the dial of the 1410 A.D. astrolabe of the Prague astronomical clock. The astrolabe was set to display May 30<sup>th</sup>, 2017, 2PM, the time the RNA 2017 meeting begins. Barcoding symbolizes the connection between the past and present times - barcoding is an integral part of the current state of the art high throughput analysis, while the barcode itself encodes the iconic motto "RNA forever". Credits: dsRNA NMR structure: Richard Stefl; astrolabe artwork: Jiri Solc; final design: Petr Svoboda.



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## MEETING SPONSORS

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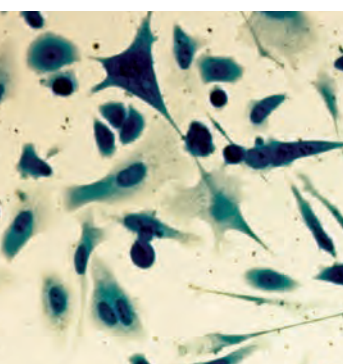
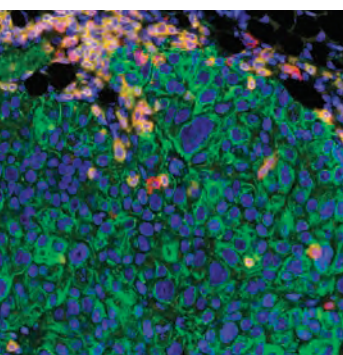
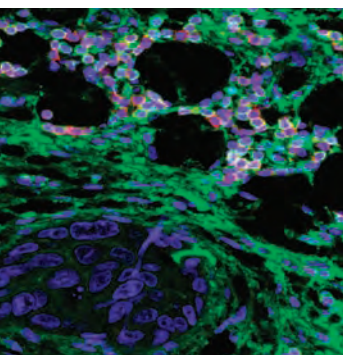
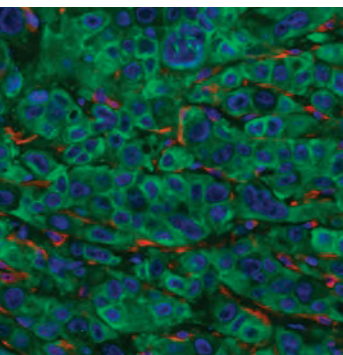
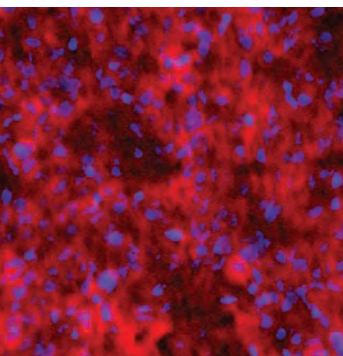
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# 2017-2018 SCIENTIFIC CONFERENCES

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

## **International Conference on Malignant Lymphoma (ICML)**

*ICML President: Franco Cavalli*

*Chair, Local Organizing Committee: Michele Ghilmini*

June 14-17, 2017 | Lugano, Switzerland

## **EACR-AACR-SIC Special Conference 2017: The Challenges of Optimizing Immuno- and Targeted Therapies: From Cancer Biology to the Clinic**

*Conference Cochairs: Anton J. M. Berns,  
Nancy E. Davidson, and Silvia Giordano*

June 24-27, 2017 | Florence, Italy

## **Third CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference**

*Conference Cochairs: Stanley Riddell,  
Robert D. Schreiber, Christoph Huber,  
and Guido Kroemer*

September 6-9, 2017 | Mainz/Frankfurt, Germany

## **Advances in Modeling Cancer in Mice: Technology, Biology, and Beyond**

*Conference Cochairs: Cory Abate-Shen,  
Kevin M. Haigis, Katerina A. Politi, and Julien Sage*

September 24-27, 2017 | Orlando, FL

## **Tenth AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved**

*Conference Cochairs: John M. Carethers,  
Rick A. Kittles, Christopher I. Li, and Electra D. Paskett*

September 25-28, 2017 | Atlanta, GA

## **Tumor Immunology and Immunotherapy**

*Conference Cochairs: James P. Allison,  
Carl H. June, Miriam Merad, and Giorgio Trinchieri*

October 1-4, 2017 | Boston, MA

## **Addressing Critical Questions in Ovarian Cancer Research and Treatment**

*Conference Cochairs: Robert C. Bast, Jr.,  
Ursula A. Matulonis, and Anil K. Sood*

October 1-4, 2017 | Pittsburgh, PA

## **Advances in Breast Cancer Research**

*Conference Cochairs: Myles A. Brown, Tak W. Mak,  
Ramon E. Parsons, and Laura J. van 't Veer*

October 7-10, 2017 | Hollywood, CA

## **AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics**

*Scientific Committee Cochairs: Antoni Ribas,  
James L. Gulley, and Charles Swanton*

October 26-30, 2017 | Philadelphia, PA

## **New Horizons in Cancer Research**

*Conference Cochairs: Nancy E. Davidson,  
Kornelia Polyak, Chi Van Dang, Hongyang Wang*

November 6-9, 2017 | Shanghai, P.R. China

## **Prostate Cancer: Advances in Basic, Translational, and Clinical Research**

*Conference Cochairs: Johann S. de Bono,  
Karen E. Knudsen, Peter S. Nelson, and Mark A. Rubin*

December 2-5, 2017 | Orlando, FL

## **Pediatric Cancer**

*Conference Cochairs: Peter C. Adamson,  
Nada Jabado, and Charles W. M. Roberts*

December 3-6, 2017 | Atlanta, GA

## **San Antonio Breast Cancer Symposium Presented by CTREC-AACR-BCM**

*Codirectors: Carlos L. Arteaga,  
Virginia G. Kaklamani, and C. Kent Osborne*

December 5-9, 2017 | San Antonio, TX

## **Obesity and Cancer**

*Conference Cochairs: Lewis C. Cantley,  
Michael N. Pollak, and Elizabeth A. Platz*

January 27-30, 2018 | Austin, TX

## **Immunobiology of Primary and Metastatic CNS Cancer: Multidisciplinary Science to Advance Cancer Immunotherapy**

*Conference Cochairs: Hideho Okada,  
Robyn S. Klein, Ignacio Melero, and Patricia S. Steeg*

February 12-15, 2018 | San Diego, CA

Learn more and register at  
[AACR.org/Calendar](http://AACR.org/Calendar)

**AACR** American Association  
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# The RNA Society

## Officers of the RNA Society FY 2017

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Juan Valcarcel  
*Centre de Regulacio Genomica*

### Past President (2015 – 2016)

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*Université de Sherbrooke*

### Membership Committee

Kristian Baker  
*Case Western Reserve University*

## RNA 2018

The 23<sup>rd</sup> Annual Meeting of the RNA Society will be held in Berkeley, California from May 29 to June 2, 2018, at the University of California, Berkeley.

### 2018 Organizers

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

9650 Rockville Pike, Bethesda, Maryland, 20814-3998

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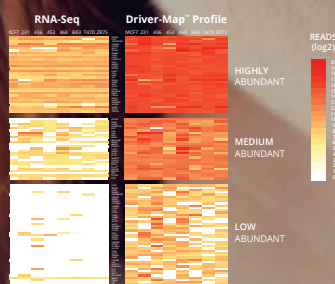
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## Invitation to Membership

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

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

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

### Benefits of RNA Society membership include:

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

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

**Please take a moment to start or renew your RNA Society membership at**

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

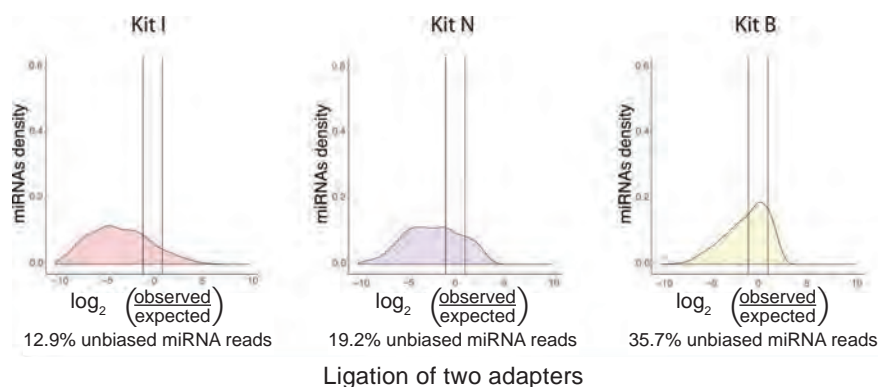
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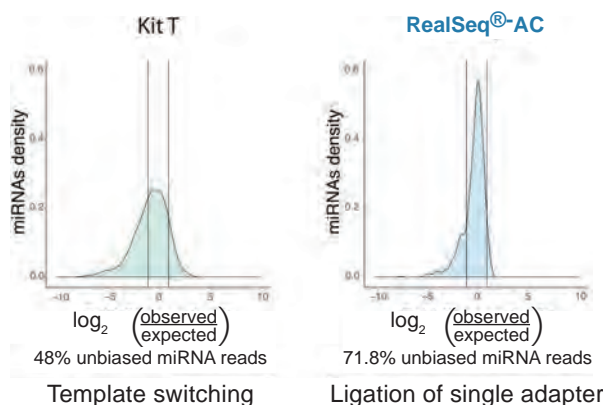
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- User-friendly, complete kit



Ratio of observed/expected reads from four popular small-RNA library preparation kits and RealSeq<sup>®</sup>-AC using one pmole of miRXPlore Universal Reference pool (Mitenyi Biotech, equimolar amounts of 963 synthetic miRNAs). Vertical lines represent a two-fold deviation from the expected values. Methods of adaptor attachment are indicated below graphs.

### miR-ID<sup>®</sup>

- Superior single nucleotide polymorphism discrimination at any position
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- Multiplexing capabilities
- Distinguishes 3' end modifications (2'-OMe/2'-OH)
- Custom and catalog assays available

### Superior isomiR discrimination by qPCR

	miR-ID <sup>®</sup> assays				AT assay
	0/0	0/+1	-1/0	-1/+1	0/0
0/0 (mature)	100%	0.09%	1.72%	0.00%	100%
0/+1 isomiR	0.09%	100%	0.20%	0.17%	37.89%
-1/0 isomiR	0.75%	0.72%	100%	0.00%	8.89%
-1/+1 isomiR	0.01%	0.05%	0.13%	100%	21.53%

Cross-discrimination assays using a synthetic reference miRNA and its isomiRs by miR-ID<sup>®</sup> and the leading competitor's advanced technology (AT). -1, 0, +1 symbolize addition to the 5', no change, or addition to 3' end with respect to the canonical miRNA.

### Superior isoform discrimination by qPCR

	miR-ID <sup>®</sup> assays		AT assays	
	miR-19a	miR-19b	miR-19a	miR-19b
miR-19a	100%	0.23%	100%	4.98%
miR-19b	1.43%	100%	3.11%	100%

hsa-miR-19a-3p UGUGCAAUUCU AUGCAAACUGA  
 hsa-miR-19b-3p UGUGCAAUUC CAUGCAAACUGA

miRNA isoform discrimination by miR-ID<sup>®</sup> (left) and leading competitor's advanced technology (AT).

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- Direct miRNA detection from biofluids
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- Inquire about custom assays

# PROGRAM—RNA 2017

## The 22<sup>nd</sup> Annual Meeting of the RNA Society Prague, Czech Republic May 30 – June 3, 2017

### Tuesday 30 May

14:00 – 19:00	Registration	Congress Hall Foyer 2B (floor 2)
18:00 – 20:00	Welcome Dinner	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3)
20:00 – 21:15	<b>Opening and Keynote 1 (page 15)</b> <i>Robert Singer, Albert Einstein College of Medicine</i>	Congress Hall
21:15 – 23:00	Social Time	Zoom Restaurant (floor 1)

### Wednesday 31 May

08:00 – 09:00	<b>Sponsored Seminar (page 18)</b> <i>Sponsored by Lexogen</i> <i>Chair: Lukas Paul</i>	Panorama Hall (floor 1)
08:00 – 19:00	Registration continues	Congress Hall Foyer 2B (floor 2)
09:00 – 12:30	<b>Plenary Session 1: RNA Editing/Modification (1-12)</b> <i>Chair: Michael Jantsch, Medical University of Vienna</i>	Congress Hall
10:30 – 11:00	Coffee Break	Congress Hall Foyer 2B (floor 2)
12:30 – 14:00	Lunch	Congress Hall Foyer 2A, 2C (floor 2)
12:30 – 14:00	Mentoring Lunch <i>Coordinator: Nancy Greenbaum, Hunter College, CUNY</i>	North Hall, Terrace 1, Terrace 2, Foyer 3A (floors 2 & 3)
14:00 – 16:15	<b>Concurrent Session 1: RNA Processing (13-21)</b> <i>Chair: Yongsheng Shi, University of California, Irvine</i>	Congress Hall
	<b>Concurrent Session 2: RNA Chemistry/Methods (22-31)</b> <i>Chair: Roland K. O. Sigel, University of Zürich</i>	Meeting Hall 1 (floor 1)
16:15 – 16:45	Coffee Break	Congress Hall Foyer 1B, 2B (floors 1 & 2)
16:45 – 17:45	<b>Keynote 2 (page 15)</b> <i>Eric Sontheimer, University of Massachusetts Medical School</i>	Congress Hall
17:45 – 19:15	<b>Concurrent Session 3: Splicing (32-38)</b> <i>Chair: David Stanek, Institute of Molecular Genetics, CAS</i>	Congress Hall
	<b>Concurrent Session 4: Retro Non-coding RNA (39-45)</b> <i>Chair: Stepanka Vanacova, Masaryk University, CEITEC</i>	Meeting Hall 1 (floor 1)
19:15 – 20:15	Junior Scientists Social	Zoom Restaurant (floor 1)
19:15 – 21:00	Dinner	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3), North Hall, Terrace 1, Terrace 2 (floor 2)
	RNA Society Meetings Committee Dinner Meeting	Club E (floor 1)

20:30 – 23:00	<b>Poster Session 1 (even numbers)</b>	Forum Hall (floor 2)
	<i>Sponsored by Biochemistry/ACS Publications</i>	
140 – 146	Biology and Mechanism of RNA Transcription	
147 – 167	Biology of Small RNAs	
168 – 184	Emerging and High-throughput Techniques	
185 – 186	Extracellular RNAs	
187 – 203	Interconnections Between Gene Expression Processes	
204 – 232, 740	Long Non-coding RNAs	
233 – 249	Mechanisms of Small RNAs	
250 – 258	Regulatory RNAs in Eukaryotes (including circular RNA)	
259 – 271	Regulatory RNAs in Prokaryotes	
272 – 291	Ribosome Biogenesis	
292 – 297	RNA and Epigenetics	
298 – 320, 741	RNA Bioinformatics	
321 – 330	RNA Catalysis and Riboswitches	
331 – 336	RNA Chemistry	
337 – 370	RNA Editing and Modification	
371 – 382	RNA in Neurobiology	
383 – 410	RNA Processing	
411 – 455	RNA Structure and Folding	
456 – 456	RNA Synthetic Biology and Systems Biology	
457 – 466	RNA Transport and Localization	
467 – 496	RNA Turnover	
497 – 528	RNA-protein Interactions	
529 – 567	RNAs in Disease	
568 – 582, 739	RNP Structure, Function and Biosynthesis	
583 – 605	Splicing Mechanisms	
606 – 639, 738	Splicing Regulation	
640 – 654	Therapeutic RNAs	
655 – 666	Translation Mechanisms	
667 – 704	Translational Regulation	
705 – 722	tRNA, snRNA, snoRNA, rRNA	
723 – 737	Viral RNAs	
23:00 – 23:30	Social Time	Zoom Restaurant (floor 1)

## Thursday 1 June

08:00 – 13:30	Registration continues	Congress Hall Foyer 2B (floor 2)
09:00 – 12:30	<b>Plenary Session 2: RNA/RNP Structure (46-57)</b> <i>Chair: Martin Jinek, University of Zürich</i>	Congress Hall
10:30 – 11:00	Coffee Break	Congress Hall Foyer 2B (floor 2)
12:30 – 13:30	<b>Keynote 3 (page 16)</b> <i>Marina Rodnina, Max Planck Institute for Biophysical Chemistry</i>	Congress Hall
13:30	Free afternoon and evening	

## Friday 2 June

08:00 – 19:00	Registration continues	Congress Hall Foyer 2B (floor 2)
09:00 – 12:30	<b>Plenary Session 3: Splicing (58-70)</b> <i>Chair: Kristen Lynch, University of Pennsylvania</i>	Congress Hall
10:30 – 11:00	Coffee Break	Congress Hall Foyer 2B (floor 2)



12:30 – 14:00	Lunch	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3), North Hall, Terrace 1, Terrace 2 (floor 2)	
14:00 – 16:15	<b>Concurrent Session 5: RNA Localization/Transport (71-78)</b>		Congress Hall
	<i>Chair: Maria Carmo-Fonseca, Instituto de Medicina Molecular Lisboa</i>		
	<b>Concurrent Session 6: Translation Regulation (79-87)</b>		Meeting Hall 1 (floor 1)
	<i>Chair: Daniel Wilson, University of Hamburg</i>		
	<b>Career Development Workshop: Combating Imposter Syndrome</b>		Panorama Hall (floor 1)
	<i>Organized by the RNA Society Junior Scientist Committee</i>		
	<i>Chair: Fadi Marayati, Wake Forest University</i>		
16:15 – 16:45	Coffee Break	Congress Hall Foyer 1B, 2B (floors 1 & 2)	
16:45 – 17:45	<b>Science and Society Lecture (page 16)</b>		Congress Hall
	<i>Adrian Krainer, Cold Spring Harbor Laboratory</i>		
17:45 – 19:15	<b>Concurrent Session 7: RNA Therapeutics (88-92,94)</b>		Meeting Hall 1 (floor 1)
	<i>Chair: Adrian Krainer, Cold Spring Harbor Laboratory</i>		
	<b>Concurrent Session 8: Non-coding RNA (95-101)</b>		Congress Hall
	<i>Chair: Stefan Ameres, Institute of Molecular Biotechnology - IMBA</i>		
19:15 – 21:00	Dinner	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3), North Hall, Terrace 1, Terrace 2 (floor 2)	
19:15 – 21:00	RNA Society Board of Directors Dinner Meeting		Club E (floor 1)
20:30 – 23:00	<b>Poster Session 2 (odd numbers)</b>		Forum Hall (floor 2)
	140 – 146	Biology and Mechanism of RNA Transcription	
	147 – 167	Biology of Small RNAs	
	168 – 184	Emerging and High-throughput Techniques	
	185 – 186	Extracellular RNAs	
	187 – 203	Interconnections Between Gene Expression Processes	
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	233 – 249	Mechanisms of Small RNAs	
	250 – 258	Regulatory RNAs in Eukaryotes (including circular RNA)	
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	298 – 320, 741	RNA Bioinformatics	
	321 – 330	RNA Catalysis and Riboswitches	
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	337 – 370	RNA Editing and Modification	
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	456 – 456	RNA Synthetic Biology and Systems Biology	
	457 – 466	RNA Transport and Localization	
	467 – 496	RNA Turnover	
	497 – 528	RNA-protein Interactions	
	529 – 567	RNAs in Disease	
	568 – 582, 739	RNP Structure, Function and Biosynthesis	
	583 – 605	Splicing Mechanisms	
	606 – 639, 738	Splicing Regulation	
	640 – 654	Therapeutic RNAs	
	655 – 666	Translation Mechanisms	
	667 – 704	Translational Regulation	
	705 – 722	tRNA, snRNA, snoRNA, rRNA	
	723 – 737	Viral RNAs	

23:00 – 23:30	Social Time	Zoom Restaurant (floor 1)
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## Saturday 3 June

08:00 – 18:00	Registration continues	Congress Hall Foyer 2B (floor 2)
09:00 – 12:30	<b>Plenary Session 4: Non-coding RNA (102-113)</b> <i>Sponsored by RNA-TRAIN</i> <i>Chair: Ling-Ling Chen, Shanghai Institute of Biochemistry and Cell Biology, CAS</i>	Congress Hall
10:30 – 11:00	Coffee Break	Congress Hall Foyer 2B (floor 2)
12:30 – 13:30	Lunch	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3)
13:30 – 14:30	<b>Keynote 4 (page 17)</b> <i>Juan Valcarcel, Center for Genomic Regulation - CRG</i>	Congress Hall
14:30 – 17:15	<b>Concurrent Session 9: RNA in Disease (114-121)</b> <i>Chair: Javier Martinez, Institute of Molecular Biotechnology - IMBA</i>  <b>Concurrent Session 10: Regulation &amp; Mechanisms of RNA Turnover (122-130)</b> <i>Chair: Kristian Baker, Case Western Reserve University</i>  <b>Concurrent Session 11: RNA Catalysis/Folding (131-139)</b> <i>Chair: Jeffrey Kieft, University of Colorado</i>	Panorama Hall (floor 1)          Congress Hall          Meeting Hall 1 (floor 1)
15:30 – 16:00	Coffee Break	Congress Hall Foyer 1B, 2B (floors 1 & 2)
17:30 – 18:30	Awards Ceremony	Congress Hall
19:00 – 24:00	Reception, Gala Dinner, Dance	Zofin Palace

## Sunday 4 June

Conference Concludes

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## KEYNOTE SPEAKER ABSTRACTS

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### Keynote 1

#### Following Single mRNAs from Birth to Death in Living Cells

Robert H. Singer

Albert Einstein College of Medicine, Bronx, NY, USA

Janelia Research Campus of the HHMI, Ashburn, VA, USA

Imaging has been an essential tool to analyze the dynamic properties of RNA. Newly developed new technologies in optical microscopy and novel methods for RNA tagging have allowed us to detect and track individual mRNA molecules in single living cells, yielding insights that could not have been obtained through any ensemble measurement. These approaches employ a plethora of imaging techniques, ranging from multiphoton microscopy, high-speed real-time widefield microscopy, single molecule tracking, super-registration microscopy and fluorescence fluctuation analysis. The kinetics of the key elements of RNA metabolism: initiation, elongation, termination, export, cytoplasmic trafficking, localization, translation and decay are now tractable at the single molecule level in real time in living cells. For instance, it was possible to measure the initiation frequency and elongation rates of single mRNA transcription or translation, or the probability and time to be transported through a nuclear pore. We have been dedicated most recently to developing and implementing these technologies to further the understanding of dynamics of mRNA regulation in living tissues, particularly neural tissues of a transgenic mouse where the mRNAs synthesized from targeted genes contain stem-loops from a phage bound by a fluorescent capsid. By observing RNA in its native environment, we may discover new regulatory mechanisms. Supported by funding from NIH.

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### Keynote 2

#### Small RNAs and Genetic Interference Pathways

Erik Sontheimer

RNA Therapeutics Institute, UMass Medical School, Worcester, MA, USA

In 2008, following several years of work on the mechanisms of RNA interference (RNAi) in *Drosophila melanogaster* and other eukaryotes, my laboratory turned its attention to a distinct set of genetic interference systems that had just been discovered the previous year in bacteria and archaea. Clustered, regularly interspaced, short, palindromic repeats (CRISPR) loci, along with proteins encoded by CRISPR-associated (*cas*) genes, encode RNA-guided, adaptive immune systems that protect the host from viral genomes and other invasive nucleic acids. These systems are widespread and mechanistically diverse, and most of them use their CRISPR RNA (crRNA) guides to target DNA molecules for destruction. CRISPR's RNA-guided DNA targeting capability, which my lab identified in 2008 in genetic studies in *Staphylococcus epidermidis*, has since been exploited for genome editing and other applications, spawning a revolution in the life sciences and biotechnology.

Our most recent focus has been on a CRISPR-Cas system found in the human pathogen *Neisseria meningitidis*. This is an example of a "Type II" CRISPR system that uses a single protein, Cas9, as its RNA-guided endonuclease. There are three subtypes of Type II systems (II-A, II-B and II-C), and the meningococcal system has served as a II-C prototype. In collaboration with others, we have used this system to document a natural role for CRISPR in limiting horizontal gene transfer, to define an unusual pathway for crRNA biogenesis, to uncover an unexpected enzymatic activity for *N. meningitidis* Cas9 (NmeCas9), and to identify the first naturally occurring Cas9 inhibitors [anti-CRISPR (Acr) proteins]. We have also validated and developed the relatively compact (1,082 amino acids) NmeCas9 for genome editing applications, demonstrated a consistent and nearly complete absence of off-target effects during NmeCas9 human genome editing, and developed Acr proteins for exerting spatial, temporal and conditional "off-switch" control over NmeCas9 editing activity. I will present our latest explorations of Type II-C CRISPR-Cas systems and their applications.



### Keynote 3

#### Translational Recoding as a Kinetic Event

*M. V. Rodnina*

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

The translation of the genetic code is normally very accurate. However, during the expression of certain genes the information is recoded to yield an alternative protein product. Recoding occurs in response to special signals in mRNA. Frameshifting or bypassing are best-known examples of such exceptions to the canonical co-linear information flow. To understand molecular mechanisms that govern -1 programmed ribosomal frameshifting and translational bypassing, we monitored the movement of the ribosome over the respective recoding sites. Recoding is explained in terms of pausing followed by a kinetic bifurcation event leading to the commitment to the alternative coding. Ribosome pausing is a key regulator of recoding.

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### Science and Society Lecture

#### Nusinersen (Spinraza<sup>TM</sup>): The First FDA-Approved Treatment for SMA

*Adrian R. Krainer*

Cold Spring Harbor Laboratory, NY, USA

Spinal muscular atrophy (SMA) is a motor-neuron disease, caused by mutations in SMN1. Patients retain one or more copies of the nearly identical SMN2 gene, which mainly expresses mRNA lacking exon 7, coding for an unstable protein isoform. The small amount of full-length mRNA and protein expressed from SMN2 only partially compensates for the loss of SMN1. Together with Ionis Pharmaceuticals, we developed nusinersen, an antisense oligonucleotide (ASO) that efficiently promotes exon 7 inclusion and restores SMN protein levels. Nusinersen hybridizes to intron 7 of the SMN2 pre-mRNA, preventing binding of the splicing repressors hnRNPA1/A2 to a bipartite intronic splicing silencer, ISS-N1; this in turn facilitates binding of U1 snRNP to the intron 7 5' splice site, resulting in enhanced exon 7 inclusion. Clinical trials of nusinersen in SMA patients began five years ago; based on the results of two phase-3 trials in infants with the most severe form of SMA, and in children with an intermediate form of SMA, respectively, nusinersen was recently approved by the FDA for all types of SMA.

We are continuing to explore aspects of SMA pathogenesis and treatment, using ASO therapy in SMA mouse models. We found that SMA is not motor-neuron cell-autonomous in the mouse models, such that correcting SMN2 splicing in peripheral tissues exclusively is necessary and sufficient for full phenotypic rescue. We are also exploring prenatal ASO treatment, as it is likely that early intervention can have the greatest clinical benefit.

Forty years after the discovery of RNA splicing, nusinersen exemplifies a successful path from basic studies of cellular mechanisms to an effective treatment for a devastating disease.

**Keynote 4****Networks of Alternative Splicing Regulation in Cancer***Juan Valcárcel***Centre de Regulació Genòmica (CRG); Barcelona Institute of Science and Technology (BIST); Universitat Pompeu Fabra (UPF); ICREA; Barcelona, Spain**

Alternative splicing of mRNA precursors allows regulation of gene function and physiology in higher eukaryotes, from the speciation of vampire bats to the selection of eatable quinoa seeds in South America. Alterations in alternative splicing can impact every hallmark of cancer and provide biomarkers of prognostic value. For example, activation of alternative splice sites in the oncogene BRAF confers resistance to vemurafenib and facilitate the frequent relapse of melanoma tumors. Splicing alterations can be caused by cancer-associated mutations in splicing regulatory sequences or by other genetic alterations, including mutations or changes in expression levels of splicing factors. Splicing factor mutations are particularly common in hematological tumors, including myelodysplastic syndromes and chronic lymphocytic leukemia. While advantageous for cancer progression, splicing alterations appear to make cancer cells particularly sensitive to splicing inhibitory drugs. These and other observations suggest that mis-regulation of alternative splicing networks contributes to tumor progression and at the same time can confer vulnerability to cancer cells.

I will summarize our recent efforts to systematically reveal splicing regulatory circuits altered in cancer cells and the potential of this knowledge to design novel anti-cancer therapies. These include methods for saturation mutagenesis of alternative exons, genome-wide identification of regulatory factors and reconstruction of splicing regulatory networks via profiling of alternative splicing after systematic knock down of spliceosomal components. Our results reveal highly dense regulatory content of alternative exon sequences and extensive regulatory potential of core splicing factors. They also uncover circuits of cell cycle and apoptosis control by spliceosomal components, reveal detailed molecular mechanisms of versatile splicing modulation by anti-tumor drugs and by modified antisense oligonucleotides, and provide insights into the impact of signaling pathways important for cancer cell proliferation on alternative splicing.

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## SPONSORED SEMINAR

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**Date & Time:** May 31, 2017 08:00 – 09:00

**Theme:** Adapting QuantSeq 3' RNA-Seq for Digital Gene Expression profiling of mRNAs and targeted RNAs

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

**Sponsored by:** Lexogen

Lexogen's QuantSeq technology provides a fast, cost-efficient protocol for generating strand-specific NGS libraries either close to the 3' end of polyadenylated RNAs (QuantSeq 3' mRNA-seq) or targeting user-defined sets of RNAs (QuantSeq-Flex). The versatile kit family also enables detection and quantification of poly(A) sites as well as automated screening of (differential) gene expression in high-throughput experiments with thousands of samples. In all cases, users benefit from low input requirements (starting from 100 pg total RNA), accurate quantification also of degraded and FFPE-sourced RNA and a free, cloud-based gene expression data analysis.

In this morning seminar, one academic speaker will present her research on uridylation-triggered RNA decay and an elegant use of QuantSeq-Flex to characterize co-immunoprecipitated RNAs transcriptome-wide. The other talk combines the analytical powers of individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP), QuantSeq 3' mRNA-Seq and Lexogen's SENSE mRNA-Seq to study the function of the RNA-binding protein TDP-43 and its role in the development of motor neuron disease.

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### Molecular basis for cytoplasmic RNA surveillance by uridylation-triggered decay in *Drosophila*

*Madalena M Reimão-Pinto<sup>1</sup>, Raphael A Manzenreither<sup>1</sup>, Thomas R Burkard<sup>1</sup>, Pawel Sledz<sup>2</sup>, Martin Jinek<sup>2</sup>, Karl Mechtler<sup>1</sup> & Stefan L Ameres<sup>1</sup>*

<sup>1</sup>Institute of Molecular Biotechnology, IMBA, Vienna Biocenter Campus (VBC), Vienna, Austria;

<sup>2</sup>Department of Biochemistry, University of Zurich, Zurich, Switzerland

The posttranscriptional addition of nucleotides to the 3' end of RNA regulates the maturation, function, and stability of RNA species in all domains of life. Here, we show that in flies, 3' terminal RNA uridylation triggers the processive, 3'-to-5' exoribonucleolytic decay via the RNase II/R enzyme CG16940, a homolog of the human Perlman syndrome exoribonuclease Dis3l2. Together with the TUTase Tailor, dmDis3l2 forms the cytoplasmic, terminal RNA uridylation-mediated processing (TRUMP) complex that functionally cooperates in the degradation of structured RNA. RNA immunoprecipitation and high-throughput sequencing reveals a variety of TRUMP complex substrates, including abundant non-coding RNA, such as 5S rRNA, tRNA, snRNA, snoRNA, and the essential RNase MRP. Based on genetic and biochemical evidence, we propose a key function of the TRUMP complex in the cytoplasmic quality control of RNA polymerase III transcripts. Together with high-throughput biochemical characterization of dmDis3l2 and bacterial RNase R, our results imply a conserved molecular function of RNase II/R enzymes as “readers” of destabilizing posttranscriptional marks—uridylation in eukaryotes and adenylation in prokaryotes—that play important roles in RNA surveillance.

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### Use of QuantSeq to study the regulation of 3' end processing by TDP-43

*Martina Hallegger<sup>1,2</sup>, Gregor Rot<sup>3</sup>, Nejc Haberman<sup>1,2</sup>, Ina Huppertz<sup>4</sup>, Christian von Mering<sup>3</sup>, Jernej Ule<sup>1,2</sup>*

<sup>1</sup>Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK; <sup>2</sup>Current

address: The Francis Crick Institute, London, UK; <sup>3</sup>Institute of Molecular Life Sciences and

Swiss Institute of Bioinformatics, Zurich, Switzerland; <sup>4</sup>European Molecular Biology Laboratory, Heidelberg, Germany

Most motor neuron disease (MND) patients display neuronal, cytoplasmic TDP-43 protein aggregates, which give the RNA-binding protein (RBP) TDP-43 a central role in the development of this disease.

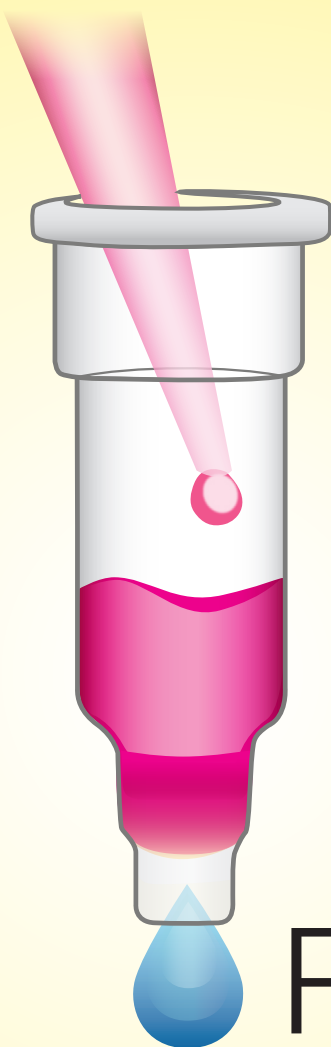


We studied the function of this protein with the use of individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP), Lexogen QuantSeq 3' mRNA-Seq and Lexogen SENSE mRNA-Seq. To integrate these data sets we developed expressRNA, a research platform for combining analysis of alternative splicing and alternative polyadenylation with the protein RNA binding sites identified by iCLIP and sequence analysis. We are visualising the regulatory principles using RNA maps, which link the binding position of RBPs to their regulatory functions. Similar to TDP-43's splicing regulation, we find that TDP 43 binds UG rich motifs close to the repressed polyA sites, and further away from the enhanced polyA sites. Aggregation of TDP-43 is promoted by its low-complexity (LC) domain, which is also the region containing most of the disease-causing mutations. While most studies suggest that the LC domain mainly mediates protein-protein interactions, its function is poorly understood. We therefore examined the role of the TDP-43 LC domain in protein-RNA interactions and the functions of these interactions in regulating pre-mRNA processing. For this purpose, we created cell lines where the endogenous RBP is replaced by mutants lacking specific portions of the LC domain. We show that deletions in the LC domain affect the function of TDP-43 in splicing and 3' end processing.

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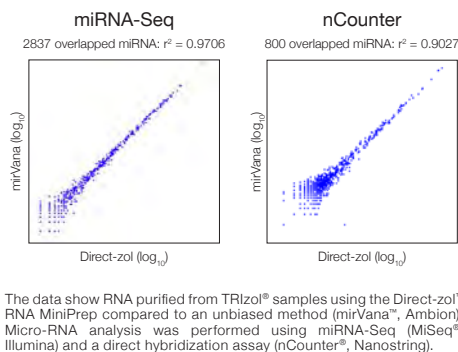
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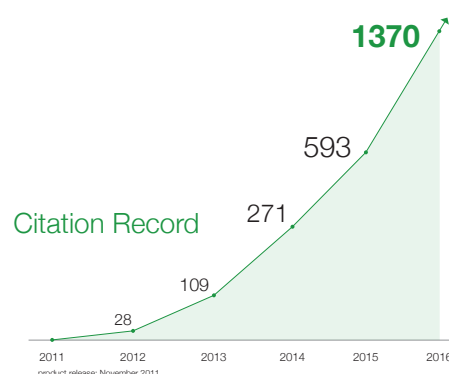
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## ADDITIONAL SCHEDULED EVENTS AT RNA 2017

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### Tuesday, 30 May

13:00 – 18:00

**Junior Scientists Pre-Conference Activities**

- Start at Prague Congress Centre

*Open to all graduate students and postdocs. Registration required, no additional charge*

The annual pre-meeting activity will give you the opportunity to explore the magnificent city of Prague with all your senses and meet other junior scientists attending the meeting. On our scavenger hunt, you will travel through time, experiencing medieval times all the way to modernity. You will be astounded by the breathtaking sights, sounds, and tastes of this multicultural city in the heart of Europe. This interactive tour will be even more fun because you will be placed on a team with fellow junior scientists.

Please register online ahead of time (by May 26<sup>th</sup>) to ensure that you are placed on a scavenger hunt team. The scavenger hunt will start at 1 p.m. and will begin at the Prague Congress Center. The scavenger hunt will end in time to make it back for the Registration and Welcome Dinner. Please bring 500 CZK (~\$20 USD) of pocket change to be prepared for any public transportation or museum/attraction admission fees on the scavenger hunt. The registration link can be found on RNA 2017 webpage (Junior Scientists tab) and through our social media accounts.

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### Wednesday, 31 May

12:45 – 14:00

**Mentor/Mentee Lunch**

- North Hall, Terrace 1 and 2, Congress Hall Foyer 3A

*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.

19:15 – 20:15

**Junior Scientists Social**

- Zoom Restaurant

*Open to all graduate students and postdocs. No additional charge, no registration required*

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

19:15 – 21:00

**Meetings Committee Meeting**

- Club E

*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, Benoit Chabot (Benoit.Chabot@USherbrooke.ca).

## Thursday, 1 June

### 13:30 onward **Free Afternoon and Evening**

*Enjoy your free time in Prague. Tour operators will have tables near registration on Wednesday to help you sign up for tours or decide what to do on your own.*

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## Friday, 2 June

### 14:00 – 15:30 **Junior Scientists Career Development Workshop**

- Panorama Hall

*Open to all attendees, but intended for graduate students and postdocs*

Have you ever felt like you're not quite as qualified as people think you are? Do you fear being exposed as a "fraud"? Our annual Career Development Workshop will address what "Impostor Syndrome" is, share stories of scientists who have struggled with and worked through it, and discuss methods to combat it.

### 19:15 – 21:00 **RNA Society Board of Directors Meeting**

- Club E

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

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

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## Saturday, 3 June

### 17:30 – 18:30 **Awards Ceremony**

- Congress Hall

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

- RNA Society Lifetime Achievement Award  
*Lynne Maquat, University of Rochester Medical Center*
- RNA Society Service Award  
*Andrew Feig, Wayne State University*
- RNA Society/Mid-career Award winners  
*Karla Neugebauer, Yale University*  
*Nils Walter, University of Michigan*
- RNA Society/Early-career Award winners  
*Wendy Gilbert, Massachusetts Institute of Technology*  
*Gene Yeo, University of California, San Diego*
- RNA Society/Scaringe Award winners  
*Malik Chaker-Margot, Rockefeller University; Graduate Student Award*  
*Zhipeng Lu, Stanford University; Post-Doctoral Award*
- Poster prize winners



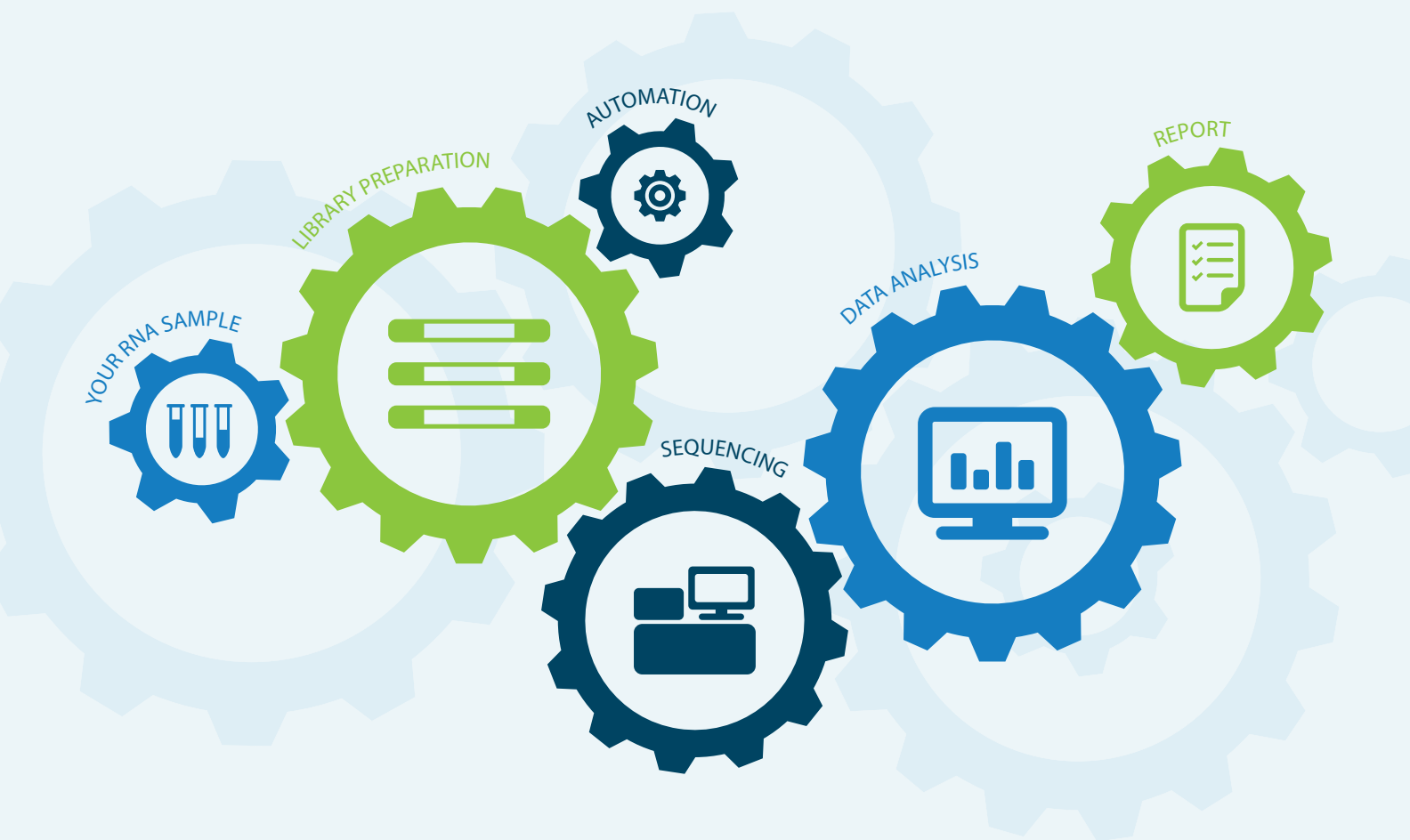
19:00 – 24:00 **Conference Closing Event - Saturday, 3 June**

- Zofin Palace

*Open to all registered attendees at no additional charge but tickets are required.*

For our closing gala, we'll break free from the Congress Center to visit one of Prague's iconic buildings, located on picturesque Slavonic Island. Palác Žofín is a neoclassical treasure where many famous composers and musicians have performed and some of the Czech Republic's most significant social and cultural events take place. Walk to the Vltava River and take Tram 17; or walk 2.8 km along the river; or take the Metro subway to the Mustek stop and then walk to the river.

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# RNA 2017 AWARDS

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## The RNA Society Lifetime Achievement Award

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The RNA Society Lifetime Achievement Award acknowledges the impact of an outstanding RNA scientist on the general scientific community. Each year beginning in 2003, the Board of Directors has selected the recipient who receives a lifetime membership in the RNA Society in recognition of their outstanding contributions. The award is presented at the Annual RNA Meeting, where the recipient gives a special address to the RNA Society. Previous winners include Joan Steitz (2003), Harry Noller (2004), John Abelson (2005), Christine Guthrie (2006), Walter Keller (2007), Norm Pace (2008), Thomas Cech (2009), Fritz Eckstein (2010), Witold Filipowicz (2011), Olke Uhlenbeck (2012), Phillip Sharp (2013), Reinhard Lührmann (2014), Anita Hopper (2015) and Eric Westhof (2016).

*Congratulations to **Lynne Maquat** who is the winner of the 2017 RNA Society Lifetime Achievement Award.*

## The RNA Society Service Award

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The RNA Society Service Award is given in appreciation of outstanding service to the RNA community. The overall mission of the RNA Society is to facilitate sharing and dissemination of experimental results and emerging concepts in RNA research. Each year, the Board of Directors identifies the recipient of this award who has made exemplary contributions to these goals. Previous winners include Tim Nilsen (2003), Chris Greer (2004), Jean Beggs (2005), Olke Uhlenbeck (2006), Marvin Wickens (2007), Eric Westhof (2008), Anita Hopper (2009), Lynne Maquat (2010), Evelyn Jabri (2011), Brenda Peculis (2012), Ann Marie Micenmacher (2014), David Lilley (2015), and Andrea Barta (2016).

*Congratulations to **Andrew Feig** who is the winner of the 2017 RNA Society Service Award.*

## The RNA Society Mid-Career Award

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

*Congratulations to **Karla Neugebauer** and **Nils Walter**, the winners of the 2017 RNA Society Mid-Career Award.*

## The RNA Society Early-Career Award

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

*Congratulations to **Wendy Gilbert** and **Gene Yeo**, the winners of the 2017 RNA Society Early-Career Award.*

## The RNA Society/Scaringe Award



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

### SCARINGE

*Supporting the Future*

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

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

*Congratulations to graduate students **Malik Chaker-Margot**, and postdoctoral fellows **Zhipeng Lu**, the winners of the 2017 RNA Society/Scaringe Award.*

## The Biochemistry Poster Prizes

### Biochemistry

The journal Biochemistry and ACS Publications are pleased to recognize junior scientists with six poster prizes to be awarded at RNA 2017. The prizes are for 'Excellence in RNA Research', and each includes a \$250 cash award.

## The New England Biolabs Poster Prizes



New England BioLabs is pleased to recognize junior scientists with four poster prizes to be awarded at RNA 2017. The prizes are for "general excellence in RNA research" and each consists of a \$250 cash award.

## The RNA Society Poster Prizes



The RNA Society is pleased to provide additional poster prizes for RNA 2017, as needed depending on the number of outstanding poster presentations. Each prize consists of a \$200 cash award.

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



# ORAL ABSTRACT LISTING

(Note: Numbers refer to abstract numbers, not page numbers)

**WEDNESDAY, MAY 31, 2017: 9:00 – 12:30; CONGRESS HALL**

**Plenary Session 1: RNA Editing/Modification**

*Chair: Michael Jantsch, Medical University of Vienna*

Abstracts 1 – 12

- 1 RNA epigenetic marks affect transcriptome switching during early embryonic development**  
*Boxuan Zhao, Xiao Wang, Alana Beadell, Robert Ho, Chuan He*
- 2 Adenosine demethylase FTO regulates nuclear pre-mRNA processing**  
*Helena Covelo-Molares, Marek Bartosovic, Pavlina Gregorova, Grzegorz Kudla, Stepanka Vanacova*
- 3 RNA fate regulation by DGCR8 through co-transcriptional methylation**  
*Philip Knuckles, Sarah Carl, Michael Musheev, Christof Niehrs, Alice Wenger, Marc Bühler*
- 4 5-methylcytosine as an epitranscriptomic mark on murine mRNA**  
*Thomas Amort, Dietmar Rieder, Alexandra Wille, Lukas Trixl, Alexandra Lusser*
- 5 Expanding the decoding capacity of the mitochondrial tRNA<sup>Met</sup> by RNA modifications**  
*Katherine E. Sloan, Sara Haag, Namit Ranjan, Ahmed S. Warda, Jens Kretschmer, Charlotte Blessing, Benedikt Hübner, Jan Seikowski, Sven Dennerlein, Peter Rehling, Marina V. Rodnina, Claudia Höbartner, Markus T. Bohnsack*
- 6 How to make EF-P sweet enough to alleviate stalled ribosomes**  
*Jakub Macošek, Ralph Krafczyk, Pravin Kumar Ankush Jagtap, Daniel Gast, Bernd Simon, Svetlana Wunder, Prithiba Mitra, Rohr Jürgen, Anja Hoffmann-Röder, Kirsten Jung, Jürgen Lassak, Janosch Hennig*
- 7 MeTH-seq: Transcriptome-wide Mapping of 2'-O-Methyl Ribose Reveals Widespread Regulated Modification of mRNAs**  
*Kristen Bartoli, Cassandra Schaening, Thomas Carlile, Wendy Gilbert*
- 8 A catalytic RNA helicase Subcomplex in the Trypanosome Holo-editosome**  
*Vikas Kumar, Pawan Doharey, Shelly Gulati, James Wohlschlegel, Achim Schnauffer, Al Ivens, Blaine Mooers, Jorge Cruz-Reyes*
- 9 The C. elegans neural editome reveals an ADAR target mRNA required for proper chemotaxis**  
*Heather Hundley, Sarah Deffit, Yee Brian, Vadlamani Pranathi, Wheeler Emily, Manning Aidan, Yeo Gene*
- 10 ADAR1 controls apoptosis of stressed cells by inhibiting Staufen1-mediated mRNA decay**  
*Masayuki Sakurai, Yusuke Shiromoto, Hiromitsu Ota, Chunzi Song, Andrew Kossenkoy, Jayamanna Wickramasinghe, Louise Showe, Emmanuel Skordalakes, Hsin-Yao Tang, David Speicher, Kazuko Nishikura*
- 11 Evolutionarily conserved biological roles of ADAR RNA editing enzymes**  
*Anzer Khan, Nagraj Sambrani, Simona Paro, Leanne McGurk, Xianghua Li, Mary A. O'Connell, Liam P. Keegan*
- 12 Separate analysis of pre-mRNA and mRNA A-to-I editing reveals surprising differences in the level of editing and suggests selective maturation of edited transcripts**  
*Konstantin Licht, Utkarsh Kapoor, Michael F Jantsch*

**WEDNESDAY, MAY 31, 2017: 14:00 – 16:15; CONGRESS HALL**

**Concurrent Session 1: RNA Processing**

*Chair: Yongsheng Shi, University of California, Irvine*

Abstracts 13 – 21

- 13 Kinetics of pre-mRNA cleavage and termination in living cells**  
*Rita Vaz-Drago, Ana C de Jesus, Robert M Martin, Célia Carvalho, José Rino, Noélia Custódio, Maria Carmo-Fonseca*

- 14 A unified activation mechanism for mRNA 3' processing and splicing**  
*Yong Zhu, Xiuye Wang, Elmira Fouromand, Yongsheng Shi*
- 15 Molecular and Structural Basis for the Integrator Cleavage Module**  
*Todd Albrecht, Yixuan Wu, David Baillat, Liang Tong, Eric Wagner*
- 16 Dynamic assembly of RNA polymerase II CTDsome**  
*Olga Jasnovidova, Tomasz Kabzinski, Pavel Brazda, Madgalena Krejcikova, Tomas Klumpler, Karel Kubicek, Richard Stefl*
- 17 Replication compartment formation increases viral mRNA transcription during lytic infection**  
*David McSwiggen, Robert Tjian, Xavier Darzacq*
- 18 Surveilling the proper processing of mRNAs for nuclear export: A coordinated effort between ZC3H14 and the THO complex**  
*Kevin Morris, Qiudong Deng, Nicholas Seyfried, Anita Corbett*
- 19 Molecular roles and charactersitics of a novel RNA-binding protein - E3 ubiquitin ligase Trim25**  
*Nila Roy Choudhury, Gregory Heikel, Maryia Trubitsyna, Jakub Stanislaw Nowak, Shaun Webb, Sander Granneman, Christos Spanos, Juri Rappsilber, Alfredo Castello, Gracjan Michlewski*
- 20 Structures and evolutionarily divergent phosphodiesterase activities of Usb1 from yeast and human**  
*Allison Didychuk, Eric Montemayor, Tucker Carrocci, Andrew DeLaitsch, Stefani Lucarelli, Ronnie Frederick, Marco Tonelli, William Westler, David Brow, Aaron Hoskins, Samuel Butche*
- 21 Acidic C-terminal domains autoregulate the RNA chaperone Hfq**  
*Andrew Santiago-Frangos, Jeliasko Jeliaskov, Jeffrey Gray, Sarah Woodson*

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WEDNESDAY, MAY 31, 2017: 14:00 – 16:15; MEETING HALL 1 (FLOOR 1)

Concurrent Session 2: RNA Chemistry/Methods

Chair: Roland K. O. Sigel, University of Zürich

Abstracts 22–31

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- 22 RNA folding nearest neighbor parameter derivation**  
*Hongying Sun, Jeffrey Zuber, David Mathews*
- 23 Predicting RNA structure based on direct co-evolutionary couplings obtained with Boltzmann learning techniques**  
*Francesca Cuturello, Guido Tiana, Giovanni Bussi*
- 24 RNA 3D Structure Prediction Using Multiple Sequence Alignment Information**  
*Marcin Magnus, Caleb Geniesse, Rhiju Das, Janusz M. Bujnicki*
- 25 RNA-protein interactions in an unstructured context**  
*Bojan Zagrovic, Anton Polyansky*
- 26 Novel 5' cap analogs as a tools for site-specific sequence-independent labeling of messenger RNA.**  
*Pawel J. Sikorski, Marcin Warminski, Adam Mamot, Zofia Warminska, Joanna Kowalska, Jacek Jemielity*
- 27 Chemical Strategies for Massively Parallel RNA Synthesis on Surfaces**  
*Matthew T. Holden, Cheng-Hsien Wu, Matthew C.D. Carter, Jameson Wolfer, Eric Codner, Caitlin M. Pavelec, Shannon K. Ting, Michael R. Sussman, David M. Lynn, Lloyd M. Smith*
- 28 ClickSeq and Poly(A)-ClickSeq: Methods for Next-Generation Sequencing library preparation**  
*Elizabeth Jaworski, Ping Ji, Zheng Xia, Wei Li, Eric Wagner, Andrew Routh*
- 29 Thermodynamically optimal riboswitches achieved through massively parallel rational design**  
*Johan Andreasson, Wipapat Kladwang, Jeehyung Lee, Adrien Treuille, John Nicol, Fernando Portela, Michelle Wu, Vineet Kosaraju, Eterna Participants, Rhiju Das, William Greenleaf*
- 30 Data-rich strategies for elucidating hammerhead ribozyme sequence-function relationships and engineering ribozyme switches**  
*Joy S. Xiang, Brent Townshend, Gabriel Manzanarez, Emily Gale, Michaela Hinks, Christina D. Smolke*

**31 Ultrahigh-throughput in vitro evolution and functional characterization of RNA using droplet-based microfluidics**

Alexis Autour, Farah Bouhedda, Stéphanie Baudrey, Ketty Pernod, Christian Rick, Sunny Jeng, Peter Unrau, Franck Martin, Eric Westhof, Michael Ryckelynck

**WEDNESDAY, MAY 31, 2017: 17:45 – 19:15; CONGRESS HALL**

**Concurrent Session 3: Splicing**

Chair: David Stanek, Institute of Molecular Genetics, CAS

Abstracts 32–38

**32 Assembly of U5-specific proteins is controlled by the HSP90/R2TP chaperone system**

Anna Malinová, Zuzana Cvačková, Daniel Matějů, Zuzana Hořejší, Claire Abeza, Franck Vandermoere, Edouard Bertrand, David Staněk, Celine Verheggen

**33 Structure and assembly of the yeast U6 snRNP**

Eric Montemayor, Allison Didychuk, Tucker Carrocci, Gurnimrat Sidhu, Allyson Yake, Aaron Hoskins, David Brow, Samuel Butcher

**34 LUC7L2 is an SR domain-containing splicing regulatory protein that is frequently mutated in bone marrow neoplasms**

Courtney Hershberger, Naoko Hosono, Jarnail Singh, Rosemary Dietrich, Xiaorong Gu, Dewen You, Hideki Makishima, Yogen Sauntharajah, Jarek Maciejewski, Richard Padgett

**35 Npl3p, the yeast SR-like mRNA binding protein, affects the fidelity of pre-mRNA splicing**

Agata Jaskulska, Magda Konarska

**36 PRP40 mediates the communication between RNA polymerase II, the microprocessor and spliceosome in plants**

Agata Stepień, Mateusz Bajczyk, Tomasz Gulanicz, Katarzyna Knop, Dariusz Smolinski, Zofia Szwejkowska-Kulinska, Artur Jarmolowski

**37 Identification of intrasplicing events in the human transcriptome and their regulatory potential**

Maximilian Radtke, Ismet Srndic, Renee Schroeder

**38 Divergent splicing of paralogous genes – the intergenic regulatory loop of RPL22**

Katerina Abrahmova, Filip Nemcko, Jiri Libus, Martin Prevorovsky, Martina Halova, Frantisek Puta, Petr Folk

**WEDNESDAY, MAY 31, 2017: 17:45 – 19:15; MEETING HALL 1 (FLOOR 1)**

**Concurrent Session 4: Retro Non-coding RNA**

Chair: Stepanka Vanacova, Masaryk University, CEITEC

Abstracts 39–45

**39 Discovery of regulators of mammalian ribosome biogenesis**

Katherine Farley-Barnes, Kathleen McCann, Janie Merkel, Yulia Surovtseva, Susan Baserga

**40 Co-transcriptional ribosome assembly in real-time**

Olivier Duss, Galina Stepanyuk, Sean O'Leary, Jody Puglisi, Jamie Williamson

**41 The Assembly Factor Rrp5 Coordinates the Assembly of 40S and 60S Ribosomal Subunits**

Sohail Khoshnevis, Maria Dattolo, Katrin Karbstein

**42 Charged tRNA-seq: Quantifying tRNA charging levels via high throughput sequencing**

Molly Evans, Wesley Clark, Guanqun Zheng, Tao Pan

**43 A novel class of minimal and RNA-free RNase P in the hypertermophilic bacterium *Aquifex aeolicus***

Astrid Nickel, Nadine B. Wäber, Markus Gößringer, Marcus Lechner, Walter Rossmann, Roland K. Hartmann

**44 Fragmentation of tRNAs in *Trypanosoma brucei* during stress**

Marina Cristodero, Roger Fricker, Rebecca Brogli, André Schneider, Norbert Polacek

**45 5'-TOG containing tiRNAs assemble into G-quadruplexes to promote translation repression**

Shawn Lyons, Dorota Gudanis, Christopher Achorn, Zofia Gdaniec, Paul Anderson, Pavel Ivanov

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THURSDAY, JUNE 1, 2017: 09:00 – 12:30; CONGRESS HALL

Plenary Session 2: RNA/RNP Structure

Chair: Martin Jinek, University of Zürich

Abstracts 46–57

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- 46 mRNA structural heterogeneity and extensive post-transcriptional regulation revealed by high-resolution SHAPE probing in living cells**  
*Anthony Mustoe, Steven Busan, Gregory Rice, Christine Hajdin, Brant Peterson, Vera Ruda, Neil Kubica, Jeremy Baryza, Kevin Weeks*
- 47 Enzymatic modification of eukaryotic mRNA for intracellular labeling and photo-crosslinking**  
*Andrea Rentmeister*
- 48 Hybrid structural modeling of PTBP1 bound to a structured RNA target using crosslinking of segmentally isotope labeled RNA and MS/MS**  
*Georg Dorn, Alexander Leitner, Boudet Julien, Christoph Gmeiner, Dedic Emil, Maxim Yulikov, Gunnar Jeschke, Ruedi Aebersold, Frédéric Allain*
- 49 Co-crystal structure RNA Mango: fluorescence activation of thiazole orange derivatives by an aptamer**  
*Robert Trachman, Natasha Demeshkina, Matthew Lau, Shanker Panchapakesan, Sunny Jeng, Peter Unrau, Adrian Ferré-D'Amaré*
- 50 Structural Basis for Ligand Recognition by a Guanidinium Specific Riboswitch**  
*Robert Battaglia, Ian Price, Ailong Ke*
- 51 Time-resolved structures of an aptamer domain of the adenine riboswitch by diffusion-deley-diffraction using X-ray free electron laser and serial femotosecond crystallography**  
*Jason Stagno, Yu Liu, Yuba Bhandari, Chalsie Conrad, Subrata Panja, Monalisa Swain, Lixin Fan, Tom White, Anton Barty, Dominic Oberthuer, Nadia Zatsepin, John Spence, Tom Grant, Adrian Ferre D'Maria, Mark Hunter, Henry Chapman, Sebastien Boutet, Xiaobing Zuo, Sarah Woodson, Yun-Xing Wang*
- 52 The structural switch in a viral RNA protects from 5'-3' exonucleolytic decay**  
*Anna-Lena Steckelberg, David Costantino, Jay Nix, Jeffrey Kieft*
- 53 Structure-Specific Recognition of a G-Quadruplex RNA by the Histone Demethylase LSD1**  
*William Martin, Alex Hirschi, Zigmund Luka, Manuel Ascano, Nick Reiter*
- 54 Recognition of RNA structures by quadruplex helicases and their functional consequences.**  
*Evan Booy, Ewan McRae, Markus Meier, Trushar Patel, Jorg Stetefeld, Sean McKenna*
- 55 Architecture of the yeast small subunit processome**  
*Malik Chaker-Margot, Jonas Barandun, Mirjam Hunziker, Sebastian Klinge*
- 56 Final maturation of the 60S ribosomal subunit: atomic structure the 60S subunit with the nuclear export adapter Nmd3 and the cpGTPase Lsg1**  
*Andrey Malyutin, Sharmishtha Musalgaonkar, Stephanie Patchett, Joachim Frank, Arlen Johnson*
- 57 Structural basis for guide RNA processing and seed-dependent DNA targeting by CRISPR-Cas12a**  
*Daan Swarts, John van der Oost, Martin Jinek*
- 

FRIDAY, JUNE 2, 2017: 09:00 – 12:30; CONGRESS HALL

Plenary Session 3: Splicing

Chair: Kristen Lynch, University of Pennsylvania

Abstracts 58–70

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- 58 Cryo-EM structure of *S. cerevisiae* U1 snRNP at 3.7Å resolution**  
*Xueni Li, Shiheng Liu, Jiansen Jiang, Lingdi Zhang, Sara Espinosa, Z. Hong Zhou, Rui Zhao*
- 59 Prp40 WW Domains are Critical for Efficient Splicing of Nonconsensus Introns in the Budding Yeast**  
*Luh Tung, Chung-Shu Yeh, Hsuan-Kai Wang, Fu-Lung Yeh, Jeffrey A. Pleiss, Tien-Hsien Chang*
- 60 Single-Molecule Fluorescence Microscopy Reveals the Dynamics and Consequences of Spliceosome E Complex Formation**  
*Joshua Larson, Aaron Hoskins*



- 61 Human spliceosome assembly and its regulation by splicing inhibitors at single-molecule resolution**  
*Joerg E Braun, Larry J Friedman, Jeff Gelles, Melissa J Moore*
- 62 Structural insight into the mechanism of splicing inhibition by modulators**  
*Vladimir Pena, Constantin Cretu*
- 63 Structure of a pre-catalytic spliceosome**  
*Clemens Plaschka, Pei-Chun Lin, Kiyoshi Nagai*
- 64 Prp18 promotes global second step splicing fidelity by suppressing non-canonical 3'-splice sites**  
*Jason Gabunilas, Kevin Roy, Samantha Edwards, Jonelle White, Guochang Lyu, Guillaume Chanfreau*
- 65 Structural insights into the mechanism of the DEAH-box helicase Prp43**  
*Marcel Tauchert, Jean-Baptiste Fourmann, Reinhard Lührmann, Ralf Ficner*
- 66 Potential role of transcription R-loops on alternative splicing regulation**  
*Gwendal Dujardin, Alvaro Castells Garcia, Maria Pia Cosma, Juan Valcárcel*
- 67 Splicing-associated pausing of RNA polymerase II is enforced by ubiquitination of the catalytic subunit**  
*Laura Milligan, Camille Sayou, Tomasz Turowski, Alex Tuck, Juri Rappsilber, Jean D. Beggs, Gregorz Kudla, David Tollervey*
- 68 m6A potentiates Sxl alternative pre-mRNA splicing for robust Drosophila sex determination**  
*Matthias Soller, Zsuzsa Bodi, Eugenio Sanchez-Moran, Nigel Mongan, Nathan Archer, Rupert Fray, Irmgard Haussmann*
- 69 Repeated transformations of the spliceosomal machinery and introns across eukaryotes**  
*Scott Roy*
- 70 A novel regulatory function in an ancestral splicing factor enabled the remodeling of neuronal proteomes by microexons**  
*Manuel Irimia*

**FRIDAY, JUNE 2, 2017: 14:00 – 16:15; CONGRESS HALL**

**Concurrent Session 5: RNA Localization/Transport**

*Chair: Maria Carmo-Fonseca, Instituto de Medicina Molecular Lisboa*

Abstracts 71–78

- 71 The p53 transcription factor regulates transcriptional burst frequency and RNA Polymerase II density in convoys on the p21 gene locus**  
*Adrien Senecal, Charles Kenworthy, Robert Coleman, Robert Singer*
- 72 Feed-forward TDP43-paraspeckle axis remodels alternative polyadenylation landscape to stabilize mutually exclusive states of pluripotency and differentiation**  
*Miha Modic, Gregor Rot, Markus Grosch, Tjasa Lepko, Dmitry Shaposhnikov, Davide Cacchiarelli, Boris Rogelj, Alexander Meissner, Micha Drukker, Jernej Ule*
- 73 Microscopically visible liquid-droplet P-bodies contribute minimally to miRNA-mediated gene silencing**  
*Sethuramasundaram Pitchaiya, Ameya Jaliha, Marcio Mourao, Santiago Schnell, Nils Walter*
- 74 Interactomic Studies of Single Transcripts During mRNA Maturation**  
*Peter Fridy, Erica Jacobs, Brian Chait, Michael Rout*
- 75 Cellular imaging of small RNAs using fluorescent RNA Mango aptamers**  
*Adam Cawte, Sunny Jeng, Alexis Autour, Michael Ryckelynck, Peter Unrau, David Rueda*
- 76 Systematic characterization of the subcellular distribution properties of human RNA binding proteins**  
*Xiaofeng Wang, Philip B. Bouvrette, Sahar Soltanieh, Balaji Sundararaman, Neal Cody, Eric Van Nostrand, Xuan-Tam Nguyen, Ashley Chin, Julie Bergalet, Steven Blue, Xintao Wei, Lijun Zhan, Brenton Graveley, Gene Yeo, Eric Lecuyer*
- 77 Export of discarded, splicing intermediates provides insight into mRNA export**  
*Yi Zeng, Jon Staley*

- 78 Oxidative Stress induces selective retrograde tRNA transport in the nucleus of human cells**  
*Hagen Schwenzer, Frank Jühling, Alexander Chu, Laura J. Pallett, Thomas F. Baumert, Mala Maini, Ariberto Fassati*

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**FRIDAY, JUNE 2, 2017: 14:00 – 16:15; MEETING HALL 1 (FLOOR 1)**

**Concurrent Session 6: Translation Regulation**

*Chair: Daniel Wilson, University of Hamburg*

Abstracts 79–87

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- 79 Transcription impacts the efficacy of mRNA translation via co-transcriptional N6-adenosine methylation**  
*Boris Slobodin, Ruiqi Han, Vittorio Calderone, Joachim A.F. Oude Vrielink, Fabricio Loayza-Puch, Ran Elkon, Reuven Agami*
- 80 The RNA helicase Ded1p suppresses translation initiation from near-cognate start codons**  
*Ulf-Peter Guenther, David E. Weinberg, Meghan M. Zubradt, Frank Tedeschi, Brittany Stawicki, Leah Zagore, Donny Licatalosi, Matthias W. Hentze, David P. Bartel, Jonathan S. Weissman, Eckhard Jankowsky*
- 81 An RNA structure mediated, post-transcriptional model of  $\alpha$ -1-antitrypsin expression**  
*Meredith Corley, Amanda Solem, Gabriela Phillips, Lela Lackey, Benjamin Ziehr, Heather Vincent, Nathaniel Moorman, Alain Laederach*
- 82 The force-sensing peptide VemP employs extreme compaction and secondary structure formation to induce ribosomal stalling**  
*Ting Su, Cheng Cheng, Daniel Sohmen, Rickard Hedman, Otto Berninghausen, Gunnar von Heijne, Daniel Wilson, Roland Beckmann*
- 83 The action of EF-G in translational bypassing**  
*Ekaterina Samatova, Mariia Klimova, Michael Pearson, Tamara Senjuschkina, Bee-Zen Peng, Frank Peske, Marina Rodnina*
- 84 Change in distinct ribose 2'-O-methylation impacts the ribosome to alter translation**  
*Martin Jansson, Sophia Hafner, Ulf Birkedal, Nicolai Krogh, Disa Tehler, Kasper Andersen, Sudeep Sahadevan, Henrik Nielsen, Anders Lund*
- 85 In vivo evidence that eIF3 stays bound to ribosomes elongating and terminating on short upstream ORFs to promote reinitiation**  
*Mahabub Pasha Mohammad, Vanda Munzarová Pondělíčková, Jakub Zeman, Stanislava Gunišová, Leoš Shivaya Valášek*
- 86 Mechanism of translational regulation induced by coding polyA tracks**  
*Laura Arthur, Hannah Keedy, Hani Zaher, Sergej Djuranovic*
- 87 A role for the ribosome in initiating no-go decay.**  
*Carrie Simms, Jessica Qiu, Leo Yan, Hani Zaher*
- 

**FRIDAY, JUNE 2, 2017: 17:45 – 19:15; MEETING HALL 1 (FLOOR 1)**

**Concurrent Session 7: RNA Therapeutics**

*Chair: Adrian Krainer, Cold Spring Harbor Laboratory*

Abstracts 88–92, 94

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- 88 FolamiRs: Ligand-targeted, vehicle-free microRNA replacement therapy**  
*Andrea Kasinski, Esteban Oreallana, Srinivasarao Tenneti, Loganathan Rangasamy, Philip Low*
- 89 Functional sequestration of microRNA-122 from Hepatitis C Virus by circular RNA sponges**  
*Isabelle Jost, Lyudmila A Shalamova, Michael Niepmann, Albrecht Bindereif, Oliver Rossbach*
- 90 Anti-FGF2 RNA aptamer is applicable to therapy for Achondroplasia and age-related macular degeneration**  
*Yoshikazu Nakamura, Ling Jin, Yusaku Matsuda, Yosuke Nonaka, Masatoshi Fujiwara*
- 91 RNA-biased small molecules and privileged RNA topologies for selective small molecule:RNA recognition**  
*Amanda Hargrove, Brittany Morgan, Christopher Eubanks, Neeraj Patwardhan, Anita Donlic, Jordan Forte*

## 93 Withdrawn

## 94 DNA Repair Profiling in T-cells Reveals Nonrandom Outcomes at Cas9-Mediated Breaks

**FRIDAY, JUNE 2, 2017: 17:45 – 19:15; CONGRESS HALL**

## Concurrent Session 8: Non-coding RNA

*Chair: Stefan Ameres, Institute of Molecular Biotechnology, Vienna*

Abstracts 95–101

Kai Zhang, Xiaorong Zhang, Zhiqiang Cai, Jie Zhou, Yu Zhou, Xiang-Dong Fu

*Alexandra Dallaire, Pierre-Marc Fr  d  rick, Martin Simard*

## Mi Seul Park, Hong-Duc Phan, Daniel Dayeh, Kotaro Nakanishi

Marie-Aude Tschopp, Taichiro Iki, Christopher Brosnan, Pauline Jullien, Olivier Voinnet, Nathan Pumplin

*Rastislav Horos, Anne-Marie Alleaume, Roos Kleinendorst, Abul K. Tarafder, Thomas Schwarzl, Elisabeth M. Zielonka, Asli Adak, Alfredo Castello, Wolfgang Huber, Carsten Sachse, Matthias Hentze*

*Jayakrishnan Nandakumar, Sherilyn Grill, Kamlesh Bisht, Valerie Tesmer*

## Yoav Lubelsky, Igor Ulitsky

## Plenary Session 4: Non-coding RNA

*Sponsored by RNA-TRAIN*

*Chair: Ling-Ling Chen, Shanghai Institute of Biochemistry and Cell Biology, CAS*

Abstracts 102–113

Alexandre Smirnov, Chuan Wang, Lisa L. Drewry, Jörg Vogel

*Franklin Wang-ngai Chow, Cesare Ovando-Vazquez, Georgios Koutsovoulos, Jose Roberto Bermúdez-Barrientos, Tuhin Maity, Mark Blaxter, Julie Claycomb, Cei Abreu-Goodger, Amy Buck*

*Kyungmin Baeg, Hiro-oki Iwakawa, Yukihide Tomari*

*Thomas Treiber, Nora Treiber, Simone Harlander, Uwe Plessmann, Henning Urlaub, Gunter Meister*

- 106 Uridylation-triggered RNA decay acts as a multiple turnover factor in antiviral RNA interference**  
*Raphael A. Manzenreither, Madalena Reimão-Pinto, Thomas Burkard, Stefan L. Ameres*
- 107 Transcription of animal small RNA source loci by coupling of RNA polymerase II PIC formation to heterochromatin marks**  
*Peter Refsing Andersen, Laszlo Tirian, Milica Vunjak, Julius Brennecke*
- 108 Transcriptional architecture and regulation of mammalian noncoding RNAs**  
*Claudia Kutter, Tim Rayner, Nenad Bartonicek, Bianca Schmitt, Aisling Radmond, Christina Ernst, Duncan Odom*
- 109 Antisense transcriptional interference as a common mean of strengthening gene repression in budding yeast**  
*Alicia Nevers, Antonia Doyen, Christophe Malabat, Thomas Kergrohen, Alain Jacquier, Gwenaël Badis*
- 110 Long Non-coding RNAs in Oocyte and Oocyte-to-Zygote Transition**  
*Shravya Ganesh, Rosa Karlic, Vedran Franke, Eliska Svobodova, Jana Urbanova, Yutaka Suzuki, Fugaku Aoki, Kristian Vlahovicek, Petr Svoboda*
- 111 Circular RNAs generated by readthrough transcription**  
*Deirdre Tatomer, Dongming Liang, Zheng Luo, Huang Wu, Li Yang, Ling-Ling Chen, Jeremy Wilusz*
- 112 The specific domains of architectural NEAT1 lncRNA induces the formation of the distinct phase-separated paraspeckle nuclear body**  
*Tomohiro Yamazaki, Taro Mannen, Sylvie Souquere, Hisanori Kiryu, Archa H. Fox, Shinichi Nakagawa, Gerard Pierron, Tetsuro Hirose*
- 113 The biogenesis and functional implication of circular RNAs**  
*Xiang Li, Chu-Xiao Liu, Wei Xue, Li Yang, Ling-Ling Chen*

**SATURDAY, JUNE 3, 2017: 14:30 – 17:15; PANORAMA HALL (FLOOR 1)**

**Concurrent Session 9: RNA in Disease**

*Chair: Javier Martinez, Max F. Perutz Labs. Medical University of Vienna*

Abstracts 114–121

- 114 Functional specificity of SR and SR-like proteins in breast cancer initiation and metastasis**  
*Olga Anczuków, Martin Akerman, Shipra Das, SungHee Park, Anil Kesarwani, Jie Wu, Kuan-Ting Lin, Senthil Muthuswamy, Adrian Krainer*
- 115 Disease mutations in splicing factor SF3b1 alter macroautophagy**  
*Susana Rodriguez-Santiago, John Christin, Andrea Yuste, Eloy Bejarano, Charles Query*
- 116 The FAM46C gene encodes a non-canonical poly(A) polymerase and acts as an onco-suppressor in multiple myeloma**  
*Seweryn Mroczek, Justyna Chlebowska, Tomasz Kulinski, Olga Gewartowska, Jakub Gruchota, Dominik Cysewski, Vladyslava Liudkovska, Ewa Borsuk, Dominika Nowis, Andrzej Dziembowski*
- 117 RNA virus infection induces a global remodelling in the host repertoire of RNA-binding proteins**  
*Manuel Garcia-Moreno, Marko Noerenberg, Shuai Ni, Esther González-Almela, Thomas Davis, Marcel Bach, Svenja Hester, Bingnan Li, Miguel A Sanz, Luis Carrasco, Vicent Pelechano, Shabaz Mohammed, Bernd Fischer, Alfredo Castello*
- 118 SR and hnRNP proteins regulate HIV-1 multiplication by modulating mRNA splicing and translation**  
*Lilia Ayadi, Georges Khoury, Jean Michel Saliou, Alain Van Dorsselaer, Sarah Sanglier-Cianferani, Isabelle Behm-Ansmant, Christiane Branlant*
- 119 SNORD116 missing in Prader-Willi syndrome regulates mRNA stability and microexon usage**  
*Justin R. Welden, Marina Falaleeva, Lawrence T. Reiter, Stefan Stamm*
- 120 ZFR coordinates crosstalk between RNA decay and transcription in innate immunity**  
*Nazmul Haque, Ryota Ouda, Keiko Ozato, J. Robert Hogg*
- 121 Regulation of translation of collagen mRNAs by binding of LARP6 to the 5' stem-loop; key mechanism to fibrosis development.**  
*Branko Stefanovic, Yujie Zhang, Lela Stefanovic*



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**SATURDAY, JUNE 3, 2017: 14:30 – 17:15; CONGRESS HALL**  
**Concurrent Session 10: Regulation & Mechanisms of RNA Turnover**  
*Chair: Kristian Baker, Case Western Reserve University*  
 Abstracts 122–130

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- 122 Nucleo-cytoplasmic shuttling as means to regulate coupling between mRNA synthesis and decay**  
*Shiladitya Chattopadhyay, Gal Haimovich, Oren Barkai, Silvia G. Chuartzman, Maya Schuldiner, Miriam Rosenberg, Katherine E. Sloan, Marcus T. Bohnsack, Mordechai Choder*
- 123 Regulation of the *Drosophila* maternal-to-zygotic transition by RNA-binding proteins and microRNAs**  
*Howard Lipshitz*
- 124 Maternal mRNA clearance via terminal uridylation during the maternal-to-zygotic transition**  
*Jinah Yeo, Hyesik Chang, Jung-Kyun Kim, Hyunjun Kim, Mihye Lee, Jaechul Lim, Hosung Jung, Kyuwon Kim, V. Narry Kim*
- 125 Identification of diverse target RNAs that are functionally regulated by human Pumilio proteins, PUM1 and PUM2**  
*Aaron Goldstrohm, Jennifer Bohn, Jamie Van Etten, Trista Schagat, Richard McEachin, Peter Freddolino*
- 126 Reconstitution of mRNA substrate selection by the Ccr4-Not deadenylase complex**  
*Michael W. Webster, James A. Stowell, Lori A. Passmore*
- 127 Mpp6 incorporation into the nuclear exosome contributes to RNA channeling through the Mtr4 helicase**  
*Sebastian Falk, Judith Ebert, Fabien Bonneau, Jerome Basquin, Elena Conti*
- 128 Comparative analysis of Arabidopsis mutants revealed distinct impact of the core non-sense mediate RNA decay factors on transcriptome homeostasis**  
*Vivek Raxwal, Jiradet Gloggnitzer, Gordon Simpson, John Brown, Karel Riha*
- 129 ATP hydrolysis by UPF1 promotes translation termination at premature stop codons**  
*Lucas Serdar, DaJuan Whiteside, Kristian Baker*
- 130 The Rapid tRNA Decay Pathway in Yeast Comprehensively Monitors the Structure of tRNAs as well as their Charging Status**  
*Matthew Payea, Isreal Moreno, Eric Phizicky*
- 

**SATURDAY, JUNE 3, 2017: 14:30 – 17:15; MEETING HALL 1 (FLOOR 1)**  
**Concurrent Session 11: RNA Catalysis/Folding**  
*Chair: Jeffrey Kieft, University of Colorado*  
 Abstracts 131–139

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- 131 Uncovering the Origins of Cooperativity in a Linked Secondary and Tertiary RNA Folding Transition**  
*Brant Gracia, Hashim Al-Hashimi, Rhiju Das, Daniel Herschlag, Rick Russell*
- 132 Molecular architecture and dynamics of an mRNA-transport complex while recognizing a *cis*-acting localization element of its target mRNA in yeast**  
*Franziska Theresia Edelmann, Andreas Schlundt, Andreas Jenner, Annika Niedner-Boblenz, Muhammad Ibrahim Syed, Jean-Christophe Paillart, Michael Sattler, Ralf-Peter Jansen, Dierk Niessing*
- 133 Folding and splicing of group II intron ribozymes at the single molecule level**  
*Roland KO Sigel*
- 134 Repuckering at the Nucleophilic Ribose of a Catalytic RNA Can Be Observed by NMR and Is Obligatory for Function**  
*Neil White, Mina Sumita, Patrick Ochieng, Charles Hoogstraten*
- 135 The structure and mechanism of the TS nucleolytic ribozyme**  
*Timothy Wilson, Yijin Liu, David Lilley*
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**136 Mechanism for activation of the 2' OH nucleophile in the *glmS* ribozyme revealed by inverse thio effects, active site mutants, and cofactor variants**

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*yuanchao xue*

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**563 The Role of PIWIL1 in Gastric Cancer**

*Shuo Shi, Na Liu, Zifeng Wang, Zhaoran Zhang, Sanhong Liu, Haifan Lin*

**564 Muscle-specific RNA biogenesis and satellite cell defects in a novel mouse model of OPMD**

*Katherine Vest, Ayan Banerjee, Luciano Apponi, Hyojung Choo, Bin Tian, Grace Pavlath, Anita Corbett*

**565 Sandwich-typed assays for microRNA quantification using pyrene-modified probes**

*Yu Warari, Jumpei Ariyoshi, Yuta Sugihara, Asako Yamayoshi, Akira Murakami, Akio Kobori*

**566 Analysis of the RNA methyltransferase EMG1 and the molecular basis of Bowen-Conradi syndrome**

*Ahmed S. Warda, Bernard Freytag, Sara Haag, Katherine E. Sloan, Dirk Görlich, Markus T. Bohnsack*

**567 The splicing factor SRSF1 associates with the 5S RNP and is important for both 5S RNP-mediated p53 signalling and the efficient incorporation of the 5S RNP into the ribosome**

*Loren Gibson, Zhao Zhao, Claudia Schneider, Nicholas Watkins*

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**568 Structural basis for the translational repression of *hunchback* mRNA by the Brat-Pumilio-Nanos complex**

*Jaelle Foot, Jakub Macosek, Pawel Masiewicz, Sophie Winter, Frank Gabel, Bernd Simon, Inga Loedige, Janosch Hennig*

**569 A Network Approach Reveals Novel Roles of Low Complexity Domains in Spliceosomal Proteins**

*Isaac Bishof, Eric Dammer, Duc Duong, Nicholas Seyfried*

**570 Structure of the 90S pre-ribosome prior to A1 pre-rRNA cleavage**

*Jingdong Cheng, Nikola Kellner, Otto Berninghausen, Ed. Hurt, Roland Beckmann*

**571 Unusual semi-extractability as the hallmark of nuclear body-associated architectural long noncoding RNAs**

*Takeshi Chujo, Tomohiro Yamazaki, Tetsuya Kawaguchi, Satoshi Kurosaka, Shinichi Nakagawa, Tetsuro Hirose*

**572 The effect of CHH-mutations on RNase MRP biochemistry**

*Merel Derksen, Ger Pruijn*

**573 The diversified role of RNA binding proteins in *Trypanosoma cruzi***

*Lysangela R. Alves, Camila Oliveira Antes, Sharon Martins, Juliane Malgarin, Helissa Wippel, Newton Vidal, Bruno Romagnoli, Samuel Goldenberg*

**574 RNA structural dynamics govern H/ACA ribonucleoprotein function**

*Gerd Hanspach, Andreas Schmidt, Martin Hengesbach*

**575 Telomerase attraction to DNA: What is behind processivity enhancement by human TPP1**

*Ctirad Hofr*

**576 U6-specific factor SART3 participate in biogenesis of Sm-class snRNPs**

*Klara Klimesova, David Stanek*

**577 A novel dsRNA-binding module from prolyl isomerase FKBP25**

*David Dilworth, Santosh Kumar Upadhyay, Pierre Bonnafoos, Amiirah Bibi Edoo, Francy Pesek-Jardim, Geoff Gudavicius, Chris Nelson, Cameron Mackereth*

**578 Components of the SMN complex associate with pre-box H/ACA RNPs during their assembly**

*Florence Schlotter, Maxime Dodré, Alexandra Huttin, Christiane Branlant, Séverine Massenet*

**579 Functions of Bcd1p in box C/D snoRNP biogenesis**

*Arnaud PAUL, Florence SCHLOTTER, Helene MARTY, Bruno CHARPENTIER, Severine MASSENET, Stéphane LABIALLE*

**580 Mechanism of PWI Domain Binding to Nucleic Acids***Harry Chanzu, Nafiseh Jandaghijafari, Elizabeth Lopez, Blair Szymczyna***581 Genome-wide identification of RNA sensors in prokaryotes and eukaryotes***Yue Wan***582 Transcript specific mRNP capture of locally translated mRNAs in *Drosophila* oogenesis***Frank Wippich, Imre Gáspár, Anne Ephrussi*

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**583 Atomic structure of the activated spliceosome***Chuangye Yan, Ruixue Wan, Rui Bai, Gaoxingyu Huang, Yigong Shi***584 Insights into the Functions of SF3b1 and Prp5 during Branchsite Recognition and in Spliceosome Fidelity***Tucker Carrocci, Joshua Paulson, David Beier, Aaron Hoskins***585 The Hydrolytic Splicing of Group II Intron Ribozymes: A Novel Two-Metal-Aided Mechanism Revealed by QM/MM MD Simulations***Lorenzo Casalino, Giulia Palermo, Ursula Rothlisberger, Alessandra Magistrato***586 Protein-RNA Interactions during Catalytic Steps of Pre-mRNA Splicing***Che-Sheng Chung, Chi-Kang Tseng, Ching-Yang Kao, Soo-Chen Cheng***587 Structure and Function of U1 snRNP***Sara Espinosa, Xueni Li, Lingdi Zhang, Rui Zhao***588 Modulation of the spliceosomal catalytic triplex by U6 snRNA and *cwc2* alleles***Katarzyna Eysmont, Magda Konarska***589 A novel type of intronic circRNA in nuclear genes of euglenids (Euglenida)***Natalia Gumińska, Rafał Milanowski***590 Impaired spermatogenesis in U12 intron splicing-defective *Zrsr1* mutant mice***Keiko Horiuchi, Serafín Perez-Cerezales, Panagiotis Papasaikas, Maria I Fabre, Belén Pintado, Alfonso Gutiérrez-Adán, Juan Valcárcel***591 The Function of the Cassette Exon 7b of the Transcription Factor ERG***Samantha Jumbe, Bahareh Vahabi, Ian Wilson, Michael Lodomery***592 Structural studies of diverse regulatory layers of U2AF in 3' splice site recognition***Hyun-Seo Kang, Carolina Sanchez-Rico, Reymond Sutandy, Stefanie Ebersberger, Sophie Bonnal, Juan Valcarcel, Julian König, Michael Sattler***593 Human variant U2 snRNAs provide diverse platforms for intron recognition***Brian Kosmyna, Varun Gupta, Charles Query***594 Capture of mRNA fragments and genic *trans*-splicing mediated by a bacterial group II intron during circularization***Felix LaRoche-Johnston, Caroline Monat, Samy Coulombe, Benoit Cousineau***595 Dissecting the Role of Dib1 in Spliceosome Assembly***Christian Schreib, Cody Hernandez, Emily Bowman, Camille Potts, Amber Lucas, Corina Maeder***596 Deep conservation of the neural-specific microexon alternative splicing program across bilaterians***Yamile Marquez, Jon Permanyer, Andre Gohr, Manuel Irimia***597 Prp8 affects conformational changes in the spliceosome between the catalytic steps***Katarzyna Matylla-Kulinska, Lukasz Widlo, Magda Konarska***598 Brr2 is a pre-mRNA splicing fidelity factor***Megan Mayerle, Christine Guthrie***599 Interrogating the Importance of RNA Constituency in Splice Site Selection***Linus F. Reichenbach, Andrew Jobbins, Ian C. Eperon, Glenn A. Burley***600 5' splice-sites selection by hnRNP L through multiple exon motifs in KLF6 and Bcl-x pre-mRNA alternative splicing***Tiing Jen Loh, Namjeong Choi, Jiyeon Ha, Jagyeong Oh, Xuexiu Zheng, Haihong Shen*

**601 Therapeutic splicing correction by U1 snRNP-mediated redefinition of a critical exon of spinal muscular atrophy gene**

*Natalia Singh, José Del Rio-Malewski, Lou Diou, Eric Ottesen, Matthew Howell, Ravindra Singh*

**602 The role of splicing factor gene mutation in craniofacial disorders**

*Huw Thomas, Moses Tandiono, Robert Baines, Cortland Linder, Kathryn Hentges, William Newman, Raymond O'Keefe*

**603 Birth of a bipartite exon by intragenic deletion**

*Kandai Nozu, Kazumoto Iijima, Jana Kralovicova, Toru Igarashi, Shiro Yamada, Yoshimi Nozu, Igor Vorechovsky*

**604 Structural investigations of the spliceosome: insights into the transition between two steps during RNA splicing**

*Ruixue Wan, Chuangye Yan, Rui Bai, Gaoxingyu Huang, Yigong Shi*

**605 Caught in Action: How DExD/H-box protein Prp28p Interacts with Spliceosome during Pre-mRNA Splicing**

*Fu-Lung Yeh, Shang-Lin Chang, Luh Tung, Tien-Hsien Chang*

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**606 Towards the understanding of the mechanisms responsible for alternative splicing modulation by mild heat shock**

*Christelle Aigueperse, Valentin Vautrot, Luisa Vigevani, Panagiotis Papasaikas, Nathalie Nicot, Tony Kaoma, Pierre de la Grange, Juan Valcarcel, Christiane Branlant, Isabelle Behm-Ansmant*

**607 In vivo dynamics of SR protein-RNA interactions**

*Serena Capozzi, Eugenia Basyuk, Stéphanie Boireau, Marie-Cécile Robert, Antoine Cléry, Frédéric Allain, Edouard Bertrand*

**608 A novel function for Exon Junction Complex as a repressor of recursive splicing of canonical exons**

*Lorea Blazquez, Warren Emmett, Chris Sibley, Jernej Ule*

**609 Unravelling the molecular determinants of splice site selection with chemical biological tools**

*Glenn Burley, Linus Reichenbach, Carika Weldon, Andrew Jobbins, Helen Lewis, Andrew Perrett, Cyril Dominguez, Ian Eperon*

**610 Spliceosome and Micro-processing interactions**

*Luba Farberov, Daphna Weissglas-Volkov, Noam Shomron*

**611 Regulation of circRNA formation by SR proteins and MALAT1 in hypoxia stress**

*Camila Freitas Stahl, Antonella Di Liddo, Nicolas Jaé, Kathi Zarnack, Stefanie Dimmeler, Michaela Müller-McNicoll*

**612 A splicing-based thermometer controls circadian gene expression in mammals**

*Marco Preussner, Gesine Goldammer, Alexander Neumann, Tom Haltenhof, Florian Heyd*

**613 SPSB1-Mediated HnRNP A1 Ubiquitylation Regulates Alternative Splicing and Cell Migration in EGF Signaling**

*Feng Wang, Xing Fu, Peng Chen, Ping Wu, Xiaojuan Fan, Na Li, Hong Zhu, Hongbin Ji, Zefeng Wang, Catherine Wong, Ronggui Hu, Jingyi Hui*

**614 VastDB: an Atlas of Alternative Splicing Profiles and Functional Associations in Vertebrate Tissues and Cell Types**

*Javier Tapial, Kevin Ha, Timothy Sterne-Weiler, Andre Gohr, Ulrich Braunschweig, Antonio Hermoso-Pulido, Mathieu Quesnel-Vallières, Jon Permanyer, Luca Cozzuto, Xinchun Wang, Melisa Gómez-Velázquez, Teresa Rayon, Miguel Manzanares, Julia Ponomarenko, Benjamin Blencowe, Manuel Irimia*

**615 A Repressor Candidate of Cancer Specific mRNA Re-splicing: A Key factor for splicing fidelity or mRNA quality control?**

*Toshiki Kameyama, Kazuhiro Fukumura, Kunio Inoue, Tetsuro Hirose, Akila Mayeda*

**616 Identification and Characterization of a Minimal Functional Splicing Regulatory Protein**

*Jordan Ontiveros, Adrian Hernandez, Archana Shankar, Justin Doan, Eric Adams, Douglas Black, Niroshika Keppetipola*

- 617 Loss of p105 function induces aberrant mRNA splicing and DNA damage**  
*Jee-San Lee, Eun-Ran Park, Hyun-Yoo Joo, Hyun-Jin Shin, Kee-Ho Lee*
- 618 Single cell sequencing of preimplantation embryos reveals early dynamic of alternative splicing independent of transcription change**  
*Jiefu Li, Zefeng Wang*
- 619 Cryptic exons are repressed by a family of dinucleotide repeat binding splicing factors**  
*Jonathan Ling, Kristen Lynch, Philip Wong*
- 620 Y14 governs p53 expression and modulates DNA damage sensitivity**  
*Chia-Chen Lu, Chi-Chieh Lee, Ching-Tzu Tseng, Woan-Yuh Tarn*
- 621 Study of the ageing-related alternative pre-mRNA splicing of the LMNA gene**  
*Evgeny Makarov, Olga Makarova*
- 622 Withdrawn**
- 623 Systematic survey of RNA binding proteins in human genome for their activity to regulate alternative splicing**  
*Miaowei Mao, Zefeng Wang*
- 624 Flipping the switch on Sex-lethal expression: Sister of Sex-lethal antagonizes Sxl-dependent alternative splicing to maintain a male-specific gene expression pattern in *Drosophila***  
*Rebecca Moschall, Oliver Rossbach, Gerhard Lehmann, Norbert Eichner, Daniela Strauss, Nicholas Strieder, Michael Krahn, Jan Medenbach*
- 625 Function of ubiquitin-fold containing Sde2 as an intron-specific pre-mRNA splicing regulator**  
*Shravan Kumar Mishra, Poonam Thakran, Prashant Pandit, Sumanjit Datta, Jeffrey Pleiss*
- 626 A system to study rate-limiting regulation by the minor spliceosome in healthy cells and cell culture models of minor spliceosomal diseases**  
*Antto J. Norppa, Mikko J. Frilander*
- 627 Development of RNA G-quadruplex Stabilizers as Splice-Switching Inducers of Apoptosis**  
*Olivia I. Paisley, Carika Weldon, Cyril Dominguez, Ian C. Eperon, Laurence H. Hurley, Glenn A. Burley*
- 628 Characterization of splicing modulator DDU40800**  
*Andrea Pawellek, Ursula Ryder, Marek Gierlinski, Andrew Woodland, David Gray, Angus Lamond*
- 629 The Splicing Program of SRSF1 transcripts is finely tuned by cell metabolism**  
*Daniela Pignataro, Morena Catillo, Antonina Di Maio, Giulia Brenna, Giuseppe Biamonti*
- 630 Global analysis of alternative splicing upon differentiation of human monocytes reveals MBNL1 as a major regulator**  
*Hongfei Liu, Paolo Lorenzini, Fan Zhang, Jie Zheng, Xavier Roca*
- 631 Exonization of an intronic Alu element alters the critical C-terminus of Survival Motor Neuron protein**  
*Joonbae See, Natalia Singh, Eric Ottesen, Brian Lee, Ravindra Singh*
- 632 High-throughput screening for splicing regulatory regions**  
*Samarth Thonta Setty, Simon Braun, Mihaela Enculescu, Anke Busch, Stefanie Ebersberger, Stefan Legewie, Julian König, Kathi Zarnack*
- 633 The regulation of alternative mRNA splicing by CDK12 supports the tumorigenic potential of breast cancer cells**  
*Jerry Tien, Alborz Mazloomian, S.-W. Grace Cheng, Christopher Hughes, Alvin Liu, Christalle Chow, Leanna Canapi, Arusha Oloumi, Genny Trigo-Gonzalez, Ali Bashashati, James Xu, Vicky Chang, Sohrab Shah, Samuel Aparicio, Gregg Morin*
- 634 PTBP2 is regulated by U11/U12 di-snRNP**  
*Bhupendra Verma, Ali Oghabian, Mikko Frilander*
- 635 Hypoxia induces changes in alternative pre-mRNA splicing that may lead to tumour cell survival**  
*Laurynas Vilys, Inga Peciuliene, Egle Jakubaskiene, Arvydas Kanopka*
- 636 Regulation of alternative splicing of U2AF1**  
*Igor Vorechovsky, Jana Kralovicova*



**637 Investigating the role of SR proteins in nuclear surveillance**

*Marius Wegener, Camila Freitags Stahl, Michaela Mueller McNicoll*

**638 hnRNP DL, or: the forgotten brother**

*Katrin Kemmerer, Sandra Fischer, Julia E. Weigand*

**639 Molecular basis of aberrant CFTR exon 9 splicing driven by TDP-43 and hnRNP A1**

*Maria Zlobina, Christelle Beaumont, Peter Lukavsky*

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**640 Induction Of Secreted Soluble Decoy EGFR Variants By Splicing Interference Inhibits Signaling And Tumor Growth In Treatment-Refractory Lung Cancer**

*Jeong Eun Park, Lee Spraggon, Elisa de Stanchina, Luca Cartegni*

**641 Antisense oligonucleotides delivered to the amniotic cavity *in utero* modulate gene expression in the postnatal mouse**

*Frederic Depreux, Lingyan Wang, Han Jiang, Francine Jodelka, Robert Rosencrans, Frank Rigo, Jennifer Lentz, John Brigande, Michelle Hastings*

**642 IL-8 as a Pharmacodynamic Biomarker of Trabectedin (OT-101) - An anti-TGF- $\beta$ 2 Antisense Drug - Results of a Phase I/II Study in Pancreatic Cancer Patients**

*Larn Hwang, Wen Wang, Sanjive Qazi, Kevin Ng, Osmond D'Cruz, David Nam, Tapas De, Hankuk Lee, Chulho Park*

**643 Preclinical Proof of Concept for Treatment for Inflammatory Bowel Disease (IBD) with Bacterial Delivery of Therapeutic Short Interfering RNAs (siRNAs)**

*Osmond D'Cruz, Kevin Ng, Wen Wang, David Nam, Joseph Ramelli, Falguni Trieu, Mihir Munsif, Larn Hwang*

**644 tkRNAi-mediated  $\beta$ -catenin knockdown in the Gastrointestinal Mucosa Familial Adenomatous Polyposis Patients. Results of START-FAP trial.**

*Larn Hwang, Kevin Ng, Osmond D'Cruz, Sanjive Qazi, Wen Wang, David Nam, Joseph Ramelli, Falguni Trieu, Mihir Munsif, Annie Fong*

**645 Modulation of MKNK2 Alternative Splicing by Antisense Oligonucleotides as Novel Cancer Therapy**

*Maxim Mogilevsky, Odelia Shimshon, Saran Kumar, Eylon Yavin, Rotem Karni*

**646 Targeting GRHL2 and CDH1 simultaneously using small artificial RNAs induces an epithelial-mesenchymal transition in MCF7 human breast cancer cells**

*Blandine Monjarret, Roqaya Imane, Nicolas Scott, Sylvie Mader, François Major*

**647 Targeted inhibition of WRN helicase, replication stress and cancer**

*Natalie Orlovetskie, Nayef Jarrous*

**648 Readthrough inducing potential of glutamine isoacceptor tRNAs lies in their primary sequence outside of the anticodon**

*Zuzana Pavlíková, Petra Beznosková, Jakub Zeman, Leoš Shivaya Valášek*

**649 Control of breast cancer metastasis by microRNAs**

*Nir Pillar, Avital Polsky, Daphna Weissglas-Volkov, Noam Shomron*

**650 Prophylactic and therapeutic potential of 5'PPP-NS1-shRNA against influenza infection**

*Neetu Singh, Jenish Patel, Weiping Cao, Shivaprakash Ganggapa, Paras Prasad, Suryaprakash Sambhara, Paul Knight, Priya Ranjan*

**651 In a search for an ideal IVT mRNA to engineer human mesenchymal stem cells**

*Justyna Rozycka, Adam Nowakowski, Piotr Walczak, Barbara Lukomska, Mirosław Janowski*

**652 Protecting brain after stroke by modulating noncoding RNAs**

*Raghu Vemuganti, Suresh Mehta, Mary Lopez, TaeHee Kim, Robert Dempsey*

**653 RNA Dendrimer Platform for Nanomedicine**

*Mario Vieweger, Farzin Haque, Peixuan Guo*



**654 Antisense oligonucleotide mediated rescue of aberrant COL17A1 splicing in junctional epidermolysis bullosa**

*Hannah Potocki, Nicole Friedl, Thomas Lettner, Monika Wimmer, Roland Zauner, Michael Ablinger, Johann W. Bauer, Julia Reichelt, Verena Wally*

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**655 RiboLace: a highly sensitive method for active ribosomes profiling**

*Massimiliano Clamer, Toma Tebaldi, Fabio Lauria, Paola Bernabò, Daniele Gubert, Graziano Guella, Alessandro Quattrone, Gabriella Viero*

**656 The IRES<sub>5'UTR</sub> of the dicistrovirus Cricket Paralysis Virus contains an essential pseudo-knot structure for its translational activity**

*Lauriane Gross, Quentin Vicens, Evelyne Einhorn, Audrey Noireterre, Laure Schaeffer, Jean-Luc Imler, Gilbert Eriani, Carine Meignin, Franck Martin*

**657 Consecutive elongation of D-amino acids in translation**

*Takayuki Katoh, Kenya Tajima, Hiroaki Suga*

**658 Ribosomal protein L9 in translational bypassing**

*Mariia Klimova, Ekaterina Samatova, Heena Sharma, Marina Rodnina*

**659 Elongation of the Nascent Peptide in the Ribosomal Exit Tunnel**

*Michal H. Kolar, Lars V. Bock, Andrea C. Vaiana, Helmut Grubmueller*

**660 Building a reporter system to assess repeat-associated translation: Application to screen genetic modulators of microsatellite expansion disorders**

*Hicham Mahboubi, Thomas Harper, Nicole Meisner-Kober, Michael Schebesta, Mark Labow*

**661 Structure of ABCE1 in the context of a native mammalian 48S initiation complex**

*Eder Mancera-Martínez, Jailson Brito Querido, Angelita Simonetti, Yaser Hashem*

**662 Characterization of an Internal Ribosome Entry Site (IRES) in p53 mRNA**

*Ana Rita Neves, Rafaela Lacerda, Ana Marques-Ramos, Luisa Romão, Michiyuki Matsuda, Marco M. Candeias*

**663 Selective recognition and transport of misfolded polypeptides toward aggresome via the CED complex**

*Yeonkyoung Park, Joori Park, Incheol Ryu, Mi-Hyun Choi, Hyo Jin Lee, Nara Oh, Kyutae Kim, Kyoung Mi Kim, Junho Choe, Cheolju Lee, Ja-Hyun Baik, Yoon Ki Kim*

**664 Analyses of cell type specific translation from IRES mRNA derived from two different poliovirus strains**

*Akitoshi Sadahiro, Akira Fukao, Kent Duncan, Osamu Takeuchi, Toshinobu Fujiwara*

**665 The Impact of N1-Methyladenosine on Translation**

*Erica Thomas, Hani Zaher*

**666 Eukaryotic translation initiation factor 3 undergoes dramatic structural changes prior to its binding to the 40S ribosomal subunit**

*Jakub Zeman, Michal Rosulek, Petr Novák, Leoš Valášek*

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**667 DDX3 regulates translation of ATF4 during stress**

*Pauline Adjibade, Valérie Grenier St-Sauveur, Anahita Bakhshizadeh-Gashti, Edouard W. Khandjian, Rachid Mazroui*

**668 Ribosome rearrangements at the onset of translational bypassing**

*Xabier Agirrezabala, Ekaterina Samatova, Mariia Klimova, Miguel Zamora, David Gil-Carton, Marina Rodnina, Mikel Valle*

**669 The application of *in vitro* translation assays to study the regulation by small bacterial RNAs and the Hfq protein**

*Marta Antoniewicz, Marcin Osuch, Zuzanna Wroblewska, Joanna Mazur, Mikolaj Olejniczak*

**670 Translational regulation in embryonic development and homeostasis**

*Deniz Bartsch, Wenjie Yao, Thai Bao Nguyen, Shashank Sathe, Marie Gramm, Stefan Frank, Gaurav Ahuja, Nicole Russ, Gene Yeo, Leo Kurian*

**671 Cellular adhesion is regulated by mRNA translation in Spreading Initiation Center**

*Jonathan Bergeman, Marc-Étienne Huot*

**672 In vivo translatoome profiling reveals early defects in ribosome biology underlying SMA pathogenesis**

*Paola Bernabò, Toma Tebaldi, Ewout JN Groen, Fiona M Lane, Elena Perenthaler, Francesca Mattedi, Helen J Newbery, Haiyan Zhou, Paola Zuccotti, Valentina Potrich, Francesco Muntoni, Alessandro Quattrone, Thomas H Gillingwater, Gabriella Viero*

**673 Cellular levels of readthrough inducing tRNAs and their consequences in yeast and mammals**

*Petra Beznoskova, Jakub Zeman, Zuzana Pavlikova, Leos Valasek*

**674 Structure of Ty1 internally initiated RNA influences restriction factor expression**

*Leszek Blaszczyk, Marcin Biesiada, Agniva Saha, David Garfinkel, Katarzyna Purzycka*

**675 Cryo-EM structure of ABCE1/40S complex reveals new insights on translation in *Trypanosoma cruzi***

*Jailson Brito Querido, Eder Mancera-Martínez, Angelita Simonetti, Yaser Hashem*

**676 Withdrawn**

**677 The novel function of PABP interacting protein 1 (Paip1) in translation initiation**

*Tomohiko Aoyama, Akira Fukao, Nahum Sonenberg, Toshinobu Fujiwara*

**678 tFRAP: A FRAP-based technique to monitor protein translation in living cells**

*Nadine Griesche, Verena Pesch, Rohit Nalavade, Stephanie Weber, Sybille Krauß*

**679 The *Schizosaccharomyces pombe* Ppr10-Mpa1 complex plays a general role in mitochondrial protein translation**

*Yirong Wang, Jianhua Qan, Qingzhen Zhang, Xuting Ma, Juan Zhang, Minghui Su, Xiaojun Wang, Yang Su, Ying Huang*

**680 HnRNP Q1 translationally increases the expression of Aurora-A and promotes tumorigenesis in colorectal cancer**

*Liang-Yi Hung, Chien-Hsien Lai*

**681 Processing bodies regulate selective translation in photomorphogenic Arabidopsis**

*Geng-Jen Jang, Shu-Hsing Wu*

**682 The human CCHC-type Zinc Finger Nucleic Acid Binding Protein binds G-rich elements in target mRNA coding sequences and promotes translation**

*Daniel Benhalevy, Sanjay Gupta, Charles Danan, Suman Ghosal, Hong-Wei Sun, Hinke Kazemier, Katrin Paeschke, Markus Hafner, Stefan Juranek*

**683 Ribosomal dysfunction and defective erythropoiesis in a zebrafish model of Diamond-Blackfan anemia**

*Tamayo Uechi, Maki Yoshihama, Yukari Nakajima, Yutaka Suzuki, Sumio Sugano, Etsuro Ito, Naoya Kenmochi*

**684 Structural determination of the human RISC-Loading Complex**

*Rasmus Kock Flygaard, Christian Kroun Damgaard, Lasse Bohl Jenner*

**685 p53 mutations influence IRES-dependent expression of p53 isoforms**

*Marco M. Candeias, Rafaela Lacerda, Ana Rita Neves, Luísa Romão, Michiyuki Matsuda*

**686 T-cell-restricted intracellular antigen 1 facilitates mitochondrial fragmentation by enhancing translation of mitochondrial fission factor mRNA**

*Hyosun Tak, Eun Kyung Lee*

**687 Conserved non-AUG uORFs revealed by a novel regression analysis of ribosome profiling data**

*Pieter Spealman, Rumi Naik, Gemma May, Scott Kuersten, Lindsay Freeberg, Robert Murphy, Joel McManus*

**688 Widespread presence of internal translation initiation sites in *Escherichia coli***

*Sezen Meydan, Dorota Klepacki, James Marks, Nora Vazquez-Laslop, Alexander S. Mankin*

- 689 Vga(A)LC from Staphylococcus haemolyticus directly protects ribosome against antibiotics in an ATP-dependent manner**  
*Victoria Murina, Vasili Hauryliuk, Gemma Atkinson*
- 690 Dicer-2 is a cytoplasmic polyadenylation factor**  
*Hima Priyanka Nadimpalli, Olga Coll, Tanit Guitart, Fátima Gebauer*
- 691 eRF1 translation termination factor is controlled by an autoregulatory circuit involving readthrough and nonsense-mediated decay in plants**  
*Tünde Nyikó, Andor Auber, Dániel Silhavy*
- 692 Functional relation of hnRNP K - rpS19 crosstalk in erythroid cell maturation**  
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- 693 Structure of modified mRNAs modulates protein expression**  
*David Mauger, Joseph Cabral, Kamal Sandhu, David Reid, Nikhil Khatwani, Stephen Su, Brooke Goodman, Kristian Link, Vladimir Presnyak, John Joyal, Melissa Moore, Iain McFadyen*
- 694 Regulatory mechanisms modulating the function of eukaryotic translation repressors**  
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- 695 Probing a translational switch involved in tumor angiogenesis**  
*Somdeb Mitra, Maya Ramachandran, Ruben Gonzalez*
- 696 Coherent mRNA Assemblies of Ion Channel Transcripts Underlying the Cardiac Action Potential**  
*Erick Rios-Perez, Catherine Eichel, Fang Liu, David Jones, Gail Robertson*
- 697 Translation of human mTOR can be mediated by a cap-independent mechanism to insure its expression and function in conditions of global translation inhibition**  
*Ana Marques-Ramos, Marco M Candeias, Juliane Menezes, Rafaela Lacerda, Margaret Willcocks, Nicolas Locker, Luísa Romão*
- 698 The mTOR pathway regulates expression of synaptic cell adhesion proteins in neurons**  
*Martin Schüle, Jennifer Krummeich, Dennis Strand, Susanne Strand, Susann Schweiger, Jennifer Winter*
- 699 Withdrawn**
- 700 Regulation of the selenoproteins by microRNAs**  
*Zoia Stoytcheva, Peggy Su, Victoria Soto*
- 701 HnRNPM Functions as an IRES-binding Protein to Selectively Activate Hypoxia-induced Translation for Colon Cancer Tumorigenesis**  
*Tsung-Ming Chen, Ming-Chih Lai, Shaw-Jenq Tsai, H. Sunny Sun*
- 702 A Global Functional Survey for Translational Regulators**  
*Kendra Swain, Nicholas Ingolia*
- 703 Translational Control of Invasion in Melanoma - A novel role for LARP6?**  
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- 704 Functional sites on Ribosome Modulation Factor (RMF) for 100S ribosome formation**  
*Hideji Yoshida, Hideki Nakayama, Yasushi Maki, Shou Furuike, Masami Ueta, Chieko Wada, Akira Wada*
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*Felix G. M. Ernst, Lieselotte Erber, Joana Sammler, Frank Jühling, Heike Betat, Mario Mörl*
- 706 tRNAscan-SE 2.0 and GtRNAdb 2.1: Improved resources for analysis of transfer RNA genes**  
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- 707 The rich spectrum of C/D box snoRNA interactions in human cells**  
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- 708 Non-coding RNA is required for DNA double-strand break repair**  
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## NOTES

## 1 RNA epigenetic marks affect transcriptome switching during early embryonic development

*Boxuan Zhao, Xiao Wang, Alana Beadell, Robert Ho, Chuan He*  
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Over 100 types of chemical modifications exist on RNA and carry out distinct functions. Among these RNA modifications, m6A is the most abundant epigenetic mark on mRNA and plays critical regulatory roles in RNA metabolism. Functioning through specific reader proteins, m6A notably increases mRNA translation efficiency or accelerates the decay of marked transcripts, both critical for the change of gene expression profile and cell state. However, the involvement of m6A in complex biological processes has not been well explored, particularly during cell differentiation and embryonic development.

Occurring at the early stage of embryonic development, the maternal-to-zygotic transition (MZT) is one of the most profound and orchestrated processes during the early life of embryos, yet factors that shape the temporal pattern of vertebrate MZT are largely unknown. We proposed the rapid clearance of mRNA during MZT may be facilitated by m6A and performed high throughput sequencing to study the m6A pattern and transcriptome dynamics during zebrafish embryogenesis. Our results showed that over one-third of zebrafish maternal mRNA can be marked by m6A, and the clearance of these maternal mRNAs is facilitated by an m6A reader protein, Ythdf2. Removal of Ythdf2 in zebrafish embryos decelerates the decay of m6A-modified maternal mRNAs and impedes zygotic genome activation. These embryos fail to initiate timely MZT, undergo cell cycle pause, and remain developmentally delayed throughout larval life. Additionally, injecting m6A reporter mRNA into individual embryos also revealed the potential involvement of m6A in single cell variation of gene expression among the differentiating cells. Our study reveals m6A-dependent RNA decay as a previously unidentified maternally driven mechanism that regulates maternal mRNA clearance during zebrafish MZT, highlighting the critical role of m6A mRNA methylation in transcriptome switching and animal development.

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## 2 Adenosine demethylase FTO regulates nuclear pre-mRNA processing

*Helena Covelo-Molares<sup>1</sup>, Marek Bartosovic<sup>1</sup>, Pavlina Gregorova<sup>1</sup>, Grzegorz Kudla<sup>2</sup>, Stepanka Vanacova<sup>1</sup>*

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N6-methyladenosine (m6A) is the most prevalent internal messenger RNA (mRNA) modification and, since the discovery of the m6A demethylase FTO, the first example of reversible RNA methylation. On the functional level, m6A regulates mRNA fate -stability, translation or splicing- and it plays a role in mammalian cell differentiation. Whereas the m6A methylase complex has been extensively studied, the m6A erasers remain largely unexplored. Here we show that FTO regulates nuclear mRNA processing and reveal its RNA targets transcriptome-wide. Using FTO cross-linking and immunoprecipitation coupled to high-throughput sequencing (CLIP-Seq) we found that FTO preferentially binds to intronic regions of pre-mRNAs, around alternative spliced (AS) exons and in the proximity of poly(A) sites. Furthermore, we performed RNA-Seq analysis of FTO knockout mammalian cell line and uncovered that depletion of FTO leads to changes in 3' end processing and pre-mRNA splicing, which are dependent of FTO catalytic activity in vivo. Our study contributes to the understanding of m6A-regulated processes pointing to regulation of nuclear mRNA processing as one of the major functions of the m6A demethylase FTO.

### 3 RNA fate regulation by DGCR8 through co-transcriptional methylation

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Eukaryotic gene expression is heavily regulated at the transcriptional and posttranscriptional levels. An additional level of regulation occurs co-transcriptionally, such as processing/decay of nascent transcripts physically associated with chromatin. This involves the RNA interference (RNAi) machinery and is well documented in yeast, but little is known about its conservation in mammals. Using murine embryonic stem (mES) cells, here we show that two RNAi factors, Dgcr8 and the RNaseIII Drosha, physically associate with chromatin. We found that these known microRNA-processing factors associate with a subset of actively transcribed genes, as well as non-coding genes, including snoRNA, and lncRNA genes. Dgcr8 recruitment to chromatin was dependent on Methyltransferase-like 3 (Mettl3), which catalyzes N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) of RNAs. Chemical inhibition of RNA polymerase II (RNAPII) disrupted the association of Dgcr8 and Mettl3 with chromatin, strongly suggesting RNA methylation and processing events occur co-transcriptionally. Intriguingly, we found that temperature stress causes a radical relocalization of Dgcr8 and Mettl3 to stress-induced genes including Hsp70. Genetic ablation of *Dgcr8* or *Mettl3* led to the accumulation of Hsp70 mRNA, elongation of its half-life, and increased protein levels only in cells subjected to acute heat stress. This indicates that acute heat-stress co-transcriptionally marks Hsp70 mRNAs by Mettl3 and Dgcr8 for subsequent RNA degradation to control the timing and magnitude of the heat shock response. Together, our findings elucidate a novel mode of co-transcriptional gene regulation, where m<sup>6</sup>A serves as a chemical mark that instigates **subsequent post-transcriptional RNA processing events**.

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### 4 5-methylcytosine as an epitranscriptomic mark on murine mRNA

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Recent work has identified and mapped a range of posttranscriptional modifications in mRNA, including methylation of the N6 and N1 positions in adenine, pseudouridylation and methylation of carbon 5 in cytosine (m5C). However, knowledge about prevalence and transcriptome-wide distribution of m5C is still extremely limited and thus, studies in different cell types, tissues and organisms are needed to gain insight into possible functions of this modification and implications for other regulatory processes. We have carried out a global analysis of m5C in total and nuclear poly(A)RNA of mouse embryonic stem cells and murine brain. We found intriguing differences in these samples and cell compartments with respect to the degree of methylation, functional classification of methylated transcripts and position bias within the transcript. Degree and pattern of methylation distinguish transcripts modified in both embryonic stem cells (ESC) and brain from those methylated in either one of the samples. We have also investigated the role of the RNA cytosine methyltransferase Nsun3 in ESCs and found that it plays an important role in stem cell differentiation by regulating mitochondrial translation. Together, these results expand the emerging picture of epitranscriptomic modifications in eukaryotic mRNA.

## 5 Expanding the decoding capacity of the mitochondrial tRNA<sup>Met</sup> by RNA modifications

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Mitochondrial gene expression uses a non-universal genetic code in human cells. Besides reading the conventional AUG codon, mitochondrial (mt-)tRNA<sup>Met</sup> mediates incorporation of methionine on AUA and AUU codons during translation initiation and on AUA codons during elongation. Using localisation analysis and *in vivo* UV and 5-azacytidine crosslinking we show that the RNA methyltransferase NSUN3 localises to mitochondria and specifically interacts with mt-tRNA<sup>Met</sup>. *In vitro* analyses reveal that NSUN3 methylates cytosine 34 (C34) at the wobble position of mt-tRNA<sup>Met</sup>. Our data further demonstrate that NSUN3 specifically recognises the anticodon stem loop (ASL) of the tRNA and mutations that compromise basepairing in the ASL, including a pathogenic mutation, reduce C34 methylation, implying that lack of this modification in mt-tRNA<sup>Met</sup> can lead to disease. Interestingly, it has previously been reported that the mt-tRNA<sup>Met</sup> can contain a 5-formylcytosine (f<sup>5</sup>C) modification at position 34 and we identify ALKBH1/ABH1 as the alpha-ketoglutarate and Fe(II)-dependent dioxygenase that oxidises m<sup>5</sup>C34 to generate an f<sup>5</sup>C34 modification in mt-tRNA<sup>Met</sup> *in vitro* and *in vivo*. Furthermore, *in vitro* codon recognition studies with mitochondrial translation factors reveal differential utilisation of the modification states of mt-tRNA<sup>Met</sup> in mitochondrial translation initiation and elongation. Depletion of either NSUN3 or ABH1 strongly affect mitochondrial translation *in vivo*, suggesting that both enzymes are required to install the anticodon modifications necessary for mt-tRNA<sup>Met</sup> function. Together, our data reveal how the modifications in mt-tRNA<sup>Met</sup> are generated by the sequential action of NSUN3 and ABH1, allowing recognition of the different codons encoding methionine by a single mitochondrial tRNA<sup>Met</sup>.

## 6 How to make EF-P sweet enough to alleviate stalled ribosomes

*Jakub Macošek<sup>1</sup>, Ralph Krafczyk<sup>2</sup>, Pravin Kumar Ankush Jagtap<sup>1</sup>, Daniel Gast<sup>2</sup>, Bernd Simon<sup>1</sup>, Swetlana Wunder<sup>2</sup>, Prithiba Mitra<sup>2</sup>, Rohr Jürgen<sup>2</sup>, Anja Hoffmann-Röder<sup>2</sup>, Kirsten Jung<sup>2</sup>, Jürgen Lassak<sup>2</sup>, Janosch Hennig<sup>1</sup>*

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Translation of mRNA coding for polyproline stretches often causes ribosome stalling. Bacteria are rescued by Elongation Factor P (EF-P), which binds to the stalled ribosome and alleviates the translational arrest [1]. The activity of EF-P strongly depends on post-translational modifications of a positively charged amino acid that stabilizes and optimally positions the CCA-end of the prolyl-tRNA in P-site for the peptidyl transfer reaction. In *E. coli* (as well as in many other bacterial species) EF-P is activated by  $\beta$ -lysinylation and hydroxylation of a conserved lysine 34 by the concerted action of three enzymes - EpmA, EpmB and EpmC. Interestingly orthologs to EpmAB(C) can only be found in 26% of all bacterial species. In another 10% including pathogenic bacteria (e.g. *Pseudomonas aeruginosa*), the conserved lysine of EF-P is replaced by arginine, which is rhamnosylated by a novel glycosyltransferase termed EarP using dTDP-rhamnose as substrate.

As the rhamnosylation of EF-P by EarP is only the first documented case of arginine N-glycosylation in prokaryotes, the mechanism of arginine glycosylation yet remains to be elucidated. Hence, we determined the crystal structure of EarP at 2.1 Å and further investigated its interaction with both EF-P and dTDP-rhamnose by NMR spectroscopy. Based on these findings we generated several EarP mutants and tested their activity both *in vivo* and *in vitro*. Together our results reveal the ligand binding site and the putative catalytical dyad of EarP. Since disruption of the EF-P:EarP pathway influences growth and pathogenicity of the bacteria, our results provide not only the first insights into the catalysis by a novel glycosyltransferase, but also the basis for targeted antibiotic drug design.

[1] J. Lassak, D. N. Wilson, and K. Jung, "Stall no more at polyproline stretches with the translation elongation factors EF-P and IF-5A," *Mol. Microbiol.*, Sep. 2015

## 7 MeTH-seq: Transcriptome-wide Mapping of 2'-O-Methyl Ribose Reveals Widespread Regulated Modification of mRNAs

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Non-coding RNAs contain dozens of chemically distinct modifications, of which only a few have been identified in mRNAs. The recent discovery that certain tRNA modifying enzymes also target mRNAs suggests the potential for many additional mRNA modifications. Here, we use crosslinking immunoprecipitation and sequencing (CLIP-seq) to show that conserved tRNA 2'-O-methyltransferases Trm3, Trm7, Trm13, and Trm44 and a rRNA 2'-O-methyltransferase Spb1 interact with specific mRNA sites in yeast. We also developed methylation at two prime hydroxyl sequencing (MeTH-Seq) for transcriptome-wide mapping of 2'-O-methyl ribose (Nm) with single-nucleotide resolution, and discovered thousands of potential Nm sites in mRNAs. Genetic analysis identified mRNA targets for five methyltransferases, which target distinct sequences and structural motifs for environmentally regulated modification. Our work identifies Nm as a prevalent mRNA modification that is likely to be conserved throughout life and provides methods to investigate its distribution and regulation.

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## 8 A catalytic RNA helicase Subcomplex in the Trypanosome Holo-editosome

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Trypanosomes are flagellated protozoa that split from other eukaryotic lineages over 100 Ma and exhibit extensive RNA editing by uridylyte insertion and deletion. In *T. brucei*, hundreds of small non-coding guide RNAs (gRNAs) modify over 1300 sites in the mitochondrial mRNAome. This process takes place in holo-editosomes that include the RNA editing enzyme (RECC) and auxiliary RNA-protein subcomplexes. Yet, the regulatory mechanisms of RNA editing remain obscure. We recently reported the first catalytic accessory subcomplex, termed the REH2C, which includes mRNA substrates and products, the multi-domain RNA Editing Helicase 2 (REH2) and an intriguing 8-zinc finger protein that we termed REH2-Associated Factor 1 (H2F1). Both proteins are critical for editing *in vivo*. In trypanosomes, H2F1 stabilizes REH2 and is an adaptor that tethers the helicase into editosomes. We now reconstituted a catalytically active REH2C using full-length recombinant proteins: REH2 (242 kDa) and H2F1 (62 kDa). We performed the first biochemical characterization of the REH2C subcomplex, including RNA unwinding, RNA binding, and structural proteomics. We also performed extensive mutagenesis *in vivo* and *in vitro* dissecting critical features in REH2 and H2F1 in the assembly of REH2C, docking of this subcomplex into editosomes, and overall editing function. Our RNA-seq studies indicate that the REH2 helicase controls editing fidelity. In summary, we will present comprehensive genetic, biochemical, proteomic and bioinformatic studies of the only known regulatory helicase subcomplex in holo-editosomes.



## 9 The *C. elegans* neural editome reveals an ADAR target mRNA required for proper chemotaxis

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The conversion of adenosine (A) to inosine (I) by ADAR enzymes is one of the most prevalent forms of RNA editing, occurring in thousands of transcripts across metazoa. Inosine is a biological mimic of guanosine, and thus, A-to-I editing of mRNA can alter coding potential, splicing patterns and silencing by small RNAs. In mammals, A-to-I editing is abundant in the central nervous system, where editing of ion channels and receptors creates amino acid changes that are required for proper nervous system function. Consistent with this, aberrant editing of nervous system mRNAs occurs in a number of human neuropathological diseases, including brain tumors, Amyotrophic Lateral Sclerosis and Alzheimer's disease. Loss of ADARs in model organisms such as flies and worms also results in improper neuronal function as evidenced by an inability to fly or chemotax, respectively; however, the substrates that are responsible for these phenotypes are unknown. To attempt to identify important neural targets of the *Caenorhabditis elegans* A-to-I editing enzyme, ADR-2, we performed the first unbiased assessment of editing in the *C. elegans* nervous system. After purifying neural cells from both wild-type and editing deficient worms, the neural transcriptomes were sequenced and A-to-I editing events identified as well as changes in gene expression upon loss of the editing enzyme ADR-2. Combining these two datasets, we identified a mRNA that was highly edited in the wild-type nervous system and exhibited decreased expression in neural cells deficient in *adr-2*. Interestingly, editing of this mRNA was differentially regulated in the nervous system compared to other tissues, as well as the effect on gene expression was neural cell-specific as whole worm lysates lacking *adr-2* express similar levels of this mRNA as their wildtype counterparts. Using transgenic *C. elegans*, we have specifically driven expression of this mRNA in neural cells and restored proper chemotaxis ability to *adr-2* deficient worms. This study is first of its kind in the RNA editing field to span from developing novel methodology for cell-type specific target identification to organismal behavior, significantly advancing our understanding of the importance of RNA editing in the nervous system.

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## 10 ADAR1 controls apoptosis of stressed cells by inhibiting Staufen1-mediated mRNA decay

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Both p150 and p110 isoforms of ADAR1 convert adenosine to inosine in double-stranded RNA (dsRNA). ADAR1p150 suppresses the dsRNA sensing mechanism that activates MDA5-MAVS-IFN signaling in the cytoplasm. In contrast, the biological function of the ADAR1p110 isoform, usually located in the nucleus, remains largely unknown. Here we show that stress-activated phosphorylation of ADAR1p110 by MKK6/p38/MSK1&2 MAP kinases promotes its binding to Exportin-5 and export from the nucleus. Once translocated to the cytoplasm, ADAR1p110 suppresses apoptosis of stressed cells by protecting many anti-apoptotic gene transcripts that contain 3'UTR dsRNA structures such as those consisting of inverted Alu repeats. ADAR1p110 competitively inhibits binding of Staufen1 to the 3'UTR dsRNAs and antagonizes the Staufen1-mediated mRNA decay. Our studies revealed a new stress response mechanism, in which ADAR1p110 and Staufen1 regulate surveillance of a set of mRNAs required for survival of stressed cells.

## 11 Evolutionarily conserved biological roles of ADAR RNA editing enzymes

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ADAR RNA editing enzymes deaminate adenosine bases to inosines in RNA. During translation inosine is read as guanosine and this editing diversifies the proteins present in the organism. In vertebrates, the editing by ADAR1 protein also helps to differentiate between self and non-self dsRNA. ADAR editing generates dsRNA containing I-U base pairs and recent published work from our lab on the mouse *Adar1* mutant demonstrated that ADAR1 prevents cellular dsRNA from aberrantly activating cellular innate immune responses. A mutation in human *Adar1* leads to an extreme condition known as Aicardi-Goutieres Syndrome in which children with a defective ADAR1 aberrantly express antiviral interferon and die with encephalitis. The vertebrate ADAR2 protein is involved in editing transcripts encoding ion channels subunits and other proteins, particularly in CNS, but also in pancreatic  $\beta$ -cells, where ADAR2 facilitates insulin secretion.

*Drosophila* has one *Adar* gene which is an orthologue of vertebrate *Adar2*. *Adar*<sup>SG1</sup> null mutant flies show locomotion defects, are male sterile and develop age-dependent neurodegeneration. There is also aberrant upregulation of Anti-Microbial Peptides (AMPs), in *Adar*<sup>SG1</sup> flies. This suggesting a possible role of ADAR2-type proteins in immunity; a role not identified in vertebrates. *Drosophila Adar* mutant phenotypes are rescued by human *Adar2* expression. Deciphering the role of *Adar* in *Drosophila* will help us to understand the evolutionarily conserved roles of ADAR2-type RNA editing enzymes. The main vertebrate innate immune signalling are at least partially conserved in *Drosophila melanogaster* and preliminary results indicate a potential role of CNS-associated *Toll* signalling in rescuing *Adar*<sup>SG1</sup> mutant phenotypes.

We performed a genetic screen for suppressors of the reduced viability associated with the *Adar*<sup>SG1</sup> null mutant. This screen identified a strong rescue by reduced *Tor* gene dosage of all tested *Adar* mutant phenotypes. Reduced *Tor* gene dosage leads to increased autophagy, which rescues *Adar*<sup>SG1</sup> mutant phenotypes. Increased expression of the autophagy regulators *Atg5* mimics *Tor* mutant rescue of *Adar*<sup>SG1</sup> mutant phenotypes. We propose that *Adar*<sup>SG1</sup> mutant cells aberrantly activate autophagy to clear virus RNA or aberrant intracellular dsRNA. Our current work aims to identify interactions between innate immune signalling pathways and insulin /tor signaling pathways in the *Adar*<sup>SG1</sup> mutant.

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## 12 Separate analysis of pre-mRNA and mRNA A-to-I editing reveals surprising differences in the level of editing and suggests selective maturation of edited transcripts

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Adenosine to Inosine RNA-editing (A-to-I editing) is a co-transcriptional process where a genomically encoded adenosine is deaminated and converted into inosine. The editing reaction is catalyzed by adenosine deaminases acting on RNA (ADAR) enzymes. As inosine is normally interpreted as guanosine by cellular machines, editing can have diverse consequences, ranging from changes of the coding potential to consequences for innate immunity. Editing is a highly dynamic process and editing levels range from below 1% to almost 100% editing. Editing levels generally increase during development and also vary between tissues. Moreover, de-regulation of editing has been linked to several pathologies like epilepsy, depression, or cancer.

As editing sites in protein-coding parts of transcripts are frequently defined by exon-intron basepairing, we hypothesized that the mRNA splicing efficiency should control the extent of editing. In order to analyze the impact of splicing efficiency on editing levels we used a mutation-based/reporter-construct approach and a splice-inhibitor assay. We could show that reduced splicing efficiency leads to increased editing levels when the exonic editing site is coordinated by an intron. Surprisingly, in the course of our experiments we also found that editing levels differ dramatically between pre-mRNA and mature RNA. For a series of targets we observed higher editing levels in the nuclear pre-mRNA than in the corresponding cytoplasmic mRNA. Most interestingly, promiscuous editing seen in pre-mRNAs can be completely absent in the corresponding mature mRNAs. Conceptually, differences between pre-mRNA and cytoplasmic mRNA-editing levels can be controlled by: a) selective processing, b) selective nuclear export, or c) selective degradation of nuclear or cytoplasmic edited or unedited transcripts. We could show that selective processing can explain the observed differences in part. Currently, additional experiments are on the way in order to address the underlying mechanisms.

### 13 Kinetics of pre-mRNA cleavage and termination in living cells

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Synthesis of mRNA in mammalian cells comprises several processes including transcription initiation and elongation, splicing, cleavage, polyadenylation and termination of the nascent transcript. Kinetics of transcription initiation and elongation as well as the timing of pre-mRNA splicing have been previously estimated. However, cleavage/polyadenylation and transcription termination kinetics have been less studied. Here, we directly examined with single-molecule sensitivity the timing of pre-mRNA cleavage/polyadenylation and termination in the nucleus of living human cells. Using reporter transcripts labeled with MS2 or PP7 stem loops inserted upstream of the pA site, we show that it takes 15-30 seconds to cleave and release the fully transcribed nascent RNA from the site of transcription. As expected, escape of the newly synthesized mRNA from the site of transcription is significantly delayed upon knocking down the essential cleavage and polyadenylation factor CPSF73. Analysis of reporter transcripts with  $\lambda$ N stem loops inserted downstream of the pA site reveals that these RNAs are also released from the site of transcription within 30 seconds after synthesis. Taken together, these results indicate that key steps in mRNA biogenesis including intron splicing and cleavage/polyadenylation can occur in just a few seconds, which is much faster than previously thought.

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### 14 A unified activation mechanism for mRNA 3' processing and splicing

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mRNA alternative polyadenylation (APA) plays an important role in modulating gene expression in a tissue- and developmental stage-specific manner. However, it remains poorly understood how APA is regulated. Cleavage factor I (CFI) is an essential mRNA 3' processing factor, consisting of CFI25 and two alternative large subunits, CFI59 and CFI68, both of which resemble the SR family of splicing regulators. Here we demonstrate that CFI functions as a sequence- and position-dependent activator of mRNA 3' processing, which explains how CFI regulates APA globally. Furthermore, the CFI activator function requires its arginine-serine (RS) repeat domains. Finally we provide evidence that CFI activates mRNA 3' processing through a "polar zipper" formed between its RS domains and the arginine-aspartate/glutamate repeat (RE/D) domain of the CPSF subunit Fip1. Given similar interactions between SR proteins and the core splicing factors, we propose a unified activation mechanism for mRNA 3' processing and splicing although the two processes require distinct sets of factors.

## 15 Molecular and Structural Basis for the Integrator Cleavage Module

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The metazoan Integrator complex (INT) has important functions in the 3'-end processing of noncoding RNAs, including the uridine-rich small nuclear RNA (UsnRNA) and enhancer RNA (eRNA), and the transcription of coding genes by RNA polymerase II. INT contains at least 14 subunits, but its molecular mechanism of action is poorly understood as currently there is little structural information on its subunits. The endonuclease activity of INT is mediated by its subunit 11 (IntS11), which belongs to the metallo- $\beta$ -lactamase superfamily and is a paralog of CPSF-73, the endonuclease for pre-mRNA 3'-end processing. IntS11 forms a stable complex with IntS9 through their C-terminal domains (CTDs).

Here we report the crystal structure of the IntS9-IntS11 CTD complex at 2.1 Å resolution and detailed, structure-based biochemical and functional studies. The structure of the complex is composed of a continuous nine-stranded  $\beta$ -sheet, with four strands from IntS9 and five from IntS11. Highly conserved residues are located in the extensive interface between the two CTDs. Yeast two-hybrid assays and co-immunoprecipitation experiments confirm the structural observations on the complex. Functional studies demonstrate that the IntS9-IntS11 interaction is crucial for the role of INT in snRNA 3'-end processing. We further utilized a modified yeast-two hybrid approach to screen for INT subunits that interact specifically with the IntS9/11 heterodimer and identify that IntS4 associates only with both IntS9 and IntS11 and not with either individually. Interestingly, IntS4 has many architectural similarities with Symplekin (the scaffold for CPSF73/100) but we have determined its interaction with the IntS9/11 heterodimer to be mediated by distinct regions. We carried out multiple functional assays on these three proteins including their role in UsnRNA misprocessing, Cajal body integrity, and Histone locus body formation to determine the importance of their interaction. Collectively, these results establish that the IntS4/9/11 heterotrimer constitutes the Integrator cleavage module.

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## 16 Dynamic assembly of RNA polymerase II CTDsome

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The largest subunit of RNA polymerase II (RNAPII) contains a long and flexible C-terminal domain (CTD) that consists of tandem repeats of the heptapeptide consensus  $Y_1S_2P_3T_4S_5P_6S_7$ . Repetitive nature of the CTD, its dynamic phosphorylation patterns (CTD code), and structural variability make the CTD not only a unique platform to regulate interaction network of RNAPII, but also represent a great challenge for structural biology. To visualize the structural assemblies of CTD, we combined nuclear magnetic resonance (NMR) spectroscopy, X-ray crystallography and small-angle X-ray scattering (SAXS). This hybrid approach allowed us to probe the architecture of the CTD binding platform with processing and transcription factors. We will show the structural basis for (i) reading of the CTD code by several factors involved in transcription elongation and termination, (ii) degeneration of the CTD code, (iii) dynamic exchange of the CTD binders, and (iv) formation of the CTDsome architecture.

## 17 Replication compartment formation increases viral mRNA transcription during lytic infection

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During lytic infection, Herpes Simplex Virus type 1 (HSV1) hijacks its host cell's transcriptional machinery in order to ensure the timely and robust production of its own mRNA. As the virus replicates, long concatamers of viral genome are produced and segregate apart from host chromatin, recruiting a large number of host proteins – including RNA Polymerase II (Pol II) the host transcriptional machinery— into these replication compartments (RCs). The forces that drive the striking segregation of Pol II and other host proteins into discrete nuclear domains are poorly understood, but they raise the opportunity to gain insight into the formation of other nuclear domains such as nucleoli or speckles.

Using super-resolution microscopy and single particle tracking experiments, we find that Pol II remains highly mobile, even in the context of the replication compartment. Further, we show that the increased concentration of Pol II in RCs is a consequence of a change in the association kinetics of Pol II to the viral DNA. To extend our findings, we have generated viral strains containing MS2 arrays into well-characterized early and late genes. Coupled with single molecule RNA FISH, these MS2-containing viral strains allow us to measure the kinetics of mRNA production in real time at the single-cell, single-infection level. Our quantitative approach to assessing the mechanisms underlying Pol II recruitment into RCs, and the effect this has on viral mRNA output, has allowed us to identify key advantages that such compartmentalization lends to specific viral mRNA production.

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## 18 Surveilling the proper processing of mRNAs for nuclear export: A coordinated effort between ZC3H14 and the THO complex

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Proper eukaryotic gene expression relies on the successful execution of nuclear RNA processing events that are coupled to efficient export of the resulting mRNPs. RNA-binding proteins are critical for assembling mature mRNPs and facilitating proper RNA processing. One key RNA-binding protein is the zinc finger polyadenosine RNA-binding protein, ZC3H14. Inactivating mutations in the *ZC3H14* gene lead to a severe form of intellectual disability; however, the molecular function of this protein is not yet understood. Studies in yeast analyzing the *ZC3H14* orthologue Nab2 show that depletion of Nab2 results in rapid loss of mRNA and functional mutations in *Nab2* impact production of mature mRNA. To extend the analysis to examine the function of ZC3H14 in the brain, we carried out a proteomic study to identify ZC3H14 interacting proteins. Using unbiased mass spectrometry, we identified the complete spectrum of ZC3H14 co-purifying proteins. From this approach, numerous RNA processing factors were identified including multiple splicing proteins and the entire THO complex. We focused on the THO complex because we identified all members of the complex in association with ZC3H14. We validated the interaction between ZC3H14 and the THO components THOC1, THOC2 and THOC5. We then identified several RNA targets that are coordinately affected by the loss of ZC3H14 and THO components. Furthermore, as previously shown for loss of ZC3H14, the loss of THO components alters the length of bulk poly(A) tails. Our recent work reveals that siRNA-mediated knockdown of ZC3H14 or THOC1 in cultured cells leads to the escape of pre-mRNA into the cytoplasm. Furthermore, knockdown of ZC3H14 affects the RNA target selection of the mRNA export factor, NXF1. Taken together, these results suggest a coordinated effort between ZC3H14 and the THO complex to regulate RNA processing. Loss of these proteins leads to a compromised surveillance pathway for nuclear mRNA processing. The biological importance of such a pathway is evident as mutations in both *ZC3H14* and genes encoding THO components have been linked to defects in higher order brain function.



## 19 Molecular roles and characteristics of a novel RNA-binding protein - E3 ubiquitin ligase Trim25

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Many newly identified RNA-binding proteins are poorly characterised and do not contain canonical RNA-binding domains. Trim25 is a novel RNA-binding protein and a member of the Tripartite Motif (TRIM) family of E3 ubiquitin ligases, which play a key role in the innate immune response to RNA viruses. Almost nothing is known about its RNA-related roles in cell biology and innate immunity. Furthermore, its RNA-binding domain has not been unequivocally described.

We have recently discovered that Trim25 is involved in uridylation of let-7 precursor. Here, using Clip-seq and SILAC-based co-immunoprecipitation assays we will show Trim25's endogenous RNA targets and protein binding partners. Our Clip-seq data identified hundreds of coding and non-coding RNAs bound by Trim25. Our comprehensive analyses indicated that Trim25 prefers binding G-rich sequences. We will also show that Trim25 associates with many RNA-binding proteins, thus suggesting it has a widespread role in regulating RNA metabolism.

Surprisingly, we discovered that RNA-binding is mediated by the PRY-SPRY domain of Trim25. Thus, PRY-SPRY could represent a novel RNA-binding domain. Crucially, human genome codes for forty-three Trim E3 ubiquitin ligases with highly similar PRY-SPRY domains. Thus, Trim25 could represent a novel prototypic E3 ligase with RNA-binding activity. Finally, we will show that the Trim25 binds to its own transcript and that this is important for its autoubiquitination. These results reveal new insights into how novel, non-canonical RNA-binding proteins with E3 ubiquitin ligase activities could regulate RNA metabolism and gene expression.

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## 20 Structures and evolutionarily divergent phosphodiesterase activities of Usb1 from yeast and human

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The U Six Biogenesis protein (Usb1) is a 3'-5' exonuclease responsible for U6 snRNA 3' end maturation. Mutation of human Usb1 results in the disease poikiloderma with neutropenia and predisposition to myelodysplastic syndromes. Human Usb1 cleaves multiple uridines from the 3' end of U6 snRNA and leaves a terminal cyclic phosphate. In contrast, it has been shown that U6 snRNA from *S. cerevisiae* terminates with a non-cyclic phosphate.

We have analyzed the activity of purified recombinant *S. cerevisiae* Usb1 and find that it removes a single uridine from the 3' end of U6 via a two-step mechanism: exonucleolytic nucleotide removal to produce a cyclic phosphate, and cyclic phosphodiesterase (CPDase) activity that opens the cyclic phosphate. Using <sup>31</sup>P NMR, we determined that *S. cerevisiae* Usb1 leaves a 3' phosphate, making it a unique member of the 2H phosphodiesterase family. The resulting 3' phosphate product inhibits further exonucleolytic removal of nucleotides from U6.

In order to understand the basis for differences in Usb1 activities, we determined the crystal structure of yeast Usb1 at 1.8 Å resolution. We also determined the structure of human Usb1 with the substrate analogue 5'UMP at 1.4 Å resolution. These structures provide the first direct structural evidence for the catalytic mechanism of Usb1. The overall structures of human and yeast Usb1 are similar and exhibit nearly identical active sites despite <20% sequence identity and the inability of the human enzyme to act as a CPDase.

Surprisingly, we find that residues outside of the active site influence CPDase activity. Together, these biochemical assays and new structures allow us to propose a mechanism for Usb1 activity. *In vivo*, we find that loop regions surrounding the active site play a role in substrate recognition in yeast and that the conserved N-terminal region of Usb1 is essential for viability and stability of the protein despite not being required for catalysis. This work provides insight into how subtle structural changes influence the catalytic mechanism of Usb1 to tune its activities towards producing the U6 modification required by the cell

## 21 Acidic C-terminal domains autoregulate the RNA chaperone Hfq

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Hfq is an RNA chaperone that stabilizes regulatory small RNAs (sRNAs) and anneals them to mRNAs encoding proteins involved in metabolism, stress-response and virulence pathways in many bacteria. The Sm-like core of Hfq assembles into a homo-hexameric ring that binds sRNAs and mRNAs in a sequence-specific manner to either ring face. Basic rim patches provide additional non-specific interactions to the RNA bodies and are necessary for annealing activity. Like all chaperones, Hfq must not only bind its substrates, but avoid interacting with off-target substrates, and efficiently dissociate from its products. We previously showed that the disordered C-terminal domains (CTDs) of *Escherichia coli* Hfq, which protrude from the rim, drive dynamic protein-RNA interactions and release annealed RNAs from the Hfq core, recycling the protein. However the mechanism by which this occurs has remained unknown. No sequence motifs have been previously identified in CTDs, and lengths are highly variable between bacterial families.

Two opposing models were proposed to explain autoinhibition by the CTD. The "polymer brush" model claims that the CTDs passively obstruct RNA binding sites in a non-specific manner. The "nucleic acid mimic" model suggests the CTDs specifically bind to basic core residues and actively compete against nucleic acids.

Using de novo modelling and fluorescence anisotropy assays we find that active Hfqs possess acidic CTD tips that bind to basic rim residues. Fluorescence anisotropy and gel shift assays show that the CTD competes against nucleic acids for binding to the Hfq core. Moreover, stopped-flow spectroscopy reveals that truncation of the intervening CTD sequence, increases the local concentration of the acidic tips and thereby enhances autoinhibition.

Our results suggest that the CTDs of active Hfqs act as nucleic acid mimics, accelerating product release and likely preventing the otherwise basic Hfq core from aggregating with off-target RNAs and DNA in the cell. Hfq is a representative example of an emerging paradigm of autoregulation in nucleic acid-binding domains by tethered peptide mimics. Similar de novo modelling procedures could be used to determine the sequence-function relationship of additional disordered domains, which seem to be very common in nucleic-acid binding proteins.

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## 22 RNA folding nearest neighbor parameter derivation

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The folding stability of RNA secondary structure can be estimated using a nearest neighbor model, and this model is in widespread use to predict RNA secondary structures. Nearest neighbor parameters for predicting RNA folding free energy change at 37°C are based on a database of optical melting measurements on small model systems. This work revises and expands the nearest neighbor model by including the latest experimental results on the melting stability of small model systems. Our analysis showed that a GU closing pair does not have a significant effect on the stability of an RNA helix, suggesting that it should be removed from the nearest neighbor model, consistent with another study [*Biochemistry*, 2012, 51: 3508]. A statistical model called AIC (Akaike Information Criterion) was applied to determine and select nearest neighbor parameters that are playing important roles in the stability of loops and to prevent overfitting. Surprisingly, we found that the AU helix-end penalty was removed by AIC model selection for hairpin loops, indicating that the AU end penalty should not be applied to hairpin loops. We also found that the stability of hairpin loops is independent of first mismatch sequence, which was assumed to be important in the previous nearest neighbor 2004 model. One major finding for internal loops is that, aside from internal loop initiations, the nearest neighbor parameters are not significantly different from each other for loops of different sizes. Therefore, internal loops are now fit in a single group with terms applied to all internal loops. Nearest neighbor parameters for other motifs are also updated and improved. We are now implementing these parameters in the RNAstructure software package for RNA secondary structure prediction.

## 23 Predicting RNA structure based on direct co-evolutionary couplings obtained with Boltzmann learning techniques

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Non-coding RNAs are known to play several roles in the cell, depending on their detailed structure. Their secondary structure can be predicted with reasonable accuracy by nearest-neighbor models based on thermodynamic parameters, as validated using analysis of covariant mutations. However, these models do not take into account tertiary contacts, and a different route must be followed for their prediction. Thanks to recent improvements in the sequencing technology, alignments of a large number of homologous sequences can now be obtained for each RNA family. It has been recently proposed to exploit the covariance in the mutations appearing in the alignments to predict tertiary contacts, using direct-coupling analysis within the mean field approximation [1][2], as successfully applied to proteins [3]. We perform a similar analysis using a Boltzmann-learning procedure that overcomes some of the limitations of the mean-field approach and allows for a small but detectable improvement in the prediction of secondary and tertiary contacts when applied to a dataset including several riboswitches. Moreover, within this formulation, it is straightforward to include additional constraints coming from the knowledge about RNA-specific structural motifs.

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## 24 RNA 3D Structure Prediction Using Multiple Sequence Alignment Information

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The prediction of three-dimensional structures of complex RNAs is still a challenging task. Here, we present an approach called **EvoClustRNA** (<https://github.com/mmagnus/EvoClustRNA>) that takes advantage of evolutionary information in distant sequence homologs, based on a classic strategy in protein structure prediction (Bonneau, Strauss, Baker, 2001). Using the empirical observation that RNA sequences from the same RNA family typically fold into 3D structures matching at near-atomic resolution, we test whether we might guide in silico modeling by seeking global helical arrangements for the target sequence that are shared across de novo models of numerous sequence homologs. EvoClustRNA performs a multi-step modeling process: First, for the target sequence, a subset of homologous sequences is selected using the RFAM database. Subsequently, independent folding simulations using ROSETTA/FARNA are carried out for each sequence. Structural fragments corresponding to the evolutionary conserved helical regions - determined from the alignment - are extracted from all obtained models and clustered. The model of the target sequence is selected based on the most common structural arrangement of helical.

**We tested our approach on a benchmark of RNAs of known structure and, most rigorously, on three blind RNA-Puzzles challenges. Our predictions ranked #1 (according to RMSD) of all submissions for the L-glutamine riboswitch (bound form) and #2 for the ZMP riboswitch and the Pistol ribozyme.**

We further show also that accuracy improvements from this cross-homolog modeling strategy continue when another RNA structure prediction method, SimRNA, is used instead of Rosetta, supporting its generality. EvoClustRNA, pipelined with either ROSETTA or SimRNA, is implemented as a computational workflow of user-friendly tools accompanied by the step-by-step user guide. Through this combination of parallel modeling of homologous sequences and selecting the final model based on the clustering of conserved fragments, we increase the performance of RNA structure prediction and the methodology provides useful structural information for biological problems.

## 25 RNA-protein interactions in an unstructured context

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Despite their importance, our understanding of non-covalent RNA/protein interactions remains incomplete. This especially concerns the binding between RNA and unstructured protein regions, a widespread class of such interactions. What determines specificity in RNA interactions with unstructured proteins? Can we accurately predict such interactions? How do they contribute to intracellular regulatory networks? Due to diminished structural constraints, the properties of unstructured biopolymers depend strongly on their linear-sequence features. Motivated by this, we have recently determined a comprehensive set of interaction affinities between individual nucleobases and protein sidechains using structural bioinformatics techniques and molecular dynamics simulations. Moreover, using both these scales and their experimental counterparts, we have demonstrated a surprising connection between the composition of mRNA coding sequences and the nucleobase-binding affinities of protein sequences they encode, valid proteome-wide. For example, pyrimidine-density mRNA profiles match pyrimidine-affinity profiles of their cognate proteins with a median Pearson  $|R|=0.74$  in human. This unexpected finding has allowed us to propose that many mRNAs and cognate proteins, when unstructured, may be complementary to each other and bind in a co-aligned manner. In the present talk, I will discuss our recent work in which we have detected similar complementarity for different largely unstructured proteins and non-coding RNAs. Specifically, I will focus on two paradigmatic lncRNAs, ribosomal RNA and XIST, and their bound proteomes in order to demonstrate how viewing biomolecular sequences as physicochemical profiles may help uncover signatures of their functional and evolutionary relatedness, not detectable by standard techniques.

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## 26 Novel 5' cap analogs as a tools for site-specific sequence-independent labeling of messenger RNA.

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mRNA is a template for protein biosynthesis in cells, and consequently mRNA transport, translation, and turnover are key elements in the overall regulation of gene expression. Along with growing interest in the mechanisms regulating mRNA decay and localization, there is an increasing need for tools enabling convenient fluorescent labelling or affinity tagging of mRNA molecules. Here, we report a set of new mRNA 5' cap analogue-based tools that enable site-specific labelling of RNA within the cap with labels such as biotin or fluorescein using (1) N-hydroxysuccinimide (NHS) chemistry or (2) strain-promoted azide alkyne cycloaddition (SPAAC) known as "cooper free" click chemistry. Using both methods we were able to utilize two complementary approaches: (i) co-transcriptional labelling, in which the label is first attached to cap analogue and then incorporated into RNA by transcription *in vitro*, and (ii) post-transcriptional labelling, in which a functionalized cap analogue is incorporated into RNA followed by chemical labelling of the resulting transcript. We demonstrated the utility of fluorescently labelled RNAs in decapping assays, RNA decay assays, and RNA visualization in cells. Moreover, we also demonstrated that mRNAs labelled by the reported methods are translationally active. Finally, taking advantage of "cooper free" click chemistry we present a straightforward method for bioorthogonal labeling of 5' modified mRNA in living cells.



## 27 Chemical Strategies for Massively Parallel RNA Synthesis on Surfaces

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Understanding the relationship between sequence and function is a fundamental challenge in RNA science. Although the technology for producing high-density DNA arrays was developed in the 1990s, it has been difficult to adopt this instrumentation to produce RNA arrays of similar quality. Here we present the use of a light-directed DNA array synthesizer for fabricating high-density RNA arrays. The underlying concept is to synthesize a DNA array which also contains short RNA primers covalently attached to the surface. The primers are extended by using the DNA sequences as templates for transcription. The DNA is then enzymatically digested resulting in hundreds of thousands of distinct RNA sequences, each present in a separate region on a substrate.

An advantage of this approach is its adaptability. Proof-of-concept studies were conducted showing how the synthesis process can be altered to produce arrays of modified RNA, tailor the oligonucleotide density, and utilize various substrate types. 2'-fluoro nucleoside triphosphates were used during the transcription step to produce arrays of nuclease resistant RNA. The efficiency of the overall conversion from DNA array to RNA array was measured to establish the upper limit of RNA oligonucleotide density. Within this limit, the UV-light dose was used to modulate the density during key steps of the fabrication. The in situ chemistry was also tailored to produce arrays upon flexible plastic substrates which allowed the arrays to be cut into subsections with a laser. This strategy increases throughput and facilitates utilization of the thousands of features made on each array.

Two early functional studies are also presented. In the first, a short RNA mimic of GFP was used to explore the formation of secondary structure on the surface of the arrays. In the second, the arrays were used to explore the influence of the 3'-sequence on the activity of poly(U) polymerase. Together, these studies suggest the technology is a powerful bioanalytical platform for studying RNA behavior in parallel.

## 28 ClickSeq and Poly(A)-ClickSeq: Methods for Next-Generation Sequencing library preparation

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Current methods for Next-Generation Sequencing (NGS) library preparation generally include fragmentation and ligation steps which can generate artefactual chimeric reads. This can be a major problem for the detection of rare alternative splicing variants and recombinant viral genomes. Consequently, we developed 'ClickSeq', a method that uses click-chemistry to make sequencing libraries. In ClickSeq, randomly-primed reverse-transcription reactions are supplemented with azido-2',3'-dideoxynucleotides that randomly terminate DNA synthesis generating 3'-azido-blocked cDNA fragments. Purified fragments are then 'click-ligated' via copper-catalyzed alkyne-azide cycloaddition to alkyne-functionalized DNA oligos. This generates ssDNA molecules containing a triazole-linked DNA backbone compatible with PCR amplification. Azido-blocked cDNA fragments cannot provide substrates for forced copy-choice template-switching during RT-PCR and can only be ligated to alkyne-labelled DNA oligos and not to other cDNAs. Consequently, the main suspected sources of artefactual recombination in NGS are eliminated. Using pre-mixed control RNA templates, we have found ClickSeq reduces artefactual recombination to fewer than 3 aberrant events per million reads.

We have applied ClickSeq to study the evolution of defective-interfering RNAs (DI-RNAs) of Flock House virus (FHV), which has been shown to undergo extensive recombination in vivo. DI-RNAs arise due to non-homologous recombination during viral RNA replication and have been demonstrated to attenuate viral replication; thus forming a platform for potential anti-viral therapeutics. With ClickSeq, we can capture rare, emerging, and intermediate defective RNA species that arise during serial passaging of FHV. Together with long-read MinION nanopore sequencing, this allows us to determine the pathways of DI-RNA evolution and their mechanisms of emergence.

Furthermore, we were able to adapt 'ClickSeq' to direct cDNA synthesis specifically toward the 3'UTR/poly(A) tail junction of cellular RNA. With Poly(A)-ClickSeq (PACseq), we demonstrate sensitive and specific enrichment for poly(A) site junctions without the need for complex sample preparation. As a proof-of-principle, we utilized PACseq to explore the poly(A) landscape of both human and *Drosophila* cells in culture and observed outstanding overlap with existing poly(A) databases and also identified previously unannotated poly(A) sites. Moreover, we utilized PACseq to quantify and analyze APA events regulated by CFIm25 illustrating how this technology can be harnessed to identify alternatively polyadenylated RNA.



## 29 Thermodynamically optimal riboswitches achieved through massively parallel rational design

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The rational design of riboswitches offers the prospect of creating RNA domains for in cellulo metabolite sensing, on-demand activation of gene expression, and precise in situ control of RNA therapeutics in space and time. While research into riboswitch discovery is flourishing, riboswitches found so far in Nature, through selections, or through rational design have exhibited ligand-dependent activation ratios of typically no better than 10-fold – one to three orders of magnitude less than thermodynamic optima calculated for their respective aptamers. To address this striking inefficiency in the coupling of binding to function, we have implemented an approach that integrates 1) rational functional RNA design via the internet-scale game Eterna, 2) massively parallel oligonucleotide synthesis, and 3) high-throughput functional measurements on repurposed Illumina sequencers. Through seven rounds of design for molecules that perfectly couple binding of flavin mononucleotide to binding of the MS2 viral coat protein, we report the discovery of the first riboswitches achieving the thermodynamic limit of efficiency for their aptamers. Comparison of these 85-nt riboswitches to thousands of less optimal designs reveals principles for 1) arranging and overlapping functional elements to optimize performance, 2) closing the structure and sequestering unnecessary segments, 3) and designing RNAs with multiple aptamers that can ‘beat’ the thermodynamic limit. Our ongoing work has successfully discovered riboswitches that instantiate Boolean logic of unprecedented complexity within a single molecule, as well as RNAs that bind up to five other RNAs to execute analog arithmetic needed to calculate a recently reported RNA signature of active tuberculosis.

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## 30 Data-rich strategies for elucidating hammerhead ribozyme sequence-function relationships and engineering ribozyme switches

*Joy S. Xiang, Brent Townshend, Gabriel Manzanarez, Emily Gale, Michaela Hinks, Christina D. Smolke*  
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The hammerhead ribozyme is a widespread class of self-cleaving RNA found across all kingdoms of life. Although maximal catalytic activity *in vivo* relies on a conserved core and some tertiary interactions between Stem I and Stem II, efforts to obtain a comprehensive understanding of sequence requirements for tertiary interactions to facilitate ribozyme self-cleavage are limited by the current throughput of biochemical and structural studies. We developed a massively parallel functional assay in yeast<sup>1</sup> and mammalian cells using FACS-seq to extensively interrogate sequence-function relationships of the Stem I and Stem II tertiary loop interactions of >35,000 sequences in a single experiment. A hammerhead ribozyme library with randomized loops I and II of variable sizes was integrated into the untranslated region of a fluorescent reporter, such that cleavage activity directly modulates fluorescence levels. The resulting data indicate that a wide variety of sequences can significantly enhance ribozyme cleavage. Our analysis of the large datasets identified a set of general sequence motifs that result in enhanced cleavage activity, highlighting the importance of pseudoknot-like base pairs at the base of the stem loops to mediate tertiary interactions. We also adapted an *in vitro* Cleave-seq method to quantify cleavage activity in a similarly high-throughput manner to compare sequence motif differences between *in vitro* experiments and inside cells. This *in vitro* method has been automated with a liquid handler to accelerate our ability to rapidly test various hypotheses. Employing these high throughput techniques, we generated new ribozyme switches that respond to a number of small molecule therapeutics and metabolites, which could serve as live cell genetic sensors and gene-control devices. These datasets serve as a rich resource to further enable the discovery of novel hammerhead ribozymes with bioinformatics approaches and guide the design of RNA switches for synthetic biology applications.

<sup>1</sup>Townshend, B., Kennedy, A. B., Xiang, J. S., & Smolke, C. D. (2015). High-throughput cellular RNA device engineering. *Nature methods*, 12(10), 989-994.

### 31 Ultrahigh-throughput in vitro evolution and functional characterization of RNA using droplet-based microfluidics

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Microfluidics allows strong reduction of reaction volumes by confining and manipulating liquids into micrometer scale channels. Such miniaturization already had a significant impact on biology best exemplified by next generation sequencing (NGS) technologies in which sequencing reactions are performed into nanoliter scale chambers. An additional level of miniaturization has recently been reached by compartmentalizing chemical and biological reactions into picoliter size water-in-oil droplets. Several thousands of such droplets (all of an identical volume) can be produced per second (millions per hour) and manipulated (incubation, controlled addition of reagent, droplet content analysis and sorting) by using dedicated microfluidic devices. Combining several such devices allowed us to devise cheap, fast and efficient ultrahigh-throughput screening procedures in which the genes of a mutant library are individualized, amplified, in vitro expressed and the activity of the expression products assayed at a rate of several millions of mutants in a single day. These new analytical platforms can have a profound impact on RNA biology, especially if used in combination with NGS analyses.

In this talk, I will present two main applications of these ultrahigh-throughput screening platforms. First, I will show how we used them to improve the fluorogenic properties of RNA aptamers (i.e. Spinach and Mango) by in vitro evolution. Then, I will present how combining droplet-based screening and NGS allowed us to quantitatively profile the complete set of all possible codon/anticodon pairs (4096 different pairs) and identify those readily accepted by a eukaryotic ribosome as well as some potentially toxic combinations; these findings being supported by several biological evidences (e.g. post-transcriptional modifications).

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### 32 Assembly of U5-specific proteins is controlled by the HSP90/R2TP chaperone system

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PRPF8 is a crucial component of the spliceosomal U5 small ribonucleoprotein particle (snRNP) that lies at the heart of the spliceosome. Mutations of PRPF8 cause the development of the hereditary retina degeneration - retinitis pigmentosa (RP). Here, we tested eight PRPF8 RP mutants in human cells and found that most mutations impair nuclear translocation, assembly of functional U5 snRNP and splicing. Particularly, RP mutations stall the assembly process after formation of the PRPF8-EFTUD2 complex and before addition of SNRNP200 and interaction with U5 snRNA. Using SILAC quantitative proteomics, we characterized U5 snRNP assembly intermediates, which contained U5-specific proteins PRPF8, EFTUD2 and SNRNP200, together with the chaperones AAR2 and HSP90/R2TP. Functional experiments show that HSP90 and R2TP bind unassembled U5 proteins in the cytoplasm, stabilize them and promote the formation of assembly intermediates that further associate with the core U5 snRNP (U5 snRNA + Sm proteins) in the nucleus. Furthermore, inhibition of U5 snRNP biogenesis increases the interaction of PRPF8 with the R2TP complex, and an assembly-deficient RP mutant is retained in the cytoplasm in the R2TP-dependent manner. We propose that the HSP90/R2TP chaperone system promotes and surveillances the assembly of a key module of U5 snRNP.

### 33 Structure and assembly of the yeast U6 snRNP

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The U6 snRNP is an essential spliceosomal complex consisting of U6 RNA, Prp24 protein and the Lsm2-8 protein ring. Here we report the first crystal structure of a nearly complete U6 snRNP. The structure reveals a novel interface between the Lsm2-8 ring and Prp24 that positions the Lsm ring proximal to an electropositive groove in Prp24, which we have shown guides annealing of U4 and U6 RNA. This architecture provides a potential rationale for the observed enhancement in annealing rate conferred by the Lsm2-8 ring. The structure also reveals how the Lsm2-8 complex recognizes the phosphorylated 3' end of U6 RNA, through interactions that are notably distinct from the previously reported structure of yeast Lsm2-8 bound to a short oligonucleotide lacking the native 3' phosphate. We observe secondary structure and long-range interactions across the Lsm2-8 ring that were not resolved in previous X-ray and cryo-EM maps. These features reveal unanticipated structural homology between the Lsm1 and Lsm8 proteins as well as similar quaternary structures for the Lsm1-7 and Lsm2-8 complexes.

To better understand the biological impact of the 3' phosphate found on native U6 RNA, we have investigated how the 3'-5' exonuclease Usb1 catalyzes formation of this post-transcriptional modification. We show that yeast Usb1, unlike human Usb1, has cyclic phosphodiesterase activity that generates a 3' terminal phosphate via a cyclic phosphate intermediate. We have reconstituted yeast U6 RNA processing and snRNP assembly *in vitro* from 10 purified proteins (the La homology protein Lhp1, Prp24, Usb1 and Lsm2-8) and U6 RNA. Based on these data, we propose a 4-step model for U6 snRNP assembly: 1) initial binding of Lhp1 to the unmodified 3' end of U6; 2) Prp24 binding to U6 facilitates Lhp1 dissociation through anti-cooperative interactions; 3) maturation of the U6 3' phosphate by Usb1, and 4) Lsm2-8 binding to the mature 3' end of U6 through cooperative interactions with Prp24.

### 34 LUC7L2 is an SR domain-containing splicing regulatory protein that is frequently mutated in bone marrow neoplasms

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Myelodysplastic syndrome (MDS) is a disease distinguished by the large percentage of patients that harbor a mutation or deletion in one of several genes encoding splicing factors. Exome sequencing of patient bone marrow samples recently identified frameshift mutations and deletions encompassing the putative splicing factor gene *LUC7L2* in 14% of MDS patients. Low expression of *LUC7L2* is correlated with poorer prognosis suggesting that *LUC7L2*-deficiency contributes to the pathology of MDS. While it is known that the essential orthologous yeast protein Luc7p plays a role in splicing, little is understood about the function of mammalian *LUC7L2*. To understand the pathology of *LUC7L2*-deficiency, we first characterized its role as a splicing factor using co-immunoprecipitation followed by mass spectrometry, CLIP-Seq, splicing-sensitive microarrays, RNA-Bind-N-Seq, RNA-Seq and intron splicing efficiency assays.

In this work, we demonstrate that *LUC7L2* interacts with both core and regulatory spliceosomal proteins. By CLIP-seq, *LUC7L2* binds selectively to over 300 pre-mRNA transcripts and a subset of spliceosomal snRNAs. *LUC7L2* binding on pre-mRNA is enriched 20-fold in splice site proximal regions. Knocking down *LUC7L2* in cells lines (HEK and K562) and CD34+ primary bone marrow cells results in changes to constitutive and alternative splicing. Strikingly, these alterations in splicing are 4 times more likely to be found in pre-mRNA transcripts that are bound by *LUC7L2*. Validation of *LUC7L2* target-transcripts following knockdown reveals increases in intron splicing efficiency, suggesting that *LUC7L2* normally plays a repressive role. *LUC7L2*-deficient cells also exhibit a significant number of expression changes in proteins involved in pre-mRNA splicing and RNA processing pathways, similar to the effects of SF3B1, U2AF1, SRSF2 mutations and ZRSR2 deletions also seen in MDS. Transcriptional and apoptotic regulation pathways were also enriched for gene expression changes in *LUC7L2* knockdown cells. Examination of these genes and pathways could ultimately uncover the mechanism of *LUC7L2* pathology in MDS.

To our knowledge this is the first evidence that describes *LUC7L2* as a splicing regulatory factor. This work provides insights into the biological function of *LUC7L2*, providing the framework for our intensive examination of its role in MDS in the future.

**35 Npl3p, the yeast SR-like mRNA binding protein, affects the fidelity of pre-mRNA splicing***Agata Jaskulska, Magda Konarska***Centre of New Technologies, Warsaw University, Warsaw, Poland**

To better understand the mechanism of splicing of suboptimal substrates we carried out a genetic screen for alleles that improve splicing of 5'SS-G5a introns, defective in spliceosome assembly. The screen yielded predominantly alleles of *npl3* and *mtr10*; Npl3p is a primarily nuclear, shuttling SR-like mRNA binding protein implicated in many steps of mRNA biogenesis, and Mtr10p is its karyopherin.

The majority of selected *mtr10* mutations introduce stop codons, creating truncated proteins. The identified *npl3* allele carries a point mutation, L219S, within the RRM2 domain and a 49 aa deletion within the C-terminal RGG box. As predicted from these sequence changes, *mtr10* and *npl3* alleles disrupt the nucleocytoplasmic shuttling of Npl3p, as monitored by Npl3-GFP fluorescence. Furthermore, among independently generated *npl3* mutants with various deletions in the RGG box, those that affect Npl3p shuttling also improve the splicing of suboptimal introns.

The suppression of splicing defects by *mtr10* and *npl3* alleles is not substrate-specific, as they improve splicing for various intron mutants (e.g. 5'SS-U2a, BS-g). Furthermore, improved splicing of defective introns in *mtr10* and *npl3* suppressor strains correlates with the increased cytoplasmic localization of reporter mRNA, as monitored by RNA-FISH. We hypothesize that reduced levels of nuclear Npl3p interfere with termination of transcription, polyadenylation or export of unspliced pre-mRNAs to the cytoplasm, extending their exposure to spliceosomes. The *mtr10* alleles achieve this through defective interactions with Npl3p, resulting in its decreased nuclear localization. Mutants in the Npl3-RGG domain may act in a similar way, through their reduced binding to Mtr10p. In addition, they may directly interfere with nuclear phase of mRNA biogenesis or export. Both reduced levels of nuclear Npl3p and its reduced nuclear function would increase the dwell time of pre-mRNA in the nucleus and thus improve splicing of suboptimal substrates defective in spliceosome assembly.

Previously, Kress & Guthrie, 2008, showed that Npl3p is required for the splicing of a group of yeast introns. Our results indicate that reduction of nuclear Npl3p levels leads to a decreased stringency of splice site selection, thus uncovering a previously unknown role of Npl3 in the modulation of splicing specificity.

**36 PRP40 mediates the communication between RNA polymerase II, the microprocessor and spliceosome in plants***Agata Stepień<sup>1</sup>, Mateusz Bajczyk<sup>1</sup>, Tomasz Gulanicz<sup>1</sup>, Katarzyna Knop<sup>1</sup>, Dariusz Smolinski<sup>2</sup>, Zofia Szweykowska-Kulinska<sup>1</sup>, Artur Jarmolowski<sup>1</sup>***<sup>1</sup>Adam Mickiewicz University in Poznan, Department of Gene Expression, Poznan, Poland; <sup>2</sup>Nicolaus Copernicus University, Department of Cell Biology, Torun, Poland**

MicroRNAs (miRNAs) are small non-coding RNAs of about 21 nt in length, which regulate gene expression by cleavage or translation inhibition of target mRNAs. In plants, miRNAs are encoded mostly by independent transcription units or, less frequently, miRNAs are encoded within introns of protein-coding genes. Many miRNA genes (*MIRs*) contain introns that have to be spliced from primary miRNA precursors (pri-miRNAs) by the spliceosome. We have already shown that splicing of intron-containing pri-miRNAs influences the expression levels of mature miRNAs. Moreover, we have demonstrated that the effect of introns on miRNA biogenesis mostly resides in the 5'ss rather than depends on a genuine splicing event. The goal of this study was to identify the proteins that are involved in the communication between the microprocessor and spliceosome.

In plants miRNA biogenesis takes place in highly specialized nuclear foci, and DCL1 (Dicer Like 1), HYL1 (HYPOPLASTIC LEAVES 1) and SE (SERRATE) are key factors of the plant miRNA biogenesis machinery. Interestingly, SE is also involved in pre-mRNA splicing. Our results suggest that in the communication between the spliceosome and the microprocessor the interaction between SE and U1 snRNP is involved. We identified four binding partners of SE among U1 snRNP auxiliary proteins: PRP39b, PRP40a, PRP40b and LUC7rl. The interplay between SE and PRP40 has been found to be particularly important for the plant development since triple (*se/prp40a/prp40b*) knock-out Arabidopsis plants are embryo-lethal. PRP40 proteins have been shown to interact also with the CTD of RNAPII. We have found downregulation of 50% of all Arabidopsis pri-miRNAs in the *prp40ab* mutant, suggesting a role of PRP40 in *MIR* transcription regulation. Interestingly we have also observed that SE is localized in RNAPII containing nuclear foci and forms a complex with PRP40b and CTD of RNAPII. It raises a question about the co-transcriptional character of pri-miRNA processing in plants, and a special role of SE/PRP40 interaction in this crosstalk. The molecular mechanism of the interplay between U1 snRNP and the microprocessor in plants and its role in miRNA biogenesis will be discussed.



### 37 Identification of intrasplicing events in the human transcriptome and their regulatory potential

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Alternative splicing is one of the major regulators of both, transcriptome diversity and individual isoform abundance. Therefore regulation of alternative splicing is crucial and yet, due to the diversity of the human genome (20.000 genes of which 95% can undergo alternative splicing) diverse and multileveled. Identifying and understanding the scope and variety of splicing events is still an ongoing process. We applied a novel pipeline to extract splicing events from specific RNA-seq datasets and identified numerous unusual (i.e. non-full length) events, which could be generally classified in three groups: 5'recursive (using the exonic splice donor), 3'recursive (using the exonic splice acceptor) and nested (using two intronic splice sites). The splicing events we found occurred in introns of all lengths, but generally followed the abundance scheme of all introns, i.e. most were found in introns between 500 and 5000 bps. After confirmation of these splice events by conventional methodologies, we further analyzed the impact of intrasplicing on full intron removal. For this we established a luciferase assay which showed that these splicing steps can be beneficial, deleterious or neutral for full intron removal. Thus their regulation can be crucial for determining transcriptional output. This in part confirms recent findings on recursive splicing events in humans and other vertebrates but also shows another, yet undiscovered level of flexibility and regulation of splice site selection. Ongoing research investigates the role of intrasplicing in alternative splice site selection and exon skipping.

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### 38 Divergent splicing of paralogous genes – the intergenic regulatory loop of *RPL22*

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Ribosomal protein genes (RPGs) are among the retained paralogous pairs after whole genome duplication events in both yeast and plants. RPGs were found to respond to environmental stresses and their variability may increase adaptability in natural environment. Our aim was to characterize the regulatory relationship between *RPL22A* and *RPL22B* in *Saccharomyces cerevisiae*. We generated strains with various combinations of intron and gene deletions in *RPL22A/B* and documented that both introns inhibited the expression of their genes. In brief, while the WT *RPL22A/RPL22B* ratio was 9.2/0.8, *rpl22aΔi/RPL22B* and *RPL22A/rpl22bΔi* ratios were 9.9/<0.1 and 0.6/0.4, respectively. When swapped between A and B, the introns maintained their inhibitory potential. Proteomic analyses showed that the Rpl22A/Rpl22B protein levels followed the ratios of the corresponding mRNAs. Using recombinant splicing reporters, we demonstrated that the effects were due to the inhibition of splicing by Rpl22 proteins but not their RNA binding mutants. Indeed, both Rpl22A/B proteins interacted in a yeast three-hybrid system with *RPL22B* intronic region between bp 165 and 236. Inhibition by Rpl22 was insensitive to ~10 fold accumulation of pre-mRNA in *upf1* mutants. Single *RPL22* gene from *Kluyveromyces lactis*, which did not undergo the whole genome duplication, also responded to inhibition by Rpl22 when introduced into *S. cerevisiae*. Transcriptome analyses showed that whereas increased Rpl22 B/A ratio (in *rpl22bΔi*) had almost no effect on the transcriptome, its decrease (in *rpl22bΔ*) led to the downregulation of a set of genes involved in rRNA processing, ribosome biogenesis, and proteosynthesis. This points to a paralog-specific role of Rpl22B protein. Splicing efficiency analysis of the transcriptome data revealed that minor alternative 5'ss- and 3'ss-isoforms of *RPL22B* responded differentially to *RPL22A* deletion. Our results provide evidence for intergenic regulation of splicing in *Saccharomyces cerevisiae* and suggest that the system most likely evolved from a singleton Rpl22-intron inhibitory interaction, as implicated by the *Kluyveromyces lactis* data. Ribosomal proteins may bind novel RNA structures as they occur during the evolution of the transcriptomes. The evidence for extraribosomal roles of Rpl22s in divergent taxa illustrates the exploitation of such evolutionary opportunities.



**39 Discovery of regulators of mammalian ribosome biogenesis***Katherine Farley-Barnes<sup>1</sup>, Kathleen McCann<sup>1</sup>, Janie Merkel<sup>2</sup>, Yulia Surovtseva<sup>2</sup>, Susan Baserga<sup>1</sup>*<sup>1</sup>**Yale University, New Haven, CT, USA;** <sup>2</sup>**Yale University, West Haven, CT, USA**

It is well known that in cancer an increased size and number of nucleoli correlates with increased malignant potential. Nucleoli make ribosomes, the essential protein synthesizers of the cell. Previously we have shown (PLoS Genetics 2012) that disruption of nucleolar function in mammalian cells by depletion of the ribosome biogenesis factors UTP4 or NOL11 causes a change in the number of nucleoli per cell from 2-3 to only 1. Taking advantage of this relationship between number and function, we exploited this assay to discover new regulators of nucleolar function in human cells via a high-content, high-throughput, genome-wide siRNA screen. This screen successfully identified approximately 200 proteins whose depletion caused a change in the number of nucleoli per cell from 2-3 to only 1. The function of a subset of these hits in ribosome biogenesis has been validated by oligonucleotide deconvolution. Of these high-confidence, validated hits, 22 were chosen for further study, including both nucleolar and non-nucleolar proteins. While the nucleolar proteins are directly involved in ribosome biogenesis, the non-nucleolar proteins likely reflect new pathways of nucleolar regulation in mammalian cells. Out of the 22 tested hits, we have identified new roles for 20 hits in regulating either the RNA polymerase I transcription of the ribosomal DNA (8/22), or in the processing of the pre-ribosomal RNA (19/22). These hits increase the complexity of ribosome biogenesis, extending it beyond the nucleolus to extra-nucleolar protein regulators of nucleolar function.

**40 Co-transcriptional ribosome assembly in real-time***Olivier Duss<sup>1,2</sup>, Galina Stepanyuk<sup>1</sup>, Sean O'Leary<sup>2</sup>, Jody Puglisi<sup>2</sup>, Jamie Williamson<sup>1</sup>*<sup>1</sup>**The Scripps Research Institute, La Jolla, USA;** <sup>2</sup>**Stanford University, Palo Alto, USA**

The bacterial ribosome is a large macromolecular machine composed of three large RNA molecules and more than 50 proteins, which are co-transcriptionally assembled in a living cell within 1-2 minutes.

The molecular mechanism of assembly has extensively been studied with pre-transcribed ribosomal RNA (rRNA) mainly using ensemble methods, in which binding of each ribosomal protein (r-protein) is detected as the average state of all populations in which it is present. Because active transcription at the correct speed is essential for ribosome assembly at physiological conditions, the molecular mechanism of ribosome assembly has to be understood co-transcriptionally.

Here we present a novel single-molecule fluorescence based approach in which transcription and folding of single rRNA molecules and the binding of r-proteins to them can be visualized simultaneously in real-time.

Studying the co-transcriptional binding of the primary r-protein S15 to part of the central domain of the 16S rRNA shows that besides short lived binding events (few seconds lifetime), stable S15 binding (>30 seconds lifetime) can occur shortly after transcription of its binding site. In contrast, co-transcriptional binding of primary protein S7 to the entire 3'-domain (500 nucleotides long) is much more dynamic with some RNA molecules being unable to stably bind S7 while others can eventually bind S7 specifically, yet can do so only minutes after transcription at 20 degrees Celsius. We can show that RNA structures remote from the S7 binding site are responsible for RNA misfolding but at the same time, those structures are important for stabilizing the S7 binding site.

Overall, using a novel approach to observe in real-time binding of single r-proteins to transcribing r-RNA, we see that co-transcriptional folding of the central 16S rRNA domain is efficient allowing immediate protein binding, while 3'-domain folding is inefficient, indicating that other r-proteins or assembly factors such as RNA helicases must increase folding efficiency in the cell (at least at low temperature).

## 41 The Assembly Factor Rrp5 Coordinates the Assembly of 40S and 60S Ribosomal Subunits

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Cells produce 40S and 60S ribosomal subunits in roughly equal amounts. This is ensured by co-transcription of the small and large ribosomal subunit RNAs. However, the two subunits are separated early during ribosome biogenesis and are assembled by different machineries. The separation of the two pre-rRNAs occurs co-transcriptionally and is promoted by the nuclease Rcl1. Interestingly, this cleavage step, which is required for 40S production, is delayed for nearly one minute until part of 25S rRNA are transcribed. How transcription of pre-60S rRNAs is sensed, and relayed to pre-40S subunits remains unknown.

Rrp5 is one of only three assembly factors required for assembly of both ribosomal subunits. Rrp5 joins the assembling 40S subunit early. After separation of pre-40S and pre-60S, interactions between Rrp5 and pre-40S are broken, and Rrp5 departs with pre-60S, where it is required for the first pre-60S processing event. We have used a combination of biochemical and genetic experiments to show that (i) structural domains in Rrp5 suppress premature pre-40S rRNA cleavage *in vivo* and *in vitro*; (ii) these domains also impose an rRNA binding mode on Rrp5 that prevents RNA-protein interactions required for pre-40S rRNA processing; (iii) addition of pre-60S assembly factors, rescues both the structural changes and Rcl1-dependent pre-rRNA cleavage. These data support a model in which Rrp5 blocks premature processing of pre-18S rRNA, such that 40S assembly can only proceed if initial steps of 60S assembly are successful. Our data show that Rrp5 coordinates the assembly of 40S and 60S ribosomal subunits, ensuring that the levels of the two subunits are balanced.

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## 42 Charged tRNA-seq: Quantifying tRNA charging levels via high throughput sequencing

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Charged tRNAs carry an amino acid at the 3' end and are required for protein synthesis. In proliferating cells, charged tRNAs turn over in seconds, and tRNA charging levels can be used for rapid response to cellular conditions. For example, under stress conditions in all eukaryotes, uncharged tRNAs are known to regulate global translation through the activation of the GCN2 kinase. Previous methods to determine tRNA charging fraction have relied on acidic-denaturing gels followed by Northern-blot (low throughput) or tRNA charging microarrays (low resolution). While tRNA charging microarrays are sufficient for organisms with a low number of tRNA sequences, organisms with high tRNA sequence diversity such as mammals require a method with single nucleotide resolution. Our lab recently reported a method to sequence tRNAs termed DM-tRNA-seq (1) that utilizes a demethylase treatment followed by thermostable RT to overcome the most prevalent modifications that block reverse transcription (m<sup>1</sup>A, m<sup>1</sup>G, m<sup>3</sup>C) and the extensive tRNA structure. Here, we develop a method to use sequencing to determine the charged fraction of tRNA isodecoders in mammalian cells. We employ the same periodate oxidation chemistry utilized for tRNA charging microarrays to selectively oxidize the 3' end of uncharged tRNAs. After deacylation under basic conditions, oxidized nucleotides are eliminated through  $\beta$ -elimination; therefore, the 3' end of uncharged tRNAs will end in -CC while the 3' end of charged tRNAs will end in -CCA. In this way, periodate oxidation followed by DM-tRNA-seq can be used to quantify the level of charged tRNA isodecoders in the cell. Our results show that charged tRNA-seq not only reports the charging fraction of tRNA isodecoders at single base resolution, but also enables the comparison of charging fraction and modification status (2). Our method should have wide-spread applications in the biological investigation of charged tRNA dynamics and function.

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### 43 A novel class of minimal and RNA-free RNase P in the hyperthermophilic bacterium *Aquifex aeolicus*

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Ribonuclease P (RNase P) is the essential and ubiquitous endonuclease that removes 5' extensions from tRNA precursors. In Bacteria, the enzyme consists of a catalytic RNA (~ 400 nt) and an auxiliary protein component (~ 13 kDa).

Although the genome of the hyperthermophilic bacterium *Aquifex aeolicus* became available as early as 1998, neither a gene for the RNA nor the protein component of bacterial RNase P could be identified [1]. Canonical bacterial RNase P components were nevertheless identified in related Aquificales strains [2], and RNase P activity was detected in *A. aeolicus* cell lysates [3,4]. We were now able to sufficiently enrich the RNase P activity of *A. aeolicus* to reveal its identity. Lacking an RNA subunit, the enzyme is the smallest of its kind: the 23-kDa polypeptide, which only comprises a metallonuclease domain, has RNase P activity *in vitro* and can rescue growth of *E. coli* and yeast strains with deactivations of their larger and more complex endogenous ribonucleoprotein RNase P. Homologs of *Aquifex* RNase P (HARP) were identified in many Archaea and some Bacteria, of which all Archaea and most Bacteria also encode an RNA-based RNase P. For one bacterium and one archaeon, activity of both RNase P forms could be demonstrated. Bioinformatic analyses suggest that *A. aeolicus* and related *Aquificaceae* acquired HARP by horizontal gene transfer from an archaeon.

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### 44 Fragmentation of tRNAs in *Trypanosoma brucei* during stress

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Regulation of gene expression has to be tightly controlled. In recent years tRNA-derived fragments have emerged as regulators of gene expression during stress in a plethora of organisms and have been shown to affect gene expression at different levels.

The pathogenic protozoan parasite *Trypanosoma brucei* largely lacks the ability to regulate transcription of protein coding genes and all protein-coding genes are transcribed polycistronically. Therefore they heavily rely on posttranscriptional means to regulate gene expression. We are particularly interested in the changes that happen on tRNA molecules during stress and how these might affect gene expression and parasite survival.

Analysis of tRNA molecules during nutritional stress by RNA-Seq and northern blot showed the presence of many tRNA halves. A specific tRNA fragment, the Thr tRNA 3' half, stimulates translation *in vitro* and *in vivo*, and its effect is particularly strong during recovery from nutritional stress. Depletion of the Thr tRNA half by inducible RNAi causes a decrease in translation during stress recovery.

Another discovery was the shortening of the bulk of the tRNA population during nutritional stress at the expense of the universally conserved 3'-CCA tail. This is not a consequence of lack of CCA-tail addition as the activity of the CCA-adding enzyme is not affected upon stress. tRNA shortening can be partly reversed *in vivo* by overexpressing the CCA-adding enzyme and the shortened tRNAs can be repaired by the CCA-adding enzyme *in vitro*. Analysis of cellular fractionations showed that the nuclease responsible for tRNA truncations is only present in the cytosol of stressed cells, although mitochondrial fractions purified from both stressed and exponentially growing conditions are able to remove the CCA-tail. Despite the strong decrease in polysomes observed during nutritional stress the truncated tRNAs (and other tRNA-derived RNAs) are enriched in the polysome fraction.

We propose a novel mechanism in which a nuclease is activated in the cytosol of stressed cells, where it is able to remove the CCA-tail of tRNAs, causing a reduction in overall translation. Once the stress conditions are overcome the CCA-adding enzyme can repair these tRNAs and normal translation is restored.

## 45 5'-TOG containing tiRNAs assemble into G-quadruplexes to promote translation repression

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As cells encounter adverse environmental conditions, such as hypoxia, oxidative stress or nutrient deprivation, they initiate several stress response pathways in order to protect the cells until transient stresses have passed. This is primarily accomplished by global translational arrest facilitated by phosphorylation of the translation initiation factor eIF2 $\alpha$ , consequently, leading to the formation of non-membranous cytoplasmic bodies called stress granules (SGs). Functionally, SGs play diverse roles in cell adaptation to stress and promote cell survival and perturbations in SG dynamics are implicated in human diseases. However, translational attenuation is also moderated by non-eIF2 $\alpha$  dependent mechanisms. Our lab has discovered one such mechanism that utilizes novel small non-coding RNAs that we have termed tRNA-derived stress-induced RNAs (tiRNAs). These are members of an ever-growing family of small (14 – 32 nucleotide) RNAs known as tRNA derived fragments (tRFs) that are produced through various different mechanisms, most of which have unknown function. We have identified two 5'-tiRNAs (5'-tiRNA<sup>Ala</sup> and 5'-tiRNA<sup>Cys</sup>) that are potent inhibitors of protein synthesis. This ability requires a 5' terminal oligoguanine (5'-TOG) motif. Here we show that this motif folds into parallel RNA G-quadruplex (G4) and that this structure, rather than solely 5'-TOG sequence, is required for bioactivity. By preventing the formation of G4 through ionic equilibration or through use of modified nucleic acids, we can prevent the activity of 5'-TOG tiRNAs. Further, we demonstrate that the G4 structure is required for binding to YB-1, a multifunctional RNA binding protein with documented roles in translation regulation. Using CRISPR/Cas9 mediated deletions of YB-1 in tissue culture cells, we demonstrate that YB-1 is required for 5'-TOG containing tiRNA mediated SG formation, but is dispensable for translation repression activities. *In vitro* crosslinking studies and unbiased proteomic investigation of mRNP complexes in complex with 5'-tiRNA<sup>Ala</sup> identified CNBP as a direct interacting partner of 5'-tiRNA<sup>Ala</sup>. Preliminary data suggests that interaction of CNBP with 5'-tiRNA<sup>Ala</sup> is required for translation repression activity of 5'-tiRNA<sup>Ala</sup>.

## 46 mRNA structural heterogeneity and extensive post-transcriptional regulation revealed by high-resolution SHAPE probing in living cells

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Messenger RNAs (mRNAs) can fold into complex structures that regulate gene expression. Recent experiments have provided transcriptome-average measurements of mRNA structure, but understanding of mRNA structural variation and its functional importance has been limited by the absence of high-resolution structural data for individual mRNAs. We use SHAPE-MaP experiments in living *E. coli* cells to derive nucleotide-resolution structural models for 194 endogenous transcripts encompassing approximately 400 genes. Parallel experiments performed on *E. coli* cells treated with a translation inhibiting antibiotic, and protein-free extracts allow us to examine the biological mechanisms shaping and shaped by RNA structure in unprecedented detail. Our data indicate that mRNAs adopt diverse and complex structures, including both highly structured and extended dynamic regions, frequently in the same transcript. Highly translated genes exhibit a marked increase in SHAPE reactivity in cells, consistent with transient unfolding caused by ribosome translocation. Despite this general destabilization, mRNAs adopt similar overall structures *in vitro* and *in vivo*. We uncover a much stronger relationship between mRNA structure and gene-to-gene variation in translation efficiency than previous studies of endogenous mRNAs, and validate this relationship by reporter-gene assays. mRNA structure also appears to be widely used to couple translation of adjacent genes in polycistronic transcripts. Finally, we discovered well-defined structure motifs in over 30% of examined untranslated regions (UTRs). Most of these motifs are conserved across multiple bacterial genera, and several motifs were confirmed as protein-binding elements, emphasizing that these newly identified motifs represent likely novel post-transcriptional regulatory structures. Overall, our study reveals that gene regulation via RNA structure is widespread and constitutes a fundamental component of the genetic code in bacteria.



## 47 Enzymatic modification of eukaryotic mRNA for intracellular labeling and photo-crosslinking

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Messenger RNA may not be very abundant in the cell but its central role in gene expression is indisputable. In addition to being the template for translation it can be subject for a variety of regulatory mechanisms affecting gene expression. The plethora of posttranscriptional regulatory options for eukaryotic mRNAs emphasizes the importance of labeling mRNAs to make them accessible to functional studies, particularly in living cells. The low level abundance of mRNAs at the same time denotes challenges resulting from low signal to background ratios.<sup>1</sup>

We developed a chemo-enzymatic approach for site-specific modification of mRNA at its 5' cap.<sup>2</sup> Based on the highly promiscuous methyltransferase Ecm1 and synthetic cosubstrates - so-called AdoMet analogs - we can install a range of functional moieties at the N7 position of the 5' terminal guanosine. These modifications allowed us to label mRNA via bioorthogonal click reactions in living eukaryotic cells.<sup>2a</sup> In combination with a second methyltransferase, we achieved dual labeling of the 5' cap and an improved signal-to-noise-ratio.<sup>3</sup>

We also developed a set of novel AdoMet analogs that enabled for the first time enzymatic installation of photo-crosslinkers on RNA.<sup>4</sup> These photo-crosslinkers were used to capture eIF4E as a known cap-interacting protein and may give access to identifying proteins binding in proximity to the 5' cap.

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## 48 Hybrid structural modeling of PTBP1 bound to a structured RNA target using crosslinking of segmentally isotope labeled RNA and MS/MS

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Ribonucleoprotein (RNP) complexes are key regulators involved in all steps of RNA metabolism and thus crucial for cellular function. Precise knowledge of the interaction sites on RNA and protein provides essential information for understanding RNP regulated processes in health and disease. Chemical and photo-crosslinking have been employed in the past to identify either transcriptome wide RNA targets of selected bait proteins[1, 2] or RNA-binding sites within specific proteins[3]. However, none of the present techniques is capable of localising protein-RNA interactions sites at a resolution required to support integrative structural modelling. We will present a new approach called CrossLinking of segmentally Isotope labeled RNA and MS/MS (CLIR-MS/MS)[4] that allows the detection of protein-RNA interactions with nucleotide and amino acid resolution. The method was applied to U1snRNP and an 85 kDa complex of the Polypyrimidine Tract Binding Protein 1 (PTBP1) bound to a part of the Internal Ribosomal Entry Site (IRES) of Encephalomyocarditis Virus (EMCV). The method allowed to precisely position all four RNA Recognition Motifs (RRMs) of PTBP1 on the RNA, substantially revising the previous model proposed for this complex[5]. We demonstrate that the high spatial resolution of RNA-protein interaction sites achieved by the method provides distance restraints that significantly contributed to the generation of 3D atomic models of each RRM-RNA complex by hybrid modelling. The models were validated by nuclear magnetic resonance (NMR) and combined with data derived from NMR, electron paramagnetic resonance spectroscopy and small angle neutron scattering in an integrative structural biology approach to obtain a preliminary model of full length PTBP1 in complex with the IRES-RNA.

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## 49 Co-crystal structure RNA Mango: fluorescence activation of thiazole orange derivatives by an aptamer

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Fluorescent proteins revolutionized cell biology by making it possible to visualize large complexes and even single proteins within living cells. A toolkit of fluorescent RNA tags would help answer some of the more pressing questions in RNA cell biology, facilitating *in vivo* analyses of RNA interactions, localization and traffic. Since there are no known naturally fluorescent RNAs, several groups have applied *in vitro* selection to produce aptamers that selectively bind and activate the fluorescence of small molecules (e.g., Paige et al., Dolgosheina et al.). Although not optimized for *in vivo* application, fluorescent aptamers have been used to visualize RNA within eukaryotic cells and have also been employed as reporters for biosensors. Improving existing fluorogenic RNAs will require a combination of structure based-design and reselection in order to achieve desirable traits such as high fluorophore affinity, small size, and optimized photophysical properties (e.g., brightness and emission spectrum). RNA Mango is a fluorescent aptamer that exhibits high affinity for its thiazole orange-derived ligand (~3 nM) and is currently the smallest described fluorescent RNA aptamer, making it an intriguing candidate for further engineering efforts. We have now solved the co-crystal structure of RNA Mango at 1.73 Å-resolution. The structure reveals a three-tiered, mixed-connectivity G-quadruplex forming the platform upon which the ligand binds. Unexpectedly, the thiazole orange derivative (TO1-Biotin) binds in a circular conformation, where the fluorophore is adjacent to the biotin moiety. The two heterocycles of the fluorophore are twisted by ~45°, consistent with the modest quantum yield of the RNA-ligand complex ( $\phi=0.14$ ). Alleviating steric constraints on the fluorophore within the binding pocket improves both the fluorescence lifetime and fluorescence enhancement of the Mango-TO1 complex. Thus the Mango-TO1-Biotin structure provides a detailed understanding of how an RNA enhances fluorescence of its ligand and provides the basis for future optimization.

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## 50 Structural Basis for Ligand Recognition by a Guanidinium Specific Riboswitch

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Regulation of gene expression by cis-encoded RNA structures called riboswitches is common in bacteria. The study of these RNAs reveals new facets of bacterial physiology with potential consequences for the treatment of human disease. Riboswitches are found in the 5'-UTR of mRNAs where they modulate the expression of downstream genes by changing conformation upon ligand binding. Hundreds of riboswitch families have been identified, yet the majority of them remain "orphans," without a clear ligand assignment. The ykkC orphan family was recently characterized as guanidine-sensing riboswitches (Nelson et al. 2017). By specifically binding guanidine in its positively charged guanidinium form, ykkC riboswitches are able to activate genes involved in the breakdown and removal of this toxic byproduct.

Understanding how ykkC riboswitches distinguish guanidinium from similar metabolites in the intracellular environment is critical for understanding their regulatory mechanism. Of particular interest to our lab is how the structure of this RNA allows it to both specifically bind its ligand and change conformation to affect gene expression. To this end, we have solved a 2.3 Å crystal structure of the guanidine-bound ykkC riboswitch (Battaglia et al. 2017). The riboswitch folds into two juxtaposed helices that form a boot-shaped structure via extensive cross-helix tertiary interactions. A binding pocket in the heel of the "boot" recognizes a single guanidinium molecule through co-planar hydrogen bonds and a cation- $\pi$  stacking contact. Mutagenesis targeting tertiary contacts and binding pocket residues resulted in severe binding defects.

The prevalence of guanidine-sensing riboswitches across bacteria suggests guanidine homeostasis is important to the survival of the cell and may be connected to a hitherto unappreciated metabolic process. The structural characterization of the ykkC riboswitch provides insight into the only known molecular sensor of this metabolite and opens the door to further studies of its physiological roles.

## 51 Time-resolved structures of an aptamer domain of the adenine riboswitch by diffusion-delay-diffraction using X-ray free electron laser and serial femtosecond crystallography

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Biological reactions all require interactions between biomacromolecules and their substrates, some of which involve major structural changes. In the genetic regulation by riboswitches, ligand binding to the aptamer domain triggers a signal to the downstream expression platform<sup>1</sup>. A complete understanding of the structural basis for this mechanism requires the ability to study structural changes over time. With the application of serial femtosecond crystallography (SFX) and an X-ray free electron laser (XFEL), we demonstrate advances in both time-resolved crystallography and riboswitch biology. Using a substrate diffusion-delay-diffraction by XFEL (3DX) approach, we determined four structures of the adenine riboswitch aptamer domain during the course of a four-state reaction involving two apo, one ligand-bound intermediate, and the final bound states. These structures serve as the proof of the four-state reaction kinetics model and illustrate the structural basis for signal transmission. The two distinct apo conformers differ significantly in the three-way junction and the P1 switch helix relative to the ligand-bound conformation. 3DX with a 10-second delay captured the structure of an intermediate with changes in the binding pocket that accommodate ligand. With a >10-minute delay, the RNA molecules were fully converted to the bound state, in which the substantial conformational changes resulted in conversion of the space group and unit cell. These results demonstrate the utility of 3DX to study structural transitions in real time in crystallo, including those involving large conformational changes.

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## 52 The structural switch in a viral RNA protects from 5'-3' exonucleolytic decay

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RNA viruses generally have short genomes and encode only a few proteins, thus they have evolved elegant RNA-based strategies to manipulate cellular processes and thereby enhance virus propagation. An interesting illustrative example is found in plant-infecting Dianthoviruses, which generate viral non-coding RNAs through the selective protection of their 3' UTR from 5'-3' exonucleolytic decay. We discovered that a 44-nucleotide structured RNA element at the beginning of the viral 3' UTR is sufficient to inhibit 5'-3' exonucleolytic decay in vitro. We solved the three-dimensional structure of a complete Dianthovirus nuclease-resistant RNA (nrRNA) by x-ray crystallography, revealing that it forms a stem loop structure which is held in a distorted "tilted" conformation through several long-distance base-stacking interactions. Base pairs formed between adjacent molecules in the crystal structure indicate that this Dianthovirus nrRNA can adopt two mutually exclusive conformation, and functional assays with mutant RNAs confirm that both conformations are required for nuclease resistance. These data suggest that the nrRNA element functions as a structural switch, triggered by the arrival of an exonuclease from the 5' direction. Whereas the tilted stem-loop structure likely represents a necessary folding intermediate, the actual nuclease-resistant structure contains a pseudoknot formed between the 3' end of the structure and the loop sequence, which generates a ring-like fold that protectively wraps around the 5' end of the structure. A similar ring-like shield around the 5' end of an RNA is found in the nuclease-resistant RNA structures of arthropod-borne flaviviruses (such as Dengue and Zika virus), although the folding strategy used to form the two structures are very different. Even though the overall fold of flaviviral and dianthoviral nuclease-resistant RNAs differs greatly, this observation highlights how highly divergent RNA viruses have developed similar RNA-based strategies to inhibit exoribonucleases, and suggests that manipulation of nuclease activity through RNA structure might be a widespread mechanism.

### 53 Structure-Specific Recognition of a G-Quadruplex RNA by the Histone Demethylase LSD1

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Long non-coding RNAs (lncRNAs) are increasingly linked with epigenetic regulation, but the molecular mechanisms remain largely unknown. To better understand these mechanisms we examined the recruitment of human lysine-specific histone demethylase 1 (LSD1) by the telomere repeat-containing RNA (TERRA) lncRNA, a previously identified RNA-protein interaction. LSD1 is an essential epigenetic regulator and important oncogene that affects gene expression by catalyzing the demethylation of mono- and dimethyl functional groups from lysines 4 and 9 of histone 3 (H3K4/9). LSD1 is a key component of multiple regulatory complexes that include lncRNAs such as TERRA. Here, we present evidence that TERRA acts as an inhibitor of LSD1 and that LSD1 specifically recognizes a stacked, parallel-stranded intramolecular G-quadruplex (GQ) ( $K_d \approx 96$  nM). LSD1 has dramatically weaker affinity for the same RNA when it is not folded into a GQ structure or when the same sequence is used to form a DNA GQ. This suggests that LSD1 can distinguish RNA from DNA and that LSD1 can recognize a nucleic acid tertiary structure through a novel G-quadruplex binding mechanism. Cross-linking mass spectrometry identifies a region of the SWIRM domain as the likely GQ RNA binding site. In addition, the analysis of photoactivatable ribonucleoside crosslinking immunoprecipitation (PAR-CLIP) data provides a comprehensive portrait of RNA binding by LSD1. Collectively, results indicate that LSD1 can recognize structured and G-quadruplex forming RNAs. These findings provide insight into the mechanisms of regulation by lncRNAs at chromatin and hold implications for the recruitment of LSD1 by other RNA transcripts.

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### 54 Recognition of RNA structures by quadruplex helicases and their functional consequences.

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RHAU (also known as DHX36) is an ATP-dependent helicase that binds and unwinds G-quadruplexes. We have identified novel RHAU-interacting RNAs, and the diversity of the interactions suggests that RHAU has broad implications in controlling RNA metabolism/regulation. We present structural and functional characterization of two exciting binding partners from this screen. First, RHAU interacts with RNA quadruplexes in the 3'-untranslated region of PITX1 mRNA (paired-like homeodomain transcription factor). PITX1 is known to act as a tumour suppressor via transcriptional activation of p53 and RASAL1, and as a transcriptional suppression of hTERT (telomerase enzymatic component). We present data characterizing the helicase-mRNA interaction and we detail our investigations into the functional mechanisms whereby translational regulation of PITX1 expression is achieved by RHAU. Second, BCYRN1 (brain cytoplasmic RNA 1) was identified as a RHAU-binding partner, and is a small non-coding RNA that is expressed primarily in neural tissue and germ cells but is aberrantly expressed in a variety of carcinomas. BC200 is postulated to play a role in translational regulation of a subset of mRNAs, possibly acting as a key regulator of site specific protein translation. We have discovered that BC200 binds directly to the RNA-quadruplex helicase RHAU and have demonstrated that BC200 can stabilize unwound quadruplexes through a region of 12 consecutive cytosine nucleotides at its 3' end. We have analyzed BC200 expression in a panel of eight cell lines and demonstrated a loss of cell viability and induction of apoptosis following BC200 knock-down. Further, we have developed a method to pull-down the endogenous RNA and have performed mass spectrometry analyses of the BC200 interacting proteins and NGS analysis of BC200 interacting RNAs. We conclude by presenting our studies of newly identified quadruplex helicases that may fulfill a complimentary role to RHAU.

## 55 Architecture of the yeast small subunit processome

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The small subunit (SSU) processome, a large ribonucleoprotein particle, organizes the assembly of the eukaryotic small ribosomal subunit by coordinating the folding, cleavage and modification of nascent pre-rRNA. To better understand the role of this particle, we solved the cryo-EM structure of the yeast SSU processome at 5.1 Å resolution. The structure reveals how large ribosome biogenesis complexes assist the 5' external transcribed spacer and U3 snoRNA in providing an intertwined RNA-protein assembly platform for the separate maturation of 18S rRNA domains. The strategic placement of a molecular motor at the center of the particle further suggests a mechanism for mediating conformational changes within this giant particle. This study provides a structural framework for a mechanistic understanding of eukaryotic ribosome assembly in the model organism *Saccharomyces cerevisiae*.

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## 56 Final maturation of the 60S ribosomal subunit: atomic structure the 60S subunit with the nuclear export adapter Nmd3 and the cpGTPase Lsg1

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Ribosome biogenesis in eukaryotes is a highly complex pathway that is initiated in the nucleolus and completed in the cytoplasm. While the temporal order of the steps and the associated factors are relatively well understood for the large (60S) subunit in yeast, only recently has structural information about factor-factor and factor-subunit interactions started to become available, enlightening the complex interactions and remodeling of the subunit during maturation. We are interested in the final maturation steps of the 60S subunit, the release of the export adapter Nmd3 and the anti-association factor Tif6. These events are each driven by distinct GTPases and we have previously speculated that the release of Tif6 by the translation elongation factor paralog Efl1 and Sdo1 serves as a quality control check to verify correct assembly of the large subunit. Here, we focused on the export adapter Nmd3 and the circularly permuted GTPase, Lsg1, which is required for the release of Nmd3. Using cryo-EM we achieved a 3.1 Å map for the 60S subunit in complex with Nmd3, Lsg1, GMPPNP, and Tif6. We find that Nmd3 spans the entire width of the large subunit, from the closed L1 stalk to Tif6. The central domain of Nmd3 shares structural and sequence similarity with eIF5A, probing the assembly of the L1 stalk and the E-site. Additional domains occupy the P-site and interact with Tif6. Lsg1 is centered on the joining face of the subunit and embraces helix 69, which contributes to inter-subunit bridge B2a and is involved in various steps of translation. Upon Lsg1 binding we observe a base flip within helix 69 that may be necessary for the GTPase activity. The structure of the complex suggests that the Nmd3 – Tif6 interaction stabilizes Nmd3 in the P-site. Consequently, this interaction would have to be broken to allow the L1 stalk to retract Nmd3 from the P-site and permit Sdo1 binding. Tif6 and Nmd3 may then be released independently of each other.



## 57 Structural basis for guide RNA processing and seed-dependent DNA targeting by CRISPR-Cas12a

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Cas12a (Cpf1) is a CRISPR-associated nuclease that catalyzes not only RNA-guided DNA cleavage but also the processing of its own guide RNAs. Here we report crystal structures of *Francisella novicida* Cas12a in a binary complex with a guide RNA, and in a ternary complex containing a non-cleavable guide RNA precursor and full-length target DNA. Corroborated by biochemical experiments, these structures elucidate the mechanism of guide RNA processing and pre-ordering of the guide RNA seed sequence that primes Cas12a for target DNA binding. The ternary complex structure reveals the R-loop formed upon guide-target hybridization and suggests a mechanism for target DNA cleavage. Together, these insights advance our mechanistic understanding of Cas12a enzymes that may contribute to further development of genome editing technologies.

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## 58 Cryo-EM structure of *S. cerevisiae* U1 snRNP at 3.7Å resolution

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Pre-mRNA splicing is catalyzed by the spliceosome, a huge protein-RNA complex composed of the U1, U2, U4, U5, U6 snRNPs and many non-snRNP related proteins. U1 snRNP is critical for 5' splice site (ss) recognition and is a frequent target of the action of alternative splicing factors that either facilitate or prevent U1 snRNP from binding to 5' ss. Much of what we know today about the molecular mechanism and regulation of 5' ss recognition comes from genetic, biochemical, and structural studies of two commonly used model systems, *S. cerevisiae* and human U1 snRNP. Intriguingly, the *S. cerevisiae* U1 snRNP is much more complex than the human U1 snRNP. *S. cerevisiae* U1 snRNA is 3.5 times larger than its human counterpart and contains seven additional proteins. In spite of the critical importance of *S. cerevisiae* as a model system for understanding the mechanism of splicing that is often applicable to higher eukaryotes, there has not been any structural information of *S. cerevisiae* U1 snRNP, despite the multiple high-resolution spliceosome structures solved recently. In contrast, much structural information on human U1 snRNP is available due to its compositional simplicity.

We have determined the structure of *S. cerevisiae* U1 snRNP at 3.7Å resolution using cryo electron microscopy (cryoEM). The structure reveals for the first time the three-dimensional organization of *S. cerevisiae* U1 snRNP, including common features as well as important differences from the human U1 snRNP. It provides atomic models of most of U1 snRNA and *S. cerevisiae*-associated proteins, none of which has any prior structural information. The structure offers a framework to integrate a wealth of existing genetic and biochemical data regarding the structure and function of *S. cerevisiae* U1 snRNP and the mechanism of 5' ss recognition. In addition, many of the *S. cerevisiae*-associated U1 snRNP proteins have human homologs that weakly associate with the human U1 snRNP (hence not available in the human U1 snRNP structure) and are involved in alternative splicing. The *S. cerevisiae* U1 snRNP structure provides intriguing insight into the structure and function of these auxiliary human U1 snRNP proteins in alternative splicing in higher eukaryotes.



## 59 Prp40 WW Domains are Critical for Efficient Splicing of Nonconsensus Introns in the Budding Yeast

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In the budding yeast, the intron 5' splice-site (5'SS) and branch-site (BS) sequences are largely conformed to GUAUGU and UACUAAAC signatures, respectively. Intriguingly, 40% of introns which contain nonconsensus 5'SS or BS sequences are still effectively spliced *in vivo*, suggesting that the yeast splicing system is equipped with a specific fine-tuning mechanism for recognizing and executing on them. Here we report that the WW domains in Prp40, an intrinsic U1-snRNP component, play such a role in promoting efficient splicing of the nonconsensus introns. Prp40 WW domains are well conserved across fungal species and were implicated in bridging U1 snRNP and BBP during early spliceosome assembly. Yet, the WW domains were reported to be inconsequential to splicing from analyzing a consensus intron-containing gene. To more rigorously examine this issue, we employed Synthetic Genetic Array (SGA) and uncovered synthetic interactions between WW domains and several key BS-interacting splicing factors. Subsequent splicing-sensitive microarray analysis identified a set of nonconsensus introns that are particularly sensitive to the loss of the WW domains. By varying 5'SS and BS in a splicing reporter, we further showed that the temperature-sensitive requirement of WW domains is intron specific. Detailed chromatin immunoprecipitation (ChIP) assay revealed that efficient co-transcriptional association of splicing factors to the nascent nonconsensus introns depends on the WW domains. Our data thus demonstrated a novel aspect of the nonconsensus-intron splicing in relationship to a BBP-interacting U1-snRNP protein. In mammals, the 5'SS and BS are far more diverged in sequences, and in this light, we propose that other WW-domains-containing proteins in mammals may also participate in fine-tuning the commitment step of splicing reaction in an environment- or stage-specific manner. Finally, our SGA screen also identified several protein-kinase candidates, which are reminiscent of a very recent finding that, in *S. pombe*, SR protein kinases promote splicing of nonconsensus introns via the phosphorylation state of BBP.

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## 60 Single-Molecule Fluorescence Microscopy Reveals the Dynamics and Consequences of Spliceosome E Complex Formation

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E complex forms early during spliceosome assembly and contains U1 at the 5' splice site (SS) and E Complex Proteins [ECPs; BBP/Mud2 at the branchsite (BS) and the nuclear cap binding complex (CBC)]. Yeast genetics supports U1/ECP interactions, possibly bridging sequence-separated pre-mRNA features (5' cap-5'SS-BS). Little is known about E complex formation, dynamics, or its consequences. We have used single-molecule microscopy to monitor E complex formation between U1, BBP, and pre-mRNA in yeast extract. E complex forms stochastically via a branched pathway in which U1 or BBP can first associate with pre-mRNA. E complex is transient, persists from seconds to several minutes, and does not require ATP for disassembly. As a consequence, splice sites may be defined multiple times by different pathways. This suggests that many E complexes form with little commitment of a particular pre-mRNA or a particular 5'SS/BS pair to splicing.

Nonetheless, single-molecule binding kinetics reveal that U1 is strongly influenced by ECPs. Both BBP and the CBC stabilize formation of the longest-lived U1/pre-mRNA complexes even at a consensus 5'SS. ECPs become essential for U1 binding when U1-C is mutated or at weak 5'SS. The influences of ECPs are not identical. BBP, but not the CBC, alters the relative abundance of short-and long-lived U1 interactions. This is consistent with a two-step mechanism for U1 binding in which BBP modulates the equilibrium between these steps. This mechanism provides a kinetic basis for U1 recruitment and retention to RNAs that not only contain sequences complementary to the snRNA but are also capped and contain BS. Thus, pre-mRNAs destined for splicing are more competitive for U1 than transcripts that lack a BS or cap.

Finally, ECPs can destabilize U1 interactions at 5'SS with increased snRNA complementarity. This implies that E complex formation both strengthens weak U1/5' SS interactions and weakens strong interactions to modulate U1 affinity. In conclusion, our data reveal that the U1/pre-mRNA complex is fine-tuned even at strong 5'SS by multiple *cis*- and *trans*-acting factors. This tuning may be used to control U1 dynamics during spliceosome assembly as well as regulate alternative splicing at consensus 5'SS.

## 61 Human spliceosome assembly and its regulation by splicing inhibitors at single-molecule resolution

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Human pre-mRNAs have on average nine introns, each of which is excised by a spliceosome. Spliceosomes are highly complex and dynamic macromolecular machines that must assemble and disassemble with each round of splicing. Major challenges for the human spliceosome are to accurately identify splice sites in a background of cryptic sites and to correctly connect exon ends in the context of multi-intron substrates. Which splice sites are utilized and which are not is likely a function of spliceosomal subcomplex binding and release dynamics, with active splice sites exhibiting more favorable kinetics than inactive ones. Further, spliceosome assembly must somehow distinguish exons from introns to prevent the formation of exon circles.

Here we established a colocalization single-molecule spectroscopy (CoSMoS) approach to study human spliceosome assembly in real time across introns and exons on single pre-mRNA molecules. Splice site mutational analysis and addition of small molecule inhibitors define spliceosomal subcomplex dynamics underlying splice site selection. We find that while U1 snRNP binding is independent of an adjacent U2 snRNP binding site (i.e., polypyrimidine tract), stable U2 snRNP binding is strongly enhanced by a nearby U1 snRNP binding site (i.e., 5' splice site). This 5' splice site can be either upstream or downstream of the U2 binding site, confirming the importance of studying both cross-intron and cross-exon spliceosome. Further, the splicing inhibitors spliceostatin A, meayamycin B, pladienolide B and isoginkgetin all reduce the rate of U2 snRNP binding, but they differentially affect U1 snRNP binding. These insights into human spliceosome dynamics contribute to a comprehensive understanding of splicing regulation and alternative splicing.

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## 62 Structural insight into the mechanism of splicing inhibition by modulators

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Splicing is an essential step in gene expression: it involves the excision of introns and fusion of their flanking exons into mature RNA. In humans, the exons are ligated in alternative ways for at least 95% of the genes, resulting in the vast complexity of the proteome from a limited number of genes. Splicing occurs on the spliceosome - a protein-RNA macromolecular machine that assembles step-wise on the pre-mRNA from snRNPs and proteins, starting with the early recognition of the splice sites (SS) and branch-site (BS) regions. Every spliceosome utilizes reactive groups from these three regions to catalyze ligation of one pair of exons, upon following a cyclic pathway (1).

U2 snRNP is a component of the spliceosome that enables proper BS recognition in constitutive and alternative splicing. Its core component SF3b is a heptameric protein complex acting as a 'pincer' that grasps and stabilises the U2-BS duplex, while concomitantly interacting with the intron, adjacent to the 3'SS (2). The largest SF3b subunit SF3b155/SF3B1 is the most frequently mutated spliceosomal protein in a variety of cancer types, and also represents a major drug target for therapy (3). Thus, various small-molecule compounds referred to as splicing modulators possess strong anti-tumour effects and bind SF3b. Modulators inhibit splicing *in vitro*, while *in vivo* they lead to changes in the alternative splicing of numerous genes (3).

Following up our previous work (4), we determined the first crystal structure of a modulator in complex with SF3b. The structure reveals the binding pocket, the modulator's conformation and the nature of the contacts in the binding pocket, and explains how modulators interfere with the occurrence of splicing.

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2. C. Yan *et al.*, Structure of a yeast activated spliceosome at 3.5 Å resolution. *Science* **353**, 904-911 (2016).
3. S. Bonnal *et al.*, The spliceosome as a target of novel antitumour drugs. *Nat Rev Drug Discov* **11**, 847-859 (2012).
4. C. Cretu *et al.*, Molecular Architecture of SF3b and Structural Consequences of Its Cancer-Related Mutations. *Mol Cell* **64**, 307-319 (2016).

### 63 Structure of a pre-catalytic spliceosome

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Intron removal requires assembly of the spliceosome on pre-mRNA and extensive remodelling to form the spliceosome's catalytic centre. In my talk I will report the cryo-electron microscopy structure of the yeast pre-catalytic B complex spliceosome at near-atomic resolution. The mobile U2 snRNP associates with U4/U6.U5 tri-snRNP through U2/U6 stem II and an interface between U4/U6 di-snRNP and the U2 snRNP SF3b-containing domain, which also transiently contacts the helicase Brr2. The U2 snRNP 3' region is flexibly attached to the SF3b-containing domain and protrudes over the concave surface of tri-snRNP, where the U1 snRNP may reside before its release from the pre-mRNA 5'-splice site. The U6 ACAGAGA sequence forms a hairpin which weakly tethers the 5'-splice site. B complex proteins Prp38, Snu23, and Spp381 bind the Prp8 N-terminal domain and stabilise U6 ACAGAGA stem-pre-mRNA and Brr2-U4 snRNA interactions. The B complex structure provides important insights into events leading to active site formation.

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### 64 Prp18 promotes global second step splicing fidelity by suppressing non-canonical 3'-splice sites

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Prp18 is recruited to the spliceosome during the second step of pre-mRNA splicing by stabilizing the interactions between the U5 snRNP invariant loop and exon ends. Our global RNA-Seq analysis of splice junctions in yeast strains lacking Prp18 revealed the widespread utilization of non-AG, branch point proximal alternative 3'-splice sites (SS) in over 30% of all intron-containing genes, demonstrating that Prp18 plays a global role in maintaining fidelity of 3'-SS selection. These alternative 3'-SS are located ~26 nt closer to the branch point in average than annotated 3'-SS. The use of many of these alternative 3'SS gives rise to nonsense transcripts that are subsequently degraded by the nonsense-mediated decay (NMD) pathway and accumulate upon inactivation of the NMD factor Upf1. A negative genetic interaction between the *upf1Δ* and *prp18Δ* mutants suggests that the accumulation of these nonsense transcripts may be toxic. The use of these non canonical 3'SS is specifically due to Prp18 inactivation as these are not activated in the absence of other second step splicing factors. For instance, nuclear depletion of Slu7, which interacts with Prp18 during the second step, does not trigger the alternative splicing events detected in the *prp18Δ* mutant. In addition overexpression of Slu7 in *prp18Δ* strains does not suppress use of noncanonical 3'SS, in contrast with the previously observed rescue of splicing defects upon the addition of Slu7 to *prp18Δ* extracts *in vitro*. Analysis of several *prp18* point mutants reveals that the role of Prp18 in 3' SS fidelity occurs independently of the Prp18-Slu7 interaction. We are currently investigating the pseudo-exonic sequences upstream of alternative 3'-SS sites transcriptome-wide to uncover potential relationships between exonic context and the role of Prp18 in splicing fidelity. Additionally, a recently published structure of the yeast activated C\* spliceosome complex found that Prp18 associates with the spliceosome via interactions with Prp8. As several *prp8* point mutants demonstrate reduced fidelity in 3'-splice site recognition, we aim to determine whether these mutants exhibit alternative splicing phenotypes similar those observed in *prp18* mutants. Altogether, our findings demonstrate that Prp18 is an important component for ensuring fidelity of 3'-splice site selection.

## 65 Structural insights into the mechanism of the DEAH-box helicase Prp43

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Introns are excised from eukaryotic pre-mRNAs *via* the spliceosome, a ribonucleoprotein complex being composed of five U snRNAs and numerous proteins. For one splicing event, the spliceosome undergoes fundamental rearrangements which have to be accurately orchestrated. Key players of the major rearrangement steps are RNA helicases, which belong to the DExD/H helicase family. One of these helicases is the DEAH-box protein Prp43 which is required for proper dismantling of the intron-lariat spliceosome as well as for the disassembly of defective earlier spliceosomes. Furthermore, Prp43 is also involved in rRNA biogenesis and the promotion of apoptosis.

Despite implications in numerous fundamental cellular pathways, the exact *modus operandi* of Prp43 and, in general, of all other spliceosomal DEAH-box RNA helicases has been elusive by now. We have obtained three new crystal structures of Prp43 complexes in different functional states and we analyzed structure-based mutants. The Prp43•RNA•ATP-analog complex revealed the RNA-binding site of Prp43 which is the tunnel inside the Prp43 molecule, formed by the two RecA-like and C-terminal domains. In the ATP-bound state, the tunnel can be converted into a shallow groove *via* pronounced rearrangements of the C-terminal domains. This transformation into a groove which is compatible with RNA binding allows us to rationalize how loading of complex RNAs or RNPs is facilitated by Prp43. The coupling of the ATPase activity with the RNA translocation can be deduced from conformational changes between the newly determined ATP- and the previously solved ADP-bound state. The key element for the translocation of the RNA is a  $\beta$ -hairpin in the RecA1 domain, referred to here as Hook-Turn or RF motif since its amino-acid composition is conserved in all members of the DEAH-box subfamily at least in yeast. The translocation of RNA by the Hook-Turn has not been observed in any other helicase, like NS3 or MLE, and appears to be characteristic for the DEAH-box subfamily.

## 66 Potential role of transcription R-loops on alternative splicing regulation

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R-loops are stable RNA/DNA hybrids formed during transcription *in vivo* by base pairing of the nascent RNA with the template DNA strand, thus leaving the non-template DNA strand as a single-stranded DNA loop. Growing evidence indicates that R-loops are much more frequent than expected and that their persistent formation can lead to genomic instability. On the other hand, their programmed formation was shown to regulate gene expression. Alternative splicing (AS) is a key step of gene regulation and its regulation not only depends on the interaction of splicing factors with pre-mRNAs, but also on its coupling with RNA Polymerase II (Pol II) transcription. Recently, it has been shown that DNA damage could affect AS through the modification of Pol II activity.

In this work, we hypothesized that R-loops could play a role in this effect. Genome-wide analysis of AS after induction of DNA damage by either UV irradiation or by using the DNA topoisomerase I inhibitor camptothecin, revealed a significant overlap between the two treatments, indicating a common mechanism for AS regulation. The use of super-resolution microscopy with an antibody specific for RNA/DNA hybrids allowed us to visualize an increased amount of hybrids in the nucleus after the two treatments, suggesting the stabilization of R-loops. Using minigenes, we found that R-loops rapidly formed on the template DNA of two model alternatively spliced regions affected by these treatments. Moreover, overexpression of RNase H1, which disrupts R loops, was found to partially reverse the splice site switches induced by the treatments. Finally, using an R-loop specific antibody, we mapped the localization of hybrids at a high-resolution level by high throughput sequencing, allowing us to identify endogenous alternatively splice regions enriched in R-loops after the treatments.

Our results show a rapid R-loop stabilization after UV irradiation and camptothecin treatment, in genomic regions where AS is affected, which together with the partial reversion of the effects upon RNase H1 treatment, argue for a role of R-loops in gene expression at the level of AS regulation.



## 67 Splicing-associated pausing of RNA polymerase II is enforced by ubiquitination of the catalytic subunit

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Genome wide screens were undertaken to identify factors that functionally interact with the RNA exosome nuclease complex. These uncovered negative genetic interactions between the Bre5-Ubp3 ubiquitin protease complex and several different exosome cofactors. Bre5 was shown to bind directly to RNA *in vitro* and *in vivo*. Targets were predominately in protein coding genes, with enrichment for sites over exon 2 of spliced pre-mRNAs and close to poly(A) sites. An inducible splicing-reporter construct showed that Bre5 is required for efficient *in vivo* pre-mRNA splicing, and for normal RNAPII elongation specifically on splicing-competent genes. A Bre5-Ubp3 sensitive site of RNAPII ubiquitination was mapped to Lys1246 at the entrance to the active site of the large, catalytic subunit. Ubiquitinated RNAPII was depleted close to the transcription start site but enriched at the 5' end of exon 2 and upstream of poly(A) sites, similar to Bre5. Mutation of Lys1246 to Arg reduced RNAPII occupancy upstream of the poly(A) site, consistent with reduced pausing at a potential surveillance site, but increased RNAPII residence downstream of the poly(A) site. Strains expressing RNAPII with the Lys1246Arg mutation showed increased levels of unspliced but poly(A)<sup>+</sup> RNA, indicating a reduced efficiency of cotranscriptional splicing. We propose that ubiquitination of RNAPII is induced by RNA processing events and linked to transcriptional pausing, which is released by Bre5-Ubp3 associated with the nascent transcript. Finally, we noted that ubiquitinated forms of RNAPI and RNAPIII also appeared to be correlated with the locations of RNA processing events and changes in elongation rate. This suggests the possibility that ubiquitination / de-ubiquitination may be a general feature linking RNA processing to the transcribing polymerase.

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## 68 m6A potentiates Sxl alternative pre-mRNA splicing for robust *Drosophila* sex determination

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Methylation of adenosine to N<sup>6</sup>-methyladenosine (m6A) is the most common internal modification of eukaryotic messenger RNA (mRNA) and decoded by YTH (YT521-B homology) domain proteins. The mammalian mRNA m6A methylosome is formed by a complex of nuclear proteins that include METTL3 (Methyltransferase-like 3), related METTL14, WTAP (Wilms tumour 1 associated protein) and KIAA1429, and *Drosophila* has corresponding homologues named dIME4 and dKAR4 (Inducer of meiosis-4 and Karyogamy protein-4 in yeast), and Female-lethal(2)d (Fl(2)d) and Virilizer (Vir). In *Drosophila*, *fl(2)d* and *vir* are required for sex-dependent regulation of alternative splicing of the sex determination factor *Sex-lethal* (*Sxl*). However, the functions of m6A in alternative splicing regulation remain uncertain. Here we show that *Drosophila* lacking *dIME4* do not have m6A in mRNA. In contrast to mouse and plant knock-out models, *Drosophila* *dIME4* null mutants remain viable, though flightless and show a sex bias towards maleness. This is because m6A is required for female-specific alternative splicing of *Sxl*, which determines female physiognomy, but also translationally represses *male-specific lethal2* (*msl-2*) to prevent dosage compensation normally occurring in males. We further show that lack of the m6A reader protein YT521-B decodes m6A in the sex-specifically spliced intron of *Sxl* and phenocopies lack of *dIME4*. Loss of m6A also affects alternative splicing of a number of other genes, predominantly in the 5' UTR, and has global impacts on the expression of metabolic genes. Requirement of m6A and its reader YT521-B for female-specific *Sxl* alternative splicing reveal this hitherto enigmatic mRNA modification as constituting an ancient and specific mechanism to adjust levels of gene expression.



## 69 Repeated transformations of the spliceosomal machinery and introns across eukaryotes

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Given the prohibitive cost of performing functional studies in diverse organisms, comparative genomic studies are crucial for understanding the differences in spliceosomal machinery and introns across eukaryotes. Reconstruction of the evolutionary history of intron-exon structures reveals repeated transformation: (i) widespread intron loss coupled to increased reliance on core splicing motifs (as seen in yeast) has occurred at least 9 times in eukaryotic history; (ii) evolution of splice sites with a pyrimidine at the fourth position, and thus lacking full sequence complementarity to the U1 snRNA (>10 times); (iii) movement of the branchpoint to a few nucleotides from the acceptor site (>10 times); and (iv) reduction of intron length to <30 nts (>5 times). These repeated transformations of intron sequences imply repeated transformations in the splicing machinery, however such changes have remained largely unknown. First, I summarize what is known the history of intron-exon structures and sequences across eukaryotes, with emphasis on the transformations above. Second, I report multiple complementary changes in the U1, U12 and possibly U2 snRNAs and their substrates in diverse protists. Third, I report repeated loss of the U1a splicing factor in birds. Fourth, I show convergent evolution of the region of PRP38 that binds the U6 snRNA in species with strict donor splicing motifs. Fifth, I show repeated transformation of acceptor site recognition factors in species with constrained branchpoint positions. Sixth, I show compaction of donor splice sites in species with short introns. Finally, I report a variety of anomalies arising in various transformed lineages including evolution of separate intron classes, intronic secondary structures and intronic trans-splicing. I discuss how recurrent changes in spliceosomal machinery can be used to inform our understanding of differences in the spliceosomal machinery from well characterized species.

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## 70 A novel regulatory function in an ancestral splicing factor enabled the remodeling of neuronal proteomes by microexons

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One of the major challenges for the development of complex multicellular organisms is to generate dozens of cell types from a single genomic sequence. Through differential processing of introns and exons, alternative splicing (AS) can produce cell type-specific protein isoforms that allow optimization of their specific cellular roles or even the emergence of novel functions. One of the most striking examples of this is provided by microexons in neurons. These tiny exons, which can encode as few as one or two aminoacids, are switched on during neuronal differentiation and show the highest evolutionary conservation of all AS types. They are often located in structured domains of proteins, where they subtly sculpt their interaction surfaces thereby modulating protein-protein interactions in a neuronal-specific manner. Although we are still beginning to unveil their biological roles, we already know they are crucial for proper neuritogenesis, axon guidance, and neuronal function. Remarkably, most vertebrate microexons are regulated by a single splicing factor family, *Srrm4/nSR100*. This protein is specifically expressed in neurons where it directly promotes microexon recognition and inclusion. While this factor appeared to be vertebrate-specific, we have found that nSR100-like proteins are encoded in invertebrates as alternative isoforms of the pan-eukaryotic *Srrm2/SRm300* locus. Strikingly, the neural specificity of these isoforms in Bilaterian animals is closely associated with the presence of neural-specific microexons across all studied species, and trans-phyletic swaps of nSR100-like proteins are sufficient to promote inclusion of endogenous neural microexons in human and fly cells. Our results thus suggest that the origin of a novel, cell type-specific, regulatory function in an ancestral splicing factor have allowed the origin of the program of microexons across animal nervous systems, which has in turn contributed to the origin of new molecular functions in this complex cell type.

## 71 The p53 transcription factor regulates transcriptional burst frequency and RNA Polymerase II density in convoys on the p21 gene locus

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The transcription factor p53, a tumor suppressor gene, plays a major role in the regulation of cellular responses to stress. The complex interaction between p53 and target DNA sequences tightly regulates downstream genes, such as the *p21* cell cycle arrest gene. **Here, we study how p53 binding on the endogenous *p21* promoter dictates specific transcriptional output on single living cells.**

To better understand how p53 determines cellular fate, we used CRISPR/Cas9 technology to incorporate 24 MS2 repeats into the 3'UTR of the endogenous *p21* gene in human U2OS cells. We quantified *p21* expression levels and quantitatively analyzed transcriptional bursts after different stimulations. Overexpression of p53 reduced the time interval between two bursts, while simultaneously increasing the burst duration. This suggests that p53 regulates disruption of chromatin and enhances RNA Polymerase II (Pol II) loading during transcriptional bursting events. Faster timescales analysis revealed groups of closely spaced Pol II molecules, termed convoys. Overexpression of p53 increased the number of Pol II molecules in convoys without affecting the duration of these individual convoys. Finally, Single Particle Tracking reveals that p53 binding on the *p21* promoter oscillates during transcriptional bursting, on the same time scale than Pol II convoy initiation. Taken together, we show that p53 is responsible for the activation of transcription at *p21* locus on different molecular levels involving disruption of chromatin structures to initiate a transcriptional burst, enhanced recruitment of Pol II into convoys, and increasing the number of convoys formed before a transcriptional burst is turned off. Future work will investigate the impact of chromatin modifications on p53's regulation of transcriptional bursting from the *p21* locus.

## 72 Feed-forward TDP43-paraspeckle axis remodels alternative polyadenylation landscape to stabilize mutually exclusive states of pluripotency and differentiation

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The transition from pluripotency to differentiation requires defined and rapid control of gene expression. Here, we show that global RNP rearrangement and subcellular RBP translocation dictates this transition, and show that the RNA-binding protein TDP-43 is critical in pluripotent stem cells (PSCs) for the choice between self-renewal and differentiation. We discovered that TDP-43 directly regulates an evolutionary conserved switch in alternative polyadenylation (APA) of hundreds of transcripts during early differentiation of mouse and human PSCs. Functional analysis revealed that TDP-43 promotes self-renewal by repressing the production of lengthened transcripts of the pluripotency factor SOX2, which are targeted for degradation by miR-21. TDP-43 also prevents the formation of paraspeckles, nuclear compartments found in differentiated cells, by influencing the length of the lncRNA *NEAT1*. Reduction of TDP-43 during differentiation triggers a short-to-long isoform switch of *NEAT1* and, as a consequence, paraspeckle formation and cellular differentiation. In turn, paraspeckles sequester TDP-43 to additionally deplete its RNA binding capacity. These findings reveal how the exit of pluripotency is regulated by a complex posttranscriptional network, which is functionally independent from lineage choices.

### 73 Microscopically visible liquid-droplet P-bodies contribute minimally to miRNA-mediated gene silencing

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MicroRNA (miRNA) bound mRNAs localize to cytosolic ribonucleo-protein aggregates called processing bodies (PBs) (Liu et al. 2005). Using our recently developed microinjection-based iSHiRLoc technique (intracellular Single molecule High-Resolution Localization and Counting, Pitchiaya et al., 2012), we probed the dynamics of miRNA:PB interactions and examined the contribution of PBs to miRNA-mediated gene silencing. miRNAs and PBs were labeled with fluorescent probes so that signal from single miRNAs and PBs could be localized and their diffusive motion be tracked inside live human cells with high precision, at ~30 nm spatial and 100 ms time resolution. iSHiRLoc revealed that only a small fraction of miRNAs, independent of the abundance of their mRNA targets, localize to PBs, with the majority of PBs containing only one or two fluorophore labeled miRNA molecules. Surprisingly, higher mRNA target abundance led to a smaller fraction of miRNAs stably co-localizing with PBs, suggesting that PBs may play a role in sequestration of target-free miRNAs. Moreover, 2D diffusion modeling predicted that PB aggregation into fewer, but larger particles should diminish their contribution towards gene silencing. Supporting this observation, hypertonicity-induced, rapid liquid-droplet aggregation of PB components into a more aggregated and immobile state in cultured cells was associated with suppressed miRNA mediated gene silencing. Taken together, our data suggest that microscopically visible PBs minimally contribute to gene silencing and instead play a role in target-independent miRNA sequestration. The single-molecule systems biology approach pioneered here reveals a novel function for PBs while pointing to a phase-transition-based cellular protection mechanism suppressing mRNA degradation under acute stress conditions.

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### 74 Interactomic Studies of Single Transcripts During mRNA Maturation

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mRNA processing and nuclear export require a wide range of protein factors, which interact with maturing transcripts and each other to form dynamic mRNP complexes. While there are many core mRNP factors, the pathways governing mRNA maturation are not uniform, and different transcripts can be associated with mRNP complexes of dramatically different composition or kinetics. It has proven challenging to study RNP complexes specific to any single mRNA species, as each transcript is relatively unabundant in the cell, and few robust techniques exist to specifically purify a particular mRNP for proteomic analysis. We thus developed a two-step purification method allowing native isolation of a single endogenous transcript and its associated factors. In our approach, a target transcript is tagged with MS2 hairpins - these are bound by the bacteriophage MS2 coat protein (MS2CP), which is co-expressed as a GFP fusion. In the first purification step, a chosen RNP protein known to be associated with a particular mRNA processing step of interest is tagged and affinity isolated. From this heterogeneous material, the MS2-tagged transcript of interest can then be isolated by affinity capture of MS2CP-GFP. This approach is able to efficiently and cleanly isolate a particular transcript at a chosen step of mRNP maturation, allowing us to systematically examine mRNP composition at successive processing stages.

Using this methodology for single mRNP isolations, we performed a preliminary survey of transcripts with sequence elements suspected to be associated with unique processing machinery. Mass spectrometric analysis (including label-free quantification) of proteins co-purified with these RNAs revealed transcript-specific changes in composition and stoichiometry. This was first validated by identifying known sequence-specific RNP factors, such as the She2p/She3p/Myo4p complex associated with the ASH1 transcript's 3' UTR. We have also determined that introns from either a house keeping ACT1 gene or the RPS30b ribosomal protein gene are associated with dramatically different levels of various splicing-related proteins. These differences provide mechanistic insight into changes in the kinetics of spliceosome assembly determined by intron sequence. As we extend this methodology, we can construct maps of quantitative protein compositional dynamics of individual mRNPs throughout their temporal and spatial lifecycles.

## 75 Cellular imaging of small RNAs using fluorescent RNA Mango aptamers

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In recent years, there has been an explosion of fluorescent RNA aptamers that have been isolated using SELEX. Since their discovery, fluorogenic RNA aptamers, such as Spinach and Mango [1,2] have held great potential to enable the visualisation of RNA molecules in cells. However, resolving complex RNA structures has been limited due to inefficiency in maintaining adequate aptamer stability and fluorescence [3]. Evolving new RNA aptamers with improved physicochemical properties (i.e. thermal stability, fluorescence brightness and ligand affinity) should better their use in cellular imaging.

Three new Mango-like aptamers have recently been evolved using microfluidic-assisted in vitro compartmentalization, mutagenesis and fluorescent selection [4], which improved fluorescence brightness, ligand binding affinity and thermal stability. We show that these aptamers are readily useable to image small non-coding RNAs (such as 5S rRNA and U6 snRNA) in both live and fixed human cells with improved sensitivity and resolution. The Mango tagged RNAs sub-cellular localisation pattern is conserved, as validated using immunofluorescence. Our data show that these new aptamers are significantly improved for cellular imaging over previous fluorogenic aptamers and can in principle be incorporated into a wide range of coding and non-coding RNAs. We anticipate that these new aptamers will drastically improve RNA imaging in cells.

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3. Zhang, J. et al. Tandem Spinach Array for mRNA Imaging in Living Bacterial Cells. *Sci. Rep.* 5, 17295 (2015).
4. Autour, A., Westhof, E., and Ryckelynck, M. (2016). iSpinach: a fluorogenic RNA aptamer optimized for in vitro applications. *Nucleic Acids Research* 44, 2491-2500.

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## 76 Systematic characterization of the subcellular distribution properties of human RNA binding proteins

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RNA binding proteins (RBPs) are deeply conserved regulators of gene expression implicated in all facets of RNA metabolism; consequently, they play key roles in cellular physiology and disease aetiology. Since post-transcriptional regulation processes are often carried out in distinct subcellular locations, assessing the intracellular distribution properties of an RBP can offer important insights into its function. We employed a collection of highly validated antibodies recognizing >300 distinct RBPs in order to characterize the localization features of each factor via systematic immuno-labeling in multiple cell lines. Each RBP was co-labelled with a dozen markers for specific organelles and substructures, including different subnuclear and cytoplasmic bodies. These results, have been organized within the RBP Image Database (<http://rnabiology.ircm.qc.ca/RBPImage>), which houses >200,000 images of RBP-marker pairs, as well as controlled vocabulary annotations to describe the localization features of each factor. Altogether, we find that RBPs display strikingly diverse localization patterns, demarcating subgroups of factors with similar distribution features, while also exhibiting functional coherence with their transcriptome binding properties and loss-of-function phenotypes. We have further employed this antibody resource to identify RBPs targeted to stress granules in a cell-type and stress-specific manner, as well as those that co-localize with aberrant RNA granules associated with repeat-expansion disorders. These results offer key insights to further elucidate the functions of RBPs in post-transcriptional regulatory events that occur in different subcellular compartments and how these processes are disrupted in response to stress or in different disease settings.



**77 Export of discarded, splicing intermediates provides insight into mRNA export***Yi Zeng, Jon Staley***The University of Chicago, Chicago, IL, USA**

Cell survival relies on precise removal of introns. To promote fidelity in nuclear pre-mRNA splicing, the spliceosome rejects and discards suboptimal splicing substrates after they have engaged the spliceosome. Although nuclear quality control mechanisms have been proposed to retain immature mRNPs, these discarded splicing substrates, including the 5' exon and lariat intermediate, can be exported to the cytoplasm, as indicated by the translation of these species and/or their degradation by cytoplasmic nucleases. However, it is unknown whether export of these discarded species uses a dedicated fidelity export pathway or the canonical mRNA export pathway.

Using a lacZ reporter and two-color single molecule RNA-FISH in budding yeast, we have observed nuclear export of both 5' exons and lariat intermediates and have found that their export requires the general mRNA export receptor Mex67. Further, we have found that two essential Mex67 adaptors, Nab2 and Yra1, are required for exporting suboptimal lariat intermediates, demonstrating that both mRNAs and lariat intermediates undergo a similar process to assemble export machinery. Unexpectedly, we have found evidence that efficient export of lariat intermediates requires the interaction between Nab2 and Mlp1, a nuclear basket component implicated in retaining unspliced pre-mRNA in the nucleus of budding yeast. Finally, we have shown that lariat intermediate export relies on Tom1-mediated ubiquitination of Yra1, a process proposed to function in RNA surveillance during export. Together, our findings establish the importance of the mRNA export pathway in exporting discarded splicing intermediates and implicate a dual role for Mlp1 in not only retaining unspliced transcripts but also exporting partially spliced transcripts. We propose that this dual role of Mlp1 reflects a function in docking and undocking of mRNAs at the nuclear basket during export.

**78 Oxidative Stress induces selective retrograde tRNA transport in the nucleus of human cells***Hagen Schwenzer<sup>1</sup>, Frank Jühling<sup>2</sup>, Alexander Chu<sup>1</sup>, Laura J. Pallett<sup>3</sup>, Thomas F. Baumert<sup>2,4</sup>, Mala Maini<sup>3</sup>, Ariberto Fassati<sup>1</sup>*

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Transfer RNAs (tRNAs) are key players in protein translation, delivering their cognate amino acid to the growing peptide chain at the cytosolic translation machinery. In eukaryotes, tRNAs are transcribed in the nucleus and exported to the cytosol. This movement has been believed to be unidirectional, but recent discoveries suggest an active shuttling between cytosol and nucleus, named retrograde tRNA transport (RTT). Studies in yeast and mammalian cells suggest that cellular stress activates bidirectional intracellular movement of tRNAs. In our lab, host tRNAs were shown to be imported into the nucleus in human cells by an active mechanism that also facilitates the nuclear import of HIV-1. However the exact role of RTT in human cells is still unknown. This study aims to understand the function and regulation of RTT in human cells. To do so, we employed a Fluorescence In situ Hybridization assay to survey conditions that induce tRNA nuclear accumulation in different types of human cells. Our results demonstrate that oxidative stress is the best trigger for tRNA retrograde transport in human cells, followed by Methylmethane Sulfonate and Puromycin. Nuclear accumulation of tRNAs was not due to nuclear retention of newly synthesized tRNAs because it was still detectable after treatment with Actinomycin D, which blocked synthesis of new cellular RNAs. Furthermore, we found that tRNA nuclear accumulation upon oxidative stress was dose and cell-type dependent, rapid (reaching a peak within 2 hours) and reversible. Notably, RNAseq of small RNAs imported into the nucleus from cells under oxidative stress revealed that tRNA retrograde transport is selective for certain tRNA species, mostly with shorter 3' ends. Altogether, these results indicate that RTT is an acute response to certain perturbations, mainly oxidative stress, in metabolically active cells and that this response is regulated and selective for a subset of tRNAs. This selectivity suggests that RTT is a defence mechanism, rather than a tRNA quality control system, which may gear global protein translation to survival mode.



## 79 Transcription impacts the efficacy of mRNA translation via co-transcriptional N6-adenosine methylation

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Transcription and translation, the two main processes that underlie gene expression, are typically regarded as independent because of the distinct timings, cellular locations, and action mechanisms. Nevertheless, the fact that they serve a common purpose suggests a possible flow of information between them. This study employed a large-scale genetic screen of multiple human promoters in order to test this hypothesis. Initially, this screen revealed a positive role of the TATA-box promoter element on the efficiency of mRNA translation. Following this finding, we identified a global positive correlation between expression level of mRNAs and rates of translation. Furthermore, using multiple genome-wide techniques (such as GRO-seq, RNA-seq and ribosomal footprinting) as well as precisely controlled *in vitro* experiments, we found that the rate of transcription exerts a direct and positive effect on mRNA translation. Moreover, we showed that the ability of transcription to regulate translation is mediated by N6-methylation of adenosine residues (m<sup>6</sup>A) of mRNAs, which we found to be co-transcriptional and dependent upon the dynamics of RNA polymerase II. Finally, we found that the canonical initiation of translation is indispensable for the ability of translation to respond to changing transcription rates. Overall, this study uncovers a general, robust and widespread connection between transcription and translation and suggests a mechanistic explanation for this phenomenon.

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## 80 The RNA helicase Ded1p suppresses translation initiation from near-cognate start codons

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The conserved and essential DEAD-box RNA helicase Ded1p from yeast and its mammalian ortholog DDX3 are critical for translation initiation. Mutations in DDX3 are linked to tumorigenesis and intellectual disability, and the enzyme is targeted by diverse viruses. How Ded1p and its orthologs engage RNAs to impact translation initiation has been a longstanding, unresolved question.

Here we show that Ded1p associates with the pre-initiation complex at the mRNA entry channel of the small ribosomal subunit and that the helicase unwinds mRNA structure ahead of the scanning pre-initiation complex. Defective Ded1p causes pervasive translation in 5'UTRs, which starts from near-cognate initiation codons located 5' of mRNA structures and is accompanied by decreased protein synthesis from the main ORFs. Our results show that Ded1p functions to suppress translation initiation on near-cognate codons proximal to mRNA structure and reveal how the helicase is targeted to specific RNA sites without common sequence signatures. Further data indicate that the widespread translation in 5'UTRs from near-cognate codons occurs when yeast uses respiration, as opposed to aerobic fermentation.

Our observations link repression of Ded1p activity to an activation of upstream open reading frames and suggest that mRNA structure and proximal near-cognate initiation codons encode a regulatory program for translation initiation that is sensitive to RNA helicase function. This regulatory program appears to contribute to the adaptation of yeast to different physiological growth conditions.

**81 An RNA structure mediated, post-transcriptional model of  $\alpha$ -1-antitrypsin expression**

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$\alpha$ -1-antitrypsin deficiency is a major genetic cause of Chronic Obstructive Pulmonary Disease, which affects over 65 million individuals worldwide. The  $\alpha$ -1-antitrypsin gene, *SERPINA1*, expresses an exceptional number of mRNA isoforms (eleven) generated entirely by alternative splicing in the 5' untranslated region (5'UTR). Although all *SERPINA1* mRNAs encode exactly the same protein, the individual expression levels of the mRNAs vary substantially in different human tissues. We hypothesize that these transcripts behave unequally in a post-transcriptional regulatory program via their distinct 5'UTRs and that this regulation ultimately determines  $\alpha$ -1-antitrypsin expression. Splicing in the *SERPINA1* 5'UTR determines the inclusion of long upstream open reading frames (uORFs), and using whole-transcript SHAPE chemical probing, we show that it also dictates the transcripts' local 5'UTR secondary structure. We demonstrate that disrupting the uORFs results in markedly increased translation efficiencies in luciferase reporter assays, but a quantitative model that uses uORF Kozak translation initiation sequences does not fully explain our translation efficiency data. However, when we incorporate the experimentally derived structure data, the model very accurately predicts translation efficiencies in our reporter assays and greatly improves translation efficiency predictions in primary human tissues. Additional experiments measuring structure mutants demonstrate that uORF secondary structure affects overall translation efficiency in a predictable manner. Our results reveal that 5'UTR RNA structure governs a complex post-transcriptional regulatory program of  $\alpha$ -1-antitrypsin expression. Crucially, these findings suggest a new mechanism by which genetic alterations in non-coding regions of the gene may result in  $\alpha$ -1-antitrypsin deficiency.

**82 The force-sensing peptide VemP employs extreme compaction and secondary structure formation to induce ribosomal stalling**

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Interaction between the nascent polypeptide chain and the ribosomal exit tunnel can modulate the rate of translation and induce translational arrest to regulate expression of downstream genes. The ribosomal tunnel also provides a protected environment for initial protein folding events. Despite accumulating biochemical and structural evidence for protein folding within distinct regions of the tunnel, the full extent of nascent chain compaction in the tunnel and its influence on translational activity remains to be elucidated. Here we present a 2.9 Å cryo-electron microscopy structure of a ribosome stalled during translation of the extremely compacted VemP nascent chain. The nascent chain forms two  $\alpha$ -helices connected by an  $\alpha$ -turn and a loop, enabling a total of 37 amino acids to be observed within the first 50-55 Å of the exit tunnel. The structure reveals how  $\alpha$ -helix formation directly within the peptidyltransferase center of the ribosome interferes with aminoacyl-tRNA accommodation, suggesting that during canonical translation, a major role of the exit tunnel is to prevent excessive secondary structure formation that can interfere with the peptidyltransferase activity of the ribosome.

**83 The action of EF-G in translational bypassing**

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Translational bypassing is an unusual mechanism employed by the ribosome to skip a non-coding region in the mRNA. While the best-characterized example of bypassing is found in gene 60 mRNA of bacteriophage T4, recent studies revealed about 80 examples of translational bypassing in mitochondria, suggesting that these phenomena are rather common. Furthermore, ribosome sliding along the mRNA could also play a role during regular mRNA translation, e.g. during the reading frame selection at the initiation phase or during tRNA-mRNA translocation or ribosomal frameshifting at the elongation phase. Bypassing is regulated by several elements in the mRNA, including specific mRNA sequences/structures located 5' and 3' of the take-off and landing sites, as well as by the interactions of the nascent peptide in the ribosome exit tunnel. Nevertheless, it is still unclear which event initiates the take-off, what makes the ribosome to move along the mRNA and what defines the direction of sliding. Here, we addressed these questions in the reconstituted translation system from *E. coli* by monitoring bypassing of a 50-nt segment of gene 60 mRNA *in vitro*. We found that translational bypassing requires elevated EF-G concentrations that are much higher than those needed for translation of the peptide up to the take-off codon, indicating an important, specific role of EF-G in the take-off event. By testing a collection of EF-G and ribosome mutants we identify the residues at the tip of EF-G domain 4 and in the ribosomal protein S12 that specifically affecting bypassing. We suggest that EF-G may initiate bypassing by interacting with the vacant A site of the ribosome stalled at the take-off codon and performing pseudo-translocation which disrupts the codon-anticodon interaction in the P site and thus provides the start force for bypassing. These data suggest an important, previously unanticipated role of EF-G in promoting ribosome sliding.

**84 Change in distinct ribose 2'-O-methylation impacts the ribosome to alter translation**

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Ribose 2'-O-methylation (2'-O-Me) is one of the two most abundant modifications in human rRNA and is believed to be important for ribosome biogenesis, mRNA selectivity and translational fidelity. Our development of the RiboMeth-seq method allows quantitative mapping of global 2'-O-Me sites in rRNA and permits comparison of 2'-O-Me signatures between cell types and growth conditions. We have successfully used this technique to comprehensively map and quantify 2'-O-Me sites in rRNA from human cancer cells, arriving at a set of high-confidence 2'-O-Me positions and identifying several previously uncharacterized sites (Krogh N. and Jansson M.D. *et al. Nucleic acids research* 2016). Approximately two-thirds of these sites are fully methylated, though interestingly, the remainder are fractionally modified, supporting the idea of ribosome heterogeneity at the level of RNA modification. Following on, we have used RiboMeth-seq to compare 2'-O-Me signatures of different human cell types and investigate changes occurring during various processes such as MYC oncogene activation and differentiation of human embryonic stem cells. We uncovered differences in the rRNA 2'-O-Me levels at several nucleotide positions and for the first time show dynamics at distinct positions in response to stimuli. Furthermore, in order to investigate the influence of 2'-O-methylation sites on ribosomal function and their impact on translation, we created cellular models by CRISPR knockout or overexpression of snoRDs guiding 2'-O-Me at several dynamic positions. In one example, manipulating the level of a particular MYC-responsive 2'-O-Me site altered cell proliferation, and ribosome profiling analysis revealed differences in translation that likely contribute to this phenotype. This data indicates that translation can be tuned by rRNA 2'-O-methylation and points to a potentially powerful means to regulate mRNA translational output. In addition, it re-affirms the concept that pools of specialised ribosomes exist and suggests that, during normal cellular programs and also in diseases such as cancer, mechanisms may occur to amplify certain ribosome pools that can help shape the transcriptome as required. RiboMeth-seq opens the doors for analysis and greater understanding of the function of 2'-O-Me ribose modifications on rRNA and indeed, the entire transcriptome.

## 85 *In vivo* evidence that eIF3 stays bound to ribosomes elongating and terminating on short upstream ORFs to promote reinitiation

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Translation reinitiation is a gene-specific translational control mechanism characterized by the ability of some short upstream ORFs to prevent recycling of the post-termination 40S subunit in order to resume scanning for reinitiation downstream. Its efficiency decreases with the increasing uORF length, or by the presence of secondary structures, suggesting that the time taken to translate a uORF is more critical than its length. This led to a hypothesis that some initiation factors needed for reinitiation are preserved on the 80S ribosome during early elongation. Here, using the GCN4 mRNA containing four short uORFs, we developed a novel *in vivo* RNA-protein Ni<sup>2+</sup>-pull down (Rap-Nip) assay to demonstrate for the first time that one of these initiation factors is eIF3. eIF3 but not eIF2 preferentially associates with RNA segments encompassing two GCN4 reinitiation-permissive uORFs, uORF1 and uORF2, containing cis-acting 5' reinitiation-promoting elements (RPEs). We show that the preferred association of eIF3 with these uORFs is dependent on intact RPEs and the eIF3a/TIF32 subunit and sharply declines with the extended length of uORFs. Our data thus imply that eIF3 travels with early elongating ribosomes and that the RPEs interact with eIF3 in order to stabilize the mRNA-eIF3-40S post-termination complex to stimulate efficient reinitiation downstream.

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## 86 Mechanism of translational regulation induced by coding polyA tracks

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Cellular mechanisms that control the efficiency and fidelity of mRNA translation play a large role in the regulation of gene expression. We have previously identified coding polyA tracks as a potential regulator of translation elongation for approximately 2% of human genes that endogenously contain them. By a mechanism conserved across species, polyA tracks cause both ribosomal stalling and frameshifting, leading to a predictable decrease in the stability of mRNA and protein expression<sup>1-3</sup>. Additionally, the amount of repression induced by the polyA track is directly dependent on the length of the track and conserved over large number of species<sup>4</sup>.

Here we report further on the mechanism of gene regulation by polyA tracks. We find that polyA tracks induce frameshifting independent of the charge of lysine residues translated from AAA codons in both *E. coli* and rabbit reticulocyte *in vitro* translation systems. Using mass spectrometry analysis, we identify frameshifted products indicating a directional -1 frameshifts with a tendency for multiple -1 frameshifting events on longer polyA tracks. As such, the endogenous set of polyA track genes are a good substrate for nonsense mediated decay (NMD). This prediction is supported by siRNA knockdown of key factors in this pathway and analyses of reporters and global data studies. We find strong dependence of targeting by NMD on the relative position of the putative frameshifted stop codon to the downstream exon junction complex. These results add complexity to the regulation of polyA track containing transcripts. Using reporter constructs and endogenous genes with polyA tracks, we can separate the NMD effects and poly-lysine stalling effects, controlled by the no-go decay pathway (NGD), depending on their position within the transcripts. The results of our studies provide critical insight in both NGD and NSD mechanisms and may explain differences seen in polyA induced stalling in the middle or at the end of mRNA transcripts.<sup>5,6</sup>

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## 87 A role for the ribosome in initiating no-go decay.

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mRNA surveillance pathways have evolved to protect the cell from accumulating aberrant protein products and are critical for maintaining cellular homeostasis. In particular, the quality control process of no-go decay (NGD) is responsible for eliminating mRNAs that stall ribosomes. It is characterized by an endonucleolytic cleavage of the transcript in the vicinity of the ribosome, ensuring rapid degradation of the mRNA and its removal from the translating pool; downstream events lead to ubiquitination of the nascent peptide so that it is also eliminated. Many of the trans-acting factors in the pathway have been well characterized, but the ribosome's potential role in detecting stalls and triggering a response is poorly defined. To investigate this further, we have made mutations in several ribosomal proteins and assessed their effect on the efficiency of NGD. Using high throughput sequencing, we show that cleavage sites are in fact distributed much further upstream of the ribosome than previously thought, suggesting that multiple ribosomes stack at the stall site to initiate cleavage. In agreement with our mapping data, several methods of lowering the density of ribosomes upstream of the stall sequence similarly inhibit NGD: moving a stall sequence closer to the initiation codon so as to limit the number of ribosomes that occupy the transcript, limiting initiation using an inefficient promoter, and deleting copies of ribosomal protein paralogs that reduce the density of ribosomes per mRNA all reduce cleavage efficiency. Interestingly, although these deletions lead to a global reduction in protein synthesis, NGD reporters are translated more effectively indicating a suppression of endonuclease activity. Collectively, our data suggests a model where ribosome collision functions as a trigger for NGD.

Additional insights into the ribosome's role in NGD came from our mapping data, which indicates that cleavage is likely taking place within the ribosome. Consistent with this idea, mutations deep in the mRNA entry tunnel have a profound effect on the efficiency of NGD. These mutations also promote +1 frameshifting, potentially resulting from reduced cleavage upon ribosome collision. Together, these provide evidence for a fundamental role for the ribosome in sensing damaged or defective transcripts and initiating NGD.

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## 88 FolamiRs: Ligand-targeted, vehicle-free microRNA replacement therapy

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MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression at the posttranscriptional level. Because elevations or reductions in miRNA levels can promote or maintain disease states, miRNA-based therapeutics are being evaluated extensively. Unfortunately, the therapeutic potential of miRNA replacement is limited by deficient delivery vehicles. In this work, we introduce a novel delivery platform that delivers miRNAs in the absence of a protective vehicle. The method relies on the direct attachment of a miRNA to folate (FolamiR). Folate mediates the delivery of the conjugated miRNA, into cells that overexpress the folate receptor (FR), a feature commonly found in multiple cancers. Using this strategy, we show that a specific tumor suppressive FolamiR, FolamiR-34a, is quickly taken up by human triple-negative breast cancer cells, in culture and *in vivo*, and slows their progression and demonstrate efficacy in an aggressive *Kras;p53* non-small cell lung cancer mouse model. Additional evidence indicates that a small molecule ionophore can enhance endosomal mediated escape of the miRNA increasing cytosolic concentrations and targeting. Our findings suggest that this first-in-class method of delivering miRNAs directly to tumors *in vivo*, without the use of toxic delivery vehicles, represents an exciting advance in the pursuit to develop non-toxic, targeted therapeutics with promising clinical efficacy.



**89 Functional sequestration of microRNA-122 from Hepatitis C Virus by circular RNA sponges**Isabelle Jost<sup>1</sup>, Lyudmila A Shalamova<sup>2</sup>, Michael Niepmann<sup>2</sup>, Albrecht Bindereif<sup>1</sup>, Oliver Rossbach<sup>1</sup><sup>1</sup>Institute of Biochemistry, Faculty of Biology and Chemistry, University of Giessen, Giessen, Germany;<sup>2</sup>Institute of Biochemistry, Faculty of Medicine, University of Giessen, Giessen, Germany

Circular RNAs (circRNAs) have recently emerged as a new class of ubiquitously expressed non-coding RNA. They are created by the canonical splicing machinery in a process referred to as “back-splicing”, where a donor splice site is spliced to an upstream instead of a downstream acceptor site. Two endogenous circRNAs, CDR1as/ciRS-7 and SRY, have been shown to contain conserved microRNA (miRNA)-binding sites and function as miRNA sponges in vivo. Due to their high stability compared to linear RNA, circRNAs may be an interesting tool in molecular biology.

We have designed and produced artificial circRNAs in vitro that can be used as miRNA sponges to sequester miRNA-122 from Hepatitis C Virus (HCV) and thereby affect virus RNA stability and translation. Miravirsin, the first anti-miRNA drug, had been reported to functionally sequester miRNA-122 and decrease HCV virus titers to non-detectable levels in patients after a two-week subcutaneous injection. miRNA-122 is essential for HCV propagation and protects the single-stranded RNA genome from Xrn1-mediated exonucleolytic degradation and enhances HCV translation by binding to two adjacent sites at its 5'-end. We designed artificial miRNA-122 sponges that, if transfected as an in vitro transcribed and ligated circRNA, have a similar effect in HCV cell culture systems as a Miravirsin-like antisense oligonucleotide. These circRNAs are more stable in vivo compared to their linear counterparts and found both in the cytoplasm and in the nucleus. These results demonstrate that artificial circRNA sponges are a promising tool in molecular medicine and biology with a wide range of potential applications.

**90 Anti-FGF2 RNA aptamer is applicable to therapy for Achondroplasia and age-related macular degeneration**Yoshikazu Nakamura<sup>1,2</sup>, Ling Jin<sup>2</sup>, Yusaku Matsuda<sup>2</sup>, Yosuke Nonaka<sup>2</sup>, Masatoshi Fujiwara<sup>2</sup><sup>1</sup>Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo, Japan; <sup>2</sup>Ribomic Inc., Minato-ku, Tokyo, Japan

Fibroblast growth factor 2 (FGF2) plays a crucial role in bone remodeling and disease progression. However, the potential of FGF2 antagonists for treatment of patients with bone diseases has not yet been explored. We therefore generated a novel RNA aptamer, RBM-007, specific for human FGF2 and characterized its properties in vitro and in vivo. RBM-007 blocked binding of FGF2 to each of its four cellular receptors, inhibited FGF2-induced downstream signaling and cells proliferation, and restored osteoblast differentiation blocked by FGF2. RBM-007 effectively blocked the bone disruption in mouse and rat models of arthritis and osteoporosis. To our great surprise, the severe disruption of the epiphyseal growth plate occurred in osteoporosis model (OVX) rats, and RBM-007 sharply blocked the disruption of the epiphyseal growth plate. These findings prompted us to test a novel approach to therapy for Achondroplasia, which is caused by a mutation in the gene coding for FGFR3 that leads to excess FGF signalling and shutdown of epiphyseal growth. This possibility was examined with transgenic mice carrying the Achondroplasia *fgfr3* mutation. Strikingly, the short stature was reversed upon administration of RBM-007. We trust that RBM-007 will provide us with a strong and safe opportunity to cure Achondroplasia. Moreover, FGF2 is known to possess a strong angiogenic activity and likely to be involved in diseases progression with pathologic angiogenesis. Age-related macular degeneration (AMD) is one such disease and antiangiogenic agents, i.e. anti-VEGF drugs, had been developed and approved for clinical use for a decade. However, one-third AMD patients fail to respond to these anti-VEGF drugs, leaving an area of unmet medical needs. Therapeutic potential of RBM-007 was examined in a mouse model of choroidal neovascularization (CNV). RBM-007 showed the anti-CNV efficacy equivalent or superior to anti-VEGF drugs and further exhibited the activity to block scar formation (i.e., fibrosis at retina), which cannot be prevented by anti-VEGF drugs. This dual activity will provide us with a novel therapeutic approach to AMD patients. Currently the GLP study is in progress to initiate first-in-human trials for AMD and Achondroplasia in 2018.

## 91 RNA-biased small molecules and privileged RNA topologies for selective small molecule:RNA recognition

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The ongoing discovery of functional yet non-protein coding RNA (ncRNA) sequences has led to a revolution in molecular biology that inspires novel approaches to disease diagnosis and therapy. Representative RNAs include bacterial riboswitches that control translation, viral RNA structures critical to replication, disease-dependent microRNAs that control mRNA levels and long noncoding RNAs (lncRNAs) that regulate complex gene expression networks. Despite this therapeutic potential, RNA is widely considered an “undruggable” target. Antisense oligonucleotides and related strategies are hindered by *in vivo* delivery challenges, and no small molecule drugs targeting RNAs other than the ribosome are currently available. Furthermore, our recent survey of the literature revealed less than one hundred reported chemical probes that target non-ribosomal RNA in biological systems. This slow progress is due in part to the dynamic nature and limited chemical functionality of RNA as well as to a shortage of small molecule targeting strategies. In order to gain fundamental insights into small molecule:RNA recognition, we are pursuing the parallel discovery of RNA-biased small molecule chemical space and RNA topological space privileged for differentiation. To begin, we identified chemical properties of biologically active RNA ligands that are distinct from those of protein-targeted ligands. Elaboration of four RNA binding scaffolds into a library enriched with these properties has led to improved recognition of medicinally relevant RNA targets. At the same time, we used principal component analysis to identify RNA topologies that can be differentially recognized by small molecules. We are currently expanding these studies with the ultimate goal of applying these insights to the rapid development of ligands with high affinity and specificity for a wide range of RNA targets, particularly those critical to cancer progression.

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## 92 Small Molecule Targeting of Viral and Virus-associated RNAs

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Kaposi's sarcoma herpesvirus (KSHV) is the etiological agent of Kaposi's sarcoma (KS), Primary Effusion Lymphoma and the Multicentric Castleman's Disease. In the HAART era KS remains the second most frequent tumor in HIV-infected patients worldwide, and has become the most common cancer in Sub-Saharan Africa. During lytic infection, KSHV express a highly abundant long noncoding transcript designated polyadenylated nuclear (PAN) RNA, a global regulator of viral and cellular gene expression. At its 3' terminus, PAN exhibits a unique structure - the expression and nuclear retention element (ENE) - which assumes a triple helix configuration to sequester and shield the poly(A) tail from exonucleases. While this motif imparts distinctive structural properties on PAN, the biological significance of the ENE triple helix remains unclear. We have provided proof-of-principle that structured RNA motifs can be specifically targeted with small molecules *in vivo*, potentially bypassing problems associated with “macromolecular” therapeutics. Using a small molecule microarray (SMM) strategy and PAN ENE motifs as “bait”, we identified 26 compounds that specifically bind to the ENE triple helix. A luminescence based cytotoxicity test using the specialized cell line iSLK-219 reduced the number of PAN ENE binders for further biological testing. Current studies center on defining the exact molecular mechanisms by which these chemotypes affect KSHV replication. As a simple and complementary high throughput approach, differential scanning fluorimetry (DSF) has been adapted to study RNA binding ligands, focusing on stem-loop A (SLA) of the Dengue virus genome. An update on both strategies will be presented.

#### 94 DNA Repair Profiling in T-cells Reveals Nonrandom Outcomes at Cas9-Mediated Breaks

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The repair outcomes at site-specific DNA double-strand breaks (DSBs) generated by the RNA-guided DNA endonuclease Cas9 determine how gene function is altered. Despite the widespread adoption of CRISPR-Cas9 technology to induce DSBs for genome engineering, the resulting repair products have not been examined in depth. Here, the DNA repair profiles of 223 sites in the human genome demonstrate that the pattern of DNA repair following Cas9 cutting at each site is nonrandom and consistent across experimental replicates and cell lines. Furthermore, the repair outcomes are determined by the targeted sequence rather than genomic context, enabling DNA repair profiling in cell lines to be used to anticipate repair outcomes in primary cells. In this study, the DNA repair profiles of therapeutically relevant targets in human primary T-cells are described.

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We here report the identification of a class of MREs that are exclusively function in CDS regions in mammalian cells because these MREs, while highly effective in CDS, show no effect when moved to 3'UTR. Through functional and mechanistic characterization of these "unusual" MREs, we demonstrate that CDS-targeted miRNAs require extensive base pairing with mRNA in the 3' side rather than the 5' seed. We show that Ago2 is required for these miRNAs to repress translation without inducing mRNA decay and that GW182, a key cofactor for miRNA-mediated gene silencing in 3'UTR, is completely dispensable in this process. We provide a series of evidence that CDS-targeted miRNAs repress translation by inducing ribosome drop-off. Together, these findings reveal distinct mechanisms and functional consequences for miRNA to target CDS versus 3'UTR and suggest direct competition between miRNA and translating ribosome in protein coding regions, which may be a more widespread mechanism for miRNA-mediated translational repression than previously thought.

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Our results show that germline microRNAs can block translation without initiating mRNA degradation. This study highlights an unexpected function for microRNAs in animal germline and suggests their contribution in the stability of maternal mRNAs transmitted to the embryo.



## 97 When does human Argonaute3 cleave RNAs? –Structural and functional studies of human Argonaute3

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Humans have four Argonaute paralogs. It has been thought that only Argonaute2 retains RNA-cleavage activity despite sharing the same catalytic tetrad with Argonaute3. Recent studies identified that two regions in the Argonaute2 N domain are critical to the slicer activity but different from the counterparts of Argonaute3. Therefore, why Argonaute3 has retained the complete catalytic tetrad through its molecular evolution remains an open question. Here we report that Argonaute3 indeed possesses slicer activity. When programmed with miR-20a, the recombinant Argonaute3 cleaved a 60-nucleotide (nt) target RNA harboring a fully complementary sequence, albeit with lower efficiency than that of Argonaute2. RNA cleavage was also confirmed when using Argonaute3 immunoprecipitated from HEK293T cells. However, no cleavage was detected when mutating the catalytic glutamate finger, demonstrating that the observed RNA cleavage was caused by the catalytic activity that Argonaute3 harbors. Intriguingly, the cleavage efficiency of Argonaute3 was reduced when programmed with let-7 as a guide. In addition, Argonaute3 did not cleave a short 23-nt target RNA, unlike Argonaute2. To understand the molecular basis for target recognition and cleavage, we determined the first crystal structure of human Argonaute3 in complex with guide RNA. The structure showed that the Argonaute3-specific insertion in the N domain remains as a long flexible loop, presumably participating in target recognition along with the guide 3' supplementary region rather than tightly interacting with the PIWI domain as previously proposed. Based on these structural and functional studies, we propose that only limited miRNAs can induce the cleavage activity of Argonaute3, whereas any guide strands can direct Argonaute2 for RNA cleavage if the targets contain a complementary sequence. We also discuss the structural differences amongst four human Argonaute paralogs by comparison with others including our recently determined structure of Argonaute4.

## 98 A complex of Arabidopsis DRB proteins can impair dsRNA processing

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Small RNAs play an important role in regulating gene expression through transcriptional and post-transcriptional gene silencing. Biogenesis of small RNAs from longer double-stranded (ds)RNA requires the activity of DICER-LIKE ribonucleases (DCLs), which in plants are aided by dsRNA binding proteins (DRBs). To gain insight into this pathway in the model plant Arabidopsis, we searched for interactors of DRB4 by immunoprecipitation followed by mass spectrometry-based finger printing and discovered DRB7.1. This interaction, verified by reciprocal co-immunoprecipitation and bimolecular fluorescence complementation, colocalizes with markers of cytoplasmic siRNA bodies and nuclear dicing bodies. *In vitro* experiments using tobacco BY-2 cell lysate (BYL) revealed that the complex of DRB7.1/DRB4 impairs cleavage of diverse dsRNA substrates into 24nt small interfering (si)RNAs, an action performed by DCL3. DRB7.1 also negates the action of DRB4 in enhancing accumulation of 21nt siRNAs produced by DCL4. Overexpression of DRB7.1 in Arabidopsis altered accumulation of siRNAs in a manner reminiscent of *drb4* mutant plants, suggesting that DRB7.1 can antagonize the function of DRB4 in siRNA accumulation *in vivo* as well as *in vitro*. Specifically, enhanced accumulation of siRNAs from an endogenous inverted repeat correlated with enhanced DNA methylation, suggesting a biological impact for DRB7.1 in regulating epigenetic marks. We further demonstrate that RNASE THREE-LIKE (RTL) proteins RTL1 and RTL2 cleave dsRNA when expressed in BYL, and that this activity is impaired by DRB7.1/DRB4. Investigating the DRB7.1-DRB4 interaction thus revealed that a complex of DRB proteins can antagonize, rather than promote, RNase III activity and production of siRNAs in plants.

## 99 The small non-coding vault RNA1-1 acts as a riboregulator of autophagy

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Vault RNAs (vtRNA) are small, 88-100nt non-coding RNAs found in many eukaryotes. Although they have been linked to drug resistance, apoptosis and nuclear transport, their function remains unclear. Here we show that a human vtRNA, RNA1-1, specifically binds to the autophagy receptor sequestosome-1/p62. Autophagy is a highly regulated and conserved process for recycling cellular components and degrading foreign particles in eukaryotic cells. Antisense-mediated depletion of vault RNA1-1 augments, whereas increased vault RNA1-1 expression restricts, autophagic flux in a p62-dependent manner. Bulk autophagy induced by starvation reduces the levels of vault RNA1-1 and the fraction of RNA-bound p62. These findings show that RNAs can act as riboregulators of biological processes by interacting with proteins, and assign a function to a vault RNA.

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## 100 Differential silencing of two separation-of-function isoforms of a telomeric gene by an intragenic noncoding RNA.

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Human TPP1 (hTPP1) facilitates the function of telomerase, an RNP enzyme that extends telomeres. We have discovered that the gene for hTPP1 encodes two protein isoforms [TPP1-long (L) and TPP1-short (S)] with contrasting functions. We discovered an intragenic TPP1 noncoding RNA (silencing 3'-UTR-derived noncoding RNA or sunRNA) that specifically silences the longer but not the shorter TPP1 isoform. To our knowledge this is the first report of differential isoform silencing by an intragenic noncoding RNA. TPP1-L and TPP1-S proteins share the same sequences except at the N-terminus. While TPP1-L initiates at the annotated Met1 codon, TPP1-S initiates at the annotated Met87 codon. Overexpression of TPP1-S resulted in telomere hyperelongation, while overexpression of TPP1-L resulted in severely short telomeres. Moreover, TPP1-S, but not TPP1-L, protein stimulated telomerase processivity *in vitro*. TPP1-L and TPP1-S are therefore natural separation-of-function isoforms of TPP1 with opposing functions, providing a novel mechanism for telomere length regulation. Proteomics and immunoblotting confirmed that TPP1-S protein is more abundant than TPP1-L protein in human cells. RNA expression databases revealed that two TPP1 transcripts, corresponding to RNAs coding for TPP1-L and TPP1-S proteins, respectively, exist in human cells. Although both mRNAs are transcribed at the same initial rate, TPP1-L mRNA is degraded rapidly, resulting in lower TPP1-L protein levels. We discovered a noncoding RNA derived from the 3'-UTR of TPP1 (sunRNA) that elegantly explains the efficient turnover of TPP1-L mRNA. sunRNA recognizes sequences complementary to regions of TPP1-L mRNA (but absent in TPP1-S mRNA) to trigger TPP1-L mRNA degradation. sunRNA does not act via RNAi or one specific RNA degradation pathway; rather it destabilizes TPP1-L mRNA resulting in its rapid decay. In summary, we show how a newly discovered intragenic noncoding RNA dictates differential protein isoform expression to modulate the biological function of a gene important for telomere function.

## 101 A high throughput screen identifies a transposable element-derived element driving nuclear retention of mRNAs and lncRNAs in human cells

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Long noncoding RNAs (lncRNAs) are emerging as key players in multiple cellular pathways, but their modes of action, and how those are dictated by sequence remain elusive. While they share biochemical properties with mRNAs collectively, lncRNA are more likely to be enriched in the nucleus, a feature likely to contribute to their function. The sequence and/or structure signals that lead to nuclear localization remain largely unknown.

In order to identify such elements we developed a high-throughput system for identifying elements with nuclear retention capabilities. We generated a library consisting of ~5,500 110nt fragments, tiled across 50 known nuclear transcripts. The library was cloned into the 3' UTR of an AcGFP mRNA, which is normally efficiently exported to the cytoplasm. Cells were transfected with the library, followed by fractionation and sequencing the clones from the nuclear and cytoplasmic fractions. Analysis of the resulting data identified a short conserved sequence that was present in several transcripts and was sufficient for retaining the GFP RNA in the nucleus.

After validating the retention capabilities of this sequence using high-throughput FISH imaging, we measured the contribution of individual bases and short motifs to element functionality and identified a combination of core motifs driving its function. Further, we have preliminary data on the identity of the protein binding these motifs and driving nuclear retention. Importantly, the element we identified in found in the consensus sequence of a large family of transposable elements, and increased number of its instances is globally predictive of nuclear/cytoplasm ratio of both lncRNAs and mRNAs in ENCODE cell lines.

Beyond increasing our understanding of RNA localization, we expect the discovery of an element capable of retaining long RNAs in the nucleus will have applications for synthetic biology and the ability to design RNA molecules with specific properties of interest.

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## 102 Post-transcriptional regulation of bacterial gene expression with ProQ-dependent small RNAs

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The post-transcriptional control of gene expression by small noncoding RNAs (sRNAs) in bacteria has to a great extent been associated with sRNAs that interact with the RNA chaperone Hfq. However, the recent discovery of the conserved RNA-binding protein ProQ that stabilises a distinct large class of unusual structured sRNAs suggests that additional RNA regulons exist in these organisms. The cellular functions and molecular mechanisms of these new ProQ-dependent sRNAs are largely unknown. Here we provide an update on the roles played by ProQ in sRNA-mediated regulatory events and report in *Salmonella Typhimurium* the mode-of-action of RaiZ, a ProQ-dependent sRNA that is made from the 3' end of the mRNA encoding ribosome-inactivating protein RaiA. We show that RaiZ is a base-pairing sRNA that employs a partially structured seed region to repress *in trans* the mRNA of the histone-like protein HU- $\alpha$ . Remarkably, ProQ was found to be essential not only for stability of RaiZ in the cellular milieu but also for helping the sRNA to occlude the ribosome-binding site on the mRNA by forming a stable ternary complex with both interacting RNAs and thus prevent translation initiation. This molecular mechanism combines features characteristic of both Hfq-dependent *trans*-acting and Hfq-independent *cis*-encoded antisense sRNAs, highlighting the plasticity of ProQ in mediating post-transcriptional regulation in both these modes, hitherto considered largely incompatible.

### 103 Secretion of small RNAs and an Argonaute protein by the gastrointestinal nematode *Heligmosomoides polygyrus*: at the host interface

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Extracellular RNA has been proposed as a means of cell-to-cell communication within an organism and a mechanism of cross-species communication. We previously showed that an Argonaute protein (HpWAGO) and small RNAs (miRNAs and Y-RNAs) are secreted in extracellular vesicles produced by *H. polygyrus*, a gastrointestinal nematode that infects mice. Intranasal administration of the *H. polygyrus* vesicles to mice suppressed a Type 2 innate response in an airway allergy model and our work suggests that some of the nematodes-derived miRNAs can suppress host gene expression under in vitro conditions in reporter assays. Here we describe the conservation of this Argonaute protein in Clade V nematodes and identify its protein binding partners in *H. polygyrus* as well as a *C. elegans* strain in which it has been introduced (HpWAGO::GFP). Preliminary results identify factors associated with secondary siRNA biogenesis in both organisms. Further sequencing analyses demonstrate that secondary siRNAs containing a 5' triphosphate and first nucleotide preference for guanine, consistent with generation by an RNA-dependent RNA polymerase, dominate the vesicular small RNA content rather than miRNA.

Most of the secreted 22G RNAs co-fractionate with HpWAGO and these come from numerous unannotated regions within the *H. polygyrus* genome, not clearly associated with protein-coding genes. RNA labeling results suggest the secreted RNA is indeed internalized by mouse cells and this is further supported by small RNA sequencing of mouse cells following incubation with *H. polygyrus* vesicles.

Together these results suggest further diversity in extracellular RNA from a nematode parasite and lay a foundation for understanding the origin of these exRNAs inside the parasite and the mechanisms by which they mediate cross-species communication.

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### 104 The poly(A) tail blocks RDR6 from converting self mRNAs into the substrates for gene silencing

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In order to eliminate aberrant RNAs, plants take advantage of post-transcriptional gene silencing (PTGS). The key step of PTGS is double-stranded RNA (dsRNA) synthesis by RNA-DEPENDENT RNA POLYMERASE 6 (RDR6). RDR6 converts aberrant RNAs into dsRNAs, which are processed by DICER LIKE PROTEIN 2 or 4 into 22-nt or 21-nt secondary small RNAs. These small RNAs are assembled into Argonaute proteins to form RNA-induced silencing complex (RISC), which then catalyzes the cleavage of complementary target transcripts. The cleavage fragments are further converted into dsRNA by RDR6, amplifying silencing signals. However, it remains unclear how PTGS discriminates aberrant RNAs from canonical mRNAs. Here, we investigated RDR6 activity in vitro using recombinant proteins. Our results show that recombinant RDR6 itself has an ability to select aberrant poly(A)-less mRNAs over canonical polyadenylated mRNAs as templates for complementary strand synthesis. This unique substrate specificity of RDR6 provides a mechanistic explanation for previous genetic observations and can be viewed as an innate safeguard system against "self-attack" by PTGS.



**105 A compendium of RNA-binding proteins that regulate microRNA biogenesis***Thomas Treiber<sup>1</sup>, Nora Treiber<sup>1</sup>, Simone Harlander<sup>1</sup>, Uwe Plessmann<sup>2</sup>, Henning Urlaub<sup>2</sup>, Gunter Meister<sup>1</sup>*<sup>1</sup>Laboratory of RNA biology, Universität Regensburg, Regensburg, Germany; <sup>2</sup>Bioanalytical Mass Spectrometry Group, Max-Planck-Institute of Biophysical Chemistry, Göttingen, Germany

During microRNA (miRNA) biogenesis, stem-loop-structured precursors are processed in two endonucleolytic steps, giving rise to mature miRNAs. These processing steps can be posttranscriptionally regulated by RNA-binding proteins (RBPs). However, only a small number of posttranscriptional miRNA regulators has been discovered to date. Here we have used a proteomics-based pulldown approach to map and characterize the interactome of a multitude of pre-miRNAs in 11 cell lines. We identify over 150 RBPs that interact specifically with distinct pre-miRNAs. Detailed mapping of the interactions indicates that the apical loop region as well as the lower stem segment of the miRNA precursors are the predominant binding sites for RBPs.

For validation of the functional relevance of the identified interactions, we combined RNAi and CRISPR/Cas-mediated knockout experiments to analyze RBP-dependent changes in miRNA levels. Indeed, a large number of the investigated candidates have effects on miRNA-processing, including splicing factors and other mRNA processing proteins. Moreover, we show that TRIM71/Lin41 is a potent regulator of miR-29a-processing and its inactivation directly affects miR-29a targets.

We provide an extended database of RBPs that interact with pre-miRNAs in different cell types, highlighting a widespread layer of co- and posttranscriptional regulation of miRNA biogenesis.

**106 Uridylation-triggered RNA decay acts as a multiple turnover factor in antiviral RNA interference***Raphael A. Manzenreither, Madalena Reimão-Pinto, Thomas Burkard, Stefan L. Ameres***IMBA - Institute of Molecular Biotechnology Austria, Vienna, Austria**

A dedicated small RNA silencing pathway - the RNA interference or siRNA pathway - acts as a primary antiviral innate immune response in flies. The central step in this pathway involves the efficient endonucleolytic cleavage of virus transcripts mediated by the RNA-induced silencing complex (RISC). RISC acts as a multiple-turnover enzyme, capable of catalyzing multiple rounds of cleavage, a property that requires elusive accessory protein(s) that facilitate removal of the siRNA from the cleaved target RNA after each round of cleavage.

Here, we show that RISC-mediated endonucleolytic cleavage is followed by the efficient 3' terminal uridylation of siRNA-directed cleavage products. Uridylation marks RISC-mediated cleavage products for processive degradation, thereby facilitating product release from RISC and enabling efficient multiple turnover cleavage. Uridylation and decay are mediated by the terminal RNA-uridylation-mediated processing complex (TRUMP), a novel cytoplasmic RNA decay complex consisting of the uridylyltransferase Tailor and the processive 3'-to-5' exoribonuclease Dis3L2. Based on quantitative in vitro RNAi reconstitution experiments and virus infection assays in uridylation-deficient cultured cells we propose that a novel RNA decay complex acts as the elusive multiple turnover factor in antiviral RNAi and reveal an important function of uridylation-triggered RNA decay in the innate immune response against virus infection in *Drosophila*.

## 107 Transcription of animal small RNA source loci by coupling of RNA polymerase II PIC formation to heterochromatin marks

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Nuclear small RNA pathways establish repressive heterochromatin at repeats to maintain genome integrity. This inevitably also targets the repeat-rich small RNA source loci themselves. In plants, their transcription is facilitated by an elaborate system involving two specialized RNA polymerases, but how this is achieved in animals is unknown. Here, we uncover a molecular pathway in *Drosophila* that activates transcription within heterochromatin by directly coupling the committing step of RNA polymerase II pre-initiation complex formation to chromatin marks of small RNA source loci termed piRNA clusters. At its core acts the germline-specific TFIIA-L paralog, Moonshiner, which is recruited to piRNA clusters via the H3K9me3 reader, Rhino. Moonshiner stimulates widespread transcription initiation within heterochromatin by recruiting the TATA box-binding protein (Tbp)-related factor 2 (Trf2), a poorly understood variant TFIID core protein implicated also in heterochromatin biology within the mammalian germline. We propose that direct recruitment of the core transcriptional machinery to small RNA source loci via chromatin marks rather DNA sequence is a recurring theme in evolution.

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## 108 Transcriptional architecture and regulation of mammalian noncoding RNAs

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The regulation between genetic sequence and transcriptional outcome is essential for understanding species evolution and disease progression. Identifying the divergence and conservation of functional regulatory elements that give rise to a specific transcriptome in a distinct cell at a specific time has been an area of intense investigation. Over the past years it has become apparent that noncoding RNAs can shape genomes and control gene expression programs. Mammalian genomes encode germline-specific noncoding RNAs, among them P-element induced wimpy testis (PIWI)-interacting RNAs (piRNAs) that are germ cell enriched 24-31 nucleotide small RNAs. piRNAs protect the germline by repressing genomic transposition of repeat elements and are required for the formation of mature spermatocytes. Although many features of piRNA maturation have been identified in individual species, the evolution of piRNA promoters, enhancers and gene loci remain largely unknown in mammals.

We characterized in great detail the transcriptional landscape of pachytene piRNA precursors. To this end, we combine sequencing of small RNA, total RNA and immunoprecipitated chromatin with comparative genomics in five mammals. We use this integrative approach to demonstrate that pachytene piRNA precursors originate from bi- and unidirectionally transcribed loci with features characteristic of tissue-specifically transcribed protein-coding genes. Computational analysis further reveals distinct genomic architectures, responsible for the production of these piRNAs. These architectures are conserved across mammals and suggest a possible mechanism for the birth of novel piRNA loci.

## 109 Antisense transcriptional interference as a common mean of strengthening gene repression in budding yeast

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Many studies have shown that transcription is not strictly limited to well-defined genes. Indeed, pervasive transcription occurs, arising in particular from intrinsically bidirectional promoters. In the yeast *Saccharomyces cerevisiae*, pervasive transcription generates mainly unstable non-coding transcripts, which are targeted by nuclear and/or cytoplasmic cellular RNA-quality control pathways. Although an handful example of pervasive transcription events has been described as involved in transcription interference mediated gene regulation, it is still unclear whether pervasive transcription has a more global functional role or merely represents transcriptional noise.

Here we analyzed the transcriptome in a mutant context that reveals those hidden transcripts. We characterized more than 800 antisense transcripts genome wide and analyzed the behavior of the corresponding mRNA (sense transcripts). We observed that genes most tightly repressed during the exponential phase are most often associated with high level of antisense RNAs. A substantial fraction of these corresponds to mRNAs specifically enriched in growth-arrested (G0) cells. We therefore compared transcriptomes of exponential and G0 cells. For many genes, we observed an opposite regulation on the mRNA and the associated antisense. This suggested that these antisense transcription events might participate to the tight repression of these genes during the exponential phase. In order to test this hypothesis, we specifically interrupted the antisense transcription of a subset of G0-specific genes, and found that it resulted in de-repression of the corresponding mRNAs in exponential growth. We further validated that this repression acts in *cis* and is diminished when chromatin modifiers such as set2 are absent.

In summary, we observed that antisense non-coding transcription is very commonly found associated with genes tightly repressed during exponential phase, in particular those for which mRNAs are enriched in G0. We showed that these antisense transcription events participate to the tight repression of this class of genes through a transcriptional interference mechanism. This is the first time that transcription interference is observed as a global mean of gene regulation between cellular states.

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## 110 Long Non-coding RNAs in Oocyte and Oocyte-to-Zygote Transition

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Long non-coding RNAs are non-protein coding transcripts which are longer than 200nt and mostly polyadenylated. LncRNAs are rapidly evolving group with little if not any sequence conservation; it is assumed that a relatively small fraction of these transcripts are functional while others might represent yet evolving transcripts or transcriptional noise. Nonetheless, lncRNA function in variety of physiological processes has been widely appreciated, more importantly in early development. They are shown to be involved in regulating several aspects of pluripotency and differentiation. They also form an integral part of ES cell regulatory network by directly interacting with several transcription factors and chromatin modifiers. While lncRNA contribution is thoroughly exploited in early developmental stages, their role in oocyte development and Oocyte-to-Embryo transition (OET) is the least explored.

Understanding composition of maternal and zygotic non-coding RNA pools is pre-requisite for understanding their biological roles during OET. In our study, we use NGS of total RNA from mouse oocytes and early embryos to identify and annotate 1600 lncRNA loci expressed in mouse oocytes and early embryos. We characterize OET lncRNAs expressed at these loci, including their transcriptional and post-transcriptional temporal dynamics and comparison with mRNAs. Importantly, we describe the contribution of LTR retrotransposons in oocytes and zygotes, specifically in lncRNA evolution, expression and function. Furthermore, we identified a specific class of lncRNAs, which function in small RNA biogenesis, hence coupling small RNA biology with lncRNAs. Our study puts together a comprehensive analysis of lncRNAs in oocytes and OET including their probable function.

## 111 Circular RNAs generated by readthrough transcription

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Although it was long assumed that eukaryotic pre-mRNAs are almost always spliced and polyadenylated to generate a linear mRNA, it is now clear that thousands of protein-coding genes can be non-canonically spliced (or “backspliced”) to produce circular RNAs. Most circular RNAs are rarely generated and their expression levels are often not correlated with that of the linear mRNA from the same locus. Here, we show using reporter plasmids and genome-wide nascent RNA-seq analysis that readthrough transcription from upstream genes can result in the production of circular RNAs from downstream genes, thereby uncoupling circular RNA expression from its host gene promoter. Once generated, circular RNAs accumulate as stable transcripts with long half-lives and serve as sensitive readouts of readthrough events. Using a reporter plasmid that generates a circular RNA when an upstream poly(A) signal is not used, we identified many proteins critical for transcription termination in *Drosophila* and mammalian cells. This includes well-established cleavage/polyadenylation factors (e.g. the CPSF, CstF, and CF II<sub>m</sub> complexes), the 5'-3' exonuclease Rat1 (Xrn2), and the phosphatase regulatory protein PNUTS (PPP1R10). These results reveal unexpected complexities in how endogenous circular RNAs are generated as well as provide new tools for better understanding transcription termination mechanisms.

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## 112 The specific domains of architectural NEAT1 lncRNA induces the formation of the distinct phase-separated paraspeckle nuclear body

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Liquid-liquid phase separation (LLPS) is a key mechanism for the formation of cellular bodies. These bodies are typically composed of proteins and RNAs. A class of lncRNAs plays an essential architectural role in the construction of nuclear bodies. This concept was originally derived from the finding that NEAT1 lncRNA was an essential component of nuclear body called paraspeckle. The paraspeckle is a massive, highly ordered RNP structure comprising more than 40 kinds of proteins. Many of them are RNA binding proteins (RBPs) with prion-like domains (PLDs), some of which are essential for paraspeckle formation. It has been shown that the paraspeckle functions in gene regulation by sequestering proteins and RNAs into the body, and plays crucial roles in developmental processes and disease conditions. However, it still remains unidentified what sequences in NEAT1 lncRNA function in the formation of such a huge ordered RNP body.

We applied CRISPR/Cas9 system in human haploid cell line to dissect the role of the NEAT1 sequences. Our extensive deletion analysis (over 150 mutant cell lines) identified the NEAT1 domain necessary and sufficient for the paraspeckle formation. This region is sufficient to recruit the PLD proteins essential for the paraspeckle formation. Strikingly, RNA fragments of the functional NEAT1 domain strongly facilitated LLPS *in vitro*, suggesting that the paraspeckle has a liquid-like nature in the cell. This is supported by the evidence that 1,6-Hexanediol treatment disrupts the paraspeckles. In addition, super-resolution and electron microscopic analyses using the NEAT1 mutant cell lines have revealed how NEAT1 sequences affect the organization of NEAT1 within the paraspeckle. Surprisingly, our CRISPR dissection also identified the NEAT1 region required for the segregation of the paraspeckle from another distinct nuclear body, providing the insights into the mechanism how distinct bodies can co-exist in the cell. Taken together, the specific RNA domains induce the paraspeckle through the LLPS likely by increasing local concentration of RBPs with PLDs. Furthermore, our findings would be an important step to understand the principle of the formation of cellular bodies built by the architectural RNAs and how the RNAs determine their structures, biophysical properties and functions.



### 113 The biogenesis and functional implication of circular RNAs

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Circular RNAs from back-splicing of exons (circRNAs) have been identified from thousands of genomic loci in mammals. CircRNAs are derived from Pol II transcripts together with their linear isoforms, and both *cis*-elements and *trans*-factors are involved in circRNA formation. We have demonstrated that circRNA formation is enhanced by flanking complementary sequences and that *Alu* elements in human contribute the most for the circRNA-flanking intron pairing. However, expression levels of circRNAs vary under different conditions, suggesting participation of protein factors in their biogenesis. Using genome-wide siRNA screening that targets all human unique genes and an efficient circRNA expression reporter, we identify double-stranded RNA binding domain containing immune factors NF90/NF110 as key regulators in circRNA biogenesis. NF90/NF110 promote circRNA production in the nucleus by associating with intronic RNA pairs juxtaposing the circRNA-forming exon(s); they also interact with mature circRNAs in the cytoplasm. Upon viral infection, circRNA expression is decreased, largely owing to the nuclear export of NF90/NF110 to the cytoplasm. Meanwhile, NF90/NF110 released from circRNP complexes bind to viral mRNAs as part of their functions in antiviral immune response. Our results therefore implicate a coordinated regulation of circRNA biogenesis and function by NF90/NF110 in viral infection.

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### 114 Functional specificity of SR and SR-like proteins in breast cancer initiation and metastasis

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Human tumors often exhibit alterations in splicing factors; however the functional significance of these alterations and their contribution to disease pathogenesis often remains unclear. We previously demonstrated that the splicing factor SRSF1 is upregulated in human breast tumors and promotes transformation in relevant breast cancer models. SRSF1 is a member of the SR protein family, composed of 12 structurally related proteins, yet little is known about differences and redundancies in their splicing targets and biological functions. Here, we reveal that only specific splicing factors promote mammary epithelial cell transformation and investigate their functional overlap as well as specificity.

We identified frequently amplified and/or overexpressed splicing factors by screening a collection of >800 human breast tumors, and then used 3D human mammary epithelial cell cultures to model these splicing-factor alterations. Interestingly, only a subset of splicing factors were oncogenic in mammary context, differentially affecting cell proliferation, apoptosis, and/or mammary acinar architecture, and thus suggesting non-redundant functions. We defined the global repertoire of splicing-factor regulated alternative splicing events in 3D culture using RNA-seq data, and compared the target specificities of various splicing factors. Strikingly, splicing factors promoting the same phenotype regulate a shared subset of splicing events and target common pathways in breast cancer. Furthermore, specific splicing factors promoted cell invasion and metastasis, by regulating splicing events associated with epithelial to mesenchymal transition. Finally, overexpression of the SR-like factor TRA2 $\beta$  correlated with increased metastatic incidence in breast cancer patients, and was required for the maintenance of metastatic properties of breast-cancer cells in vitro and in vivo. Finally, we are characterizing the TRA2 $\beta$  splicing targets altered in breast cancer cells, as well as in human breast tumors and patient-derived xenograft models of triple negative breast cancer.

In summary, we gained new insights into the regulatory mechanisms of SR and SR-like proteins and identified novel oncogenic TRA2 $\beta$ -regulated splicing events that represent potential biomarkers and targets for therapeutics development.

**115 Disease mutations in splicing factor SF3b1 alter macroautophagy***Susana Rodríguez-Santiago<sup>1</sup>, John Christin<sup>1</sup>, Andrea Yuste<sup>1</sup>, Eloy Bejarano<sup>2</sup>, Charles Query<sup>1</sup>*<sup>1</sup>Albert Einstein College of Medicine, Bronx, NY, USA; <sup>2</sup>Jean Mayer USDA Human Nutrition Research Center on Aging-Tufts University, Boston, MA, USA

Mutations in the U2 snRNP component SF3b1 are prominent in myelodysplastic syndromes (MDSs) and other cancers. Efforts to understand SF3b1 disease-related mutations and their effects have, thus far, been focused on the spliceosome, since SF3b1 is an essential component of this macromolecular machine. Our laboratory recently described that in the spliceosome, disease-related SF3b1 mutations alter pre-spliceosomal interactions with the ATPase Prp5p and subsequently change spliceosomal fidelity (Tang et al. 2016). Although analyses of the disease-related mutations and their consequences on spliceosomal interactions gave insights into the role of SF3b1 in splicing, paradoxically there is no clear correlation between changes in splicing patterns and MDSs (e.g. Mupo et al. 2016; Obeng et al. 2016). These conflicting observations suggest that SF3b1 disease-related mutations may have a novel non-spliceosomal role.

Using bioinformatic analysis, we identified two autophagy-related LIR (LC3-interacting regions) motifs in the SF3b1 C-terminal region, suggesting its potential role in autophagy. We focused our analysis on macroautophagy, the process in which cellular components are degraded by lysosomes (mammals) or vacuoles (yeast) after the sequestration of material by autophagosomes. The presence of LIR motifs suggests their possible interaction with LC3 (Atg8 in yeast; a critical factor for autophagosome formation) and involvement in the autophagic pathway. Indeed, immunoprecipitation assays demonstrated that SF3b1 interacts with LC3 *in vitro*. Under starvation conditions, SF3b1 is a substrate for macroautophagy, in both mammalian cells and yeast. Importantly, SF3b1 disease-related mutations K666R, K666N, and T663A alter autophagic function in yeast, increasing the formation and accumulation of low-density autophagosomes, suggestive of a defect in cargo handling. In contrast, SF3b1-K700E, the most prevalent mutation in MDS patients, inhibits autophagosome formation in yeast.

Our results, carried out in both yeast and mammalian cells, indicate that SF3b1, a known U2 snRNP component, is also involved in macroautophagy, affecting biogenesis, cargo recognition, and loading of autophagosomes into the vacuole. The disease-related mutations in SF3b1 interfere with this process, affecting either autophagosome formation (e.g. SF3b1-K700E) or their loading/fusion with the vacuole (e.g. -T663 and -K666). These data suggest a role for macroautophagy in the etiology of SF3b1 mutated disease.

**116 The FAM46C gene encodes a non-canonical poly(A) polymerase and acts as an onco-suppressor in multiple myeloma***Seweryn Mroczek<sup>1,2</sup>, Justyna Chlebowska<sup>3,4</sup>, Tomasz Kulinski<sup>1,2</sup>, Olga Gewartowska<sup>1,2</sup>, Jakub Gruchota<sup>1,2</sup>, Dominik Cysewski<sup>1,2</sup>, Vladyslava Liudkovska<sup>1,2</sup>, Ewa Borsuk<sup>5</sup>, Dominika Nowis<sup>3,4</sup>, Andrzej Dziembowski<sup>1,2</sup>*
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FAM46C is one of the most frequently mutated genes in multiple myeloma (MM). Using a combination of *in vitro* and *in vivo* approaches, we demonstrate that FAM46C encodes an active non-canonical poly(A) polymerase which enhances mRNA stability and gene expression. Moreover, we also found that the reintroduction of active FAM46C into MM cell lines, but not its catalytically-inactive mutant, leads to broad polyadenylation and stabilization of mRNAs strongly enriched with those encoding endoplasmic reticulum-targeted proteins and induces cell death. Furthermore, silencing of FAM46C in MM cells expressing WT protein enhance cell proliferation. Finally, using a FAM46C-FLAG knock-in mouse strain we show that the FAM46C protein is strongly induced during activation of primary splenocytes and that B lymphocytes isolated from newly generated FAM46C KO mice proliferate faster than those isolated from the WT littermates.

Concluding, our data clearly indicate that FAM46C works as an onco-suppressor, with the specificity for B-lymphocyte lineage from which multiple myeloma originates. This is, to our knowledge, the first report that directly associates cytoplasmic poly(A) polymerase with carcinogenesis. Furthermore, our data suggest that the human genome encodes at least eleven non-canonical poly(A) polymerases with four FAM46 family members. Since FAM46 proteins are differentially expressed during development, these proteins may positively regulate transcript stability and translational rate in a tissue-specific manner.

## 117 RNA virus infection induces a global remodelling in the host repertoire of RNA-binding proteins

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RNA Binding Proteins (RBPs) are key molecules in regulating gene expression during all viral infections, but particularly for RNA viruses whose genome is an RNA molecule. While the host cell dedicates more than 1,500 proteins to RNA metabolism, viral genomes typically encode a dozen, only a handful of which are RBPs. Viruses have thus developed sophisticated mechanisms to hijack the host resources to support viral RNA replication, translation and encapsidation. To identify the key host RBPs for viral infection, we have applied "RNA interactome capture" [1-2] to cells infected with the RNA virus, sindbis (SINV). In brief, protein-RNA interactions are immobilised by UV crosslinking and purified via oligo (dT) capture. RBPs bound to (host and viral) polyadenylated RNAs are then identified by quantitative proteomics. Our data reveal a global remodelling of the host RNA-binding proteome ("RBPome") in response to infection. In particular, association of nuclear RBPs with RNA is strongly reduced by the infection, while RBPs involved in translation, non-canonical RNA metabolism and antiviral response are enhanced. Changes in RNA-binding upon infection are almost exclusively due to differential RNA-binding activity and not linked to alterations in protein abundance. We also show that SINV infection activates important components of the 5' to 3' RNA decay pathway that results in a substantial degradation of host RNA. In parallel, viral accumulates in membrane-bound, cytoplasmic foci that are sites of viral replication. Strikingly, most of the virus-induced RBPs re-localise to these cytoplasmic foci where they interact directly with viral RNA. Functional assays demonstrate that these host RBPs have critical roles in SINV biological cycle. Extension of these experiments to other RNA viruses, such as the human immunodeficiency virus (HIV), shows that some of these RBPs may play a role as master-regulators of virus infection. In summary, our study reveals the global landscape of RBP activities operating in SINV-infected cells.

[1] Castello et al., 2012 Cell

[2] Baltz et al., 2012 Mol Cell

## 118 SR and hnRNP proteins regulate HIV-1 multiplication by modulating mRNA splicing and translation

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Human Immunodeficiency Virus type 1 (HIV-1) is a major health concern. Deciphering mechanisms that regulate HIV-1 replication will facilitate development of new therapies. Alternative splicing plays a crucial role in HIV-1 multiplication. Together with translation, it controls viral protein production all along the virus life cycle. HIV-1 primary transcripts contain five splicing donor sites (5'SSs) and nine splicing acceptor sites (3'SSs) and their combined and time scheduled utilizations generate more than 40 different mRNAs. In early phase of the virus cycle, multiply spliced mRNAs coding the Tat, Rev and Nef proteins are respectively produced. Then, singly spliced mRNAs coding the Env, Vpu, Vif and Vpr proteins, respectively, and unspliced transcripts coding the Gag and Pol protein precursors are synthesized. HIV-1 splicing regulations predominantly take place at the 3'SSs and involve SR and hnRNP proteins. We will show how Tat protein production is regulated at both the splicing and translation steps. This knowledge is as much important as the Tat transcription activator is essential for HIV-1 multiplication. In its absence only a tiny amount of full-length transcripts is produced, preventing new functional virus production. On the other hand, Tat production should be limited because of its strong toxicity for the host. The human immunodeficiency symptoms are largely due to Tat protein production. We will show how, hnRNP A1, hnRNP H, DAZAP1, SRSF1, SRSF2, SRSF5, SRSF7 and the Tat protein itself each modulate negatively or positively *tat* mRNA production. *Tat* mRNA translation, largely depends upon internal ribosome entry sites (IRESs). We established the RNA 2D structure of *tat-1* mRNA and by *in cellulo* assays using a bi-cistronic RNAs, we identified the various *tat-1* mRNA regions showing an IRES activity. Then, we tested the capability of various SR and hnRNP proteins to stimulate or to inhibit the identified IRES activities. The conclusion is that some of the SR and hnRNP proteins which modulate *tat* mRNA splicing have similar modulation effects on *tat* mRNA translation, revealing a strong implication of these proteins in Tat protein production. Targeting these splicing and translation regulations open new possibilities for HIV-1 therapy.

## 119 SNORD116 missing in Prader-Willi syndrome regulates mRNA stability and microexon usage

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Prader-Willi syndrome is a congenital disease characterized by hyperphagia and intellectual disability. Two clusters of C/D box snoRNAs, SNORD115 and SNORD116 are central to the disease etiology. SNORD115 regulates alternative splicing of the serotonin receptor 2C (1), but the function of SNORD116 has been elusive. Using our recently developed nuclear fractionation scheme (2) we found that about one third of SNORD116 forms protein complexes devoid of the methylase fibrillarin. SNORD116 binds to other RNA binding proteins (HuR, YB1, hnRNPs) showing that it has functions different from RNA methylation. SNORD116 knock-down promotes inclusion of about 50 microexons in neuroblastoma cells and alters the expression of about 800 mRNAs. These changes in gene expression were confirmed in human post-mortem tissue and neurons derived from patient's dental pulp stem cells, showing the biological significance of the findings. Using AMT (4'-Aminomethyl-trioxsalen hydrochloride) crosslink in vivo, we detected binding of SNORD116 to its mRNA targets, suggesting a transient interaction between SNORD116 and other RNAs. The influence on mRNA expression was further analyzed in transfection assays, using SNORD116 expression constructs and globin reporter constructs, driven by an inducible c-fos promoter. There are 27 copies of human SNORD116 that fall into five related clusters. Surprisingly, the SNORD116 copies have different effects on mRNA stability, suggesting sequence-specific interactions in spite of the absence of bioinformatically predicted binding sites. SNORD116 binds to the 3'UTR of regulated genes and has a stronger effect on intron-less reporter constructs than on the ones containing two introns. SNORD116's destabilizing effect is antagonized by HuR/ELAVL1, a protein known to stabilize mRNAs.

Together these findings suggest that SNORD116 binds transiently to pre-mRNAs changing splicing patterns and also binds to mRNA in the cytosol changing mRNA stability, suggesting that similar to SNORD115 (1), SNORD116 could be substituted with an oligo. The regulated genes work in appetite control and neuronal signaling, explaining some aspects of Prader-Willi syndrome. This is the first demonstration of a role for snoRNAs in mRNA stability, a mechanism that is likely to be used by other SNORDs detected in the cytosol.

(1)Zhang Z, et al. EMBO Molecular Medicine. 2016;PMID:27406820

(2)Falaleeva M, et al. PNAS;PMID:26957605

## 120 ZFR coordinates crosstalk between RNA decay and transcription in innate immunity

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Control of type-I interferon production is crucial to combat bacterial and viral infection while preventing deleterious inflammatory responses. Pathways promoting rapid transcriptional induction of type-I interferons have been intensively studied, but the contribution of post-transcriptional regulatory mechanisms to innate immune signaling is poorly understood. Here, we show that the human zinc finger RNA-binding protein (ZFR) plays a specialized role in repression of the type-I interferon response as part of its broader function as a regulator of alternative splicing.

We find that ZFR expression is under tight control during macrophage development: monocytes express a truncated form of the protein lacking zinc-finger motifs, while macrophages express a full-length isoform capable of modulating alternative splicing. In macrophages depleted of full-length ZFR, treatment with dsRNA or lipopolysaccharides causes hyper-induction of interferon  $\beta$  and downstream antiviral proteins. These findings suggest that induction of ZFR in developing macrophages helps to guard against aberrant activation of the type-I interferon response.

We show that ZFR exerts its control over type-I interferon signaling by promoting correct splicing of the histone variant macroH2A1/H2AFY pre-mRNA. Properly translated macroH2A1 protein in turn directly binds and inhibits the interferon  $\beta$  promoter. In normal cells, two major macroH2A1 isoforms are expressed via alternative splicing of mutually exclusive exons. If ZFR is depleted, both mutually exclusive exons are skipped, generating an aberrant splicing product containing a premature stop codon. We show that the isoform generated by this unusual alternative splicing event is efficiently degraded by nonsense-mediated mRNA decay, effectively abolishing macroH2A1 expression in cells lacking ZFR.

Together, our data reveal a network of mRNA processing and decay events that shapes the transcriptional response to infection.

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## 121 Regulation of translation of collagen mRNAs by binding of LARP6 to the 5' stem-loop; key mechanism to fibrosis development.

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Fibrosis is common, but untreatable, disease characterized by excessive production of type I collagen. Type I collagen is a heterotrimer composed of two  $\alpha 1(I)$  polypeptides and one  $\alpha 2(I)$  polypeptide. Translation of type I collagen mRNAs is regulated by a unique 5' stem-loop structure, which binds RNA-binding protein LARP6 with high affinity and sequence specificity. The binding of LARP6 is required to coordinate translation of type I collagen polypeptides and facilitates their folding into the heterotrimer. The LARP6 dependent mechanism is activated in fibrosis by changing the phosphorylation of LARP6 to stimulate high level of type I collagen synthesis.

To visualize formation of type I collagen in the cells and the role of LARP6 binding we developed a collagen biosensor. Full size collagen  $\alpha 1(I)$  polypeptide was tagged with BFP and full size collagen  $\alpha 2(I)$  polypeptide was tagged with GFP. The tagged  $\alpha 2(I)$  polypeptide can be expressed from mRNA with or without 5' stem-loop to visualize the effects of this sequence element on formation of type I collagen. When both polypeptides are translated from mRNAs containing the 5' stem-loop, discrete but dynamic foci of type I collagen formation are seen within the ER, but when the 5' stem-loop is mutated in the  $\alpha 2(I)$  mRNA, there is uncoupling of  $\alpha 1(I)$  and  $\alpha 2(I)$  polypeptide accumulation and disappearance of the foci. Similar pattern is observed in LARP6 knock out cells, which can be corrected by expressing LARP6. These results are the first visual demonstration of type I collagen biosynthesis and of the role of LARP6 binding to collagen mRNAs in this process.

The specificity of LARP6 regulation for type I collagen expression makes the LARP6/5' stem-loop interaction an ideal target for antifibrotic drugs. One compound that can displace LARP6 from 5' stem-loop RNA has been discovered in our lab. This compound reduced type I collagen expression, while the biosensor indicated uncoupling of production of  $\alpha 1(I)$  and  $\alpha 2(I)$  polypeptides. When administered into animals, the compound inhibited hepatic fibrosis at 1 mg/kg. Overall, these results corroborate the critical role of LARP6 in regulation of translation of collagen mRNAs and the practical implication of this finding.

## 122 Nucleo-cytoplasmic shuttling as means to regulate coupling between mRNA synthesis and decay

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The life of an mRNA starts with mRNA synthesis and culminates in degradation. Previously, we found that components of decay complex, including Xrn1, are imported to the nucleus, stimulating transcription initiation and elongation by directly interacting with the chromatin. We therefore proposed that gene expressing is a circular process [1]. As transcription and mRNA decay occur in two different compartments, shuttling of Xrn1 seems to play a key role in the balance between its mRNA synthetic and decay activities. We have found a functional "nuclear localization signal" (NLS) in Xrn1 in a domain that protrudes out of the core structure, which we named "Tail". Mutations in the NLS not only compromised the ability of Xrn1 to import but also were sufficient to block the nuclear import of some other decay components, suggesting that they are imported as a complex. Transcription rate in XRN1 $\Delta$ tail mutants is slower than wild-type, suggesting that the "Tail" plays a crucial role in regulating the nuclear function of Xrn1. When expressed independently, outside the context of the core Xrn1, Tail-GFP was found to be cytoplasmic, but some point mutations led to its nuclear localization, in an NLS-dependent manner. Screening of a yeast deletion library followed by fluorescence microscopy revealed that mutations in a few transcription and RNA export factors result in nuclear accumulation of Xrn1. Thus, we propose that shuttling of Xrn1 maintains a critical balance between mRNA synthesis and mRNA decay.

1. Haimovich, G. et al. Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis. *Cell* 153, 1000-1011 (2013).

## 123 Regulation of the *Drosophila* maternal-to-zygotic transition by RNA-binding proteins and microRNAs

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During the maternal-to-zygotic transition (MZT) metazoan embryos undergo phased clearance of a subset of maternal mRNAs together with phased activation of the zygotic genome. We have identified several RNA-binding proteins (RBPs) and multiple microRNAs (miRs) that direct maternal mRNA degradation. Smaug (SMG), which recognizes specific stem-loops in its targets, triggers the early phase of decay, prior to zygotic genome activation (ZGA), by recruiting the CCR4/NOT-deadenylase complex (*Curr. Biol.* **15**:284, 2005; *Dev. Cell* **12**:143, 2007; *Genome Biol.* **15**:R4, 2014). ZGA fails in *smg* mutants, leading to a model in which clearance of maternal mRNAs encoding transcriptional repressors is permissive for ZGA (*Dev.* **136**:923, 2009). A second, late phase of decay is carried out by zygotically synthesized miRs. In *smg* mutants, failure to produce these miRs leads to stabilization of their target mRNAs (*Dev.* **136**:923, 2009; *G3* **6**:3541, 2016). Thus, SMG directly and indirectly controls clearance of a large subset of maternal transcripts. The single-stranded RNA-binding proteins, Brain tumor (BRAT) and Pumilio (PUM), are also required for late decay and are likely to operate together with miRs in this process (*Genome Biol.* **16**:94, 2015; *G3* **6**:3541, 2016). Unexpectedly, the mRNAs bound by BRAT and PUM are largely non-overlapping; thus, while BRAT and PUM co-regulate a subset of maternal mRNAs, they independently regulate most of their targets (*Genome Biol.* **16**:94, 2015). The mRNA encoding Zelda, a transcription factor that directs the early phase of ZGA, is bound by BRAT. Zelda-dependent zygotic transcripts are produced prematurely in *brat* mutants, representing another link between regulation of maternal mRNAs by RBPs and ZGA. Together a network of RBPs and miRs regulates both maternal mRNA clearance and ZGA during the *Drosophila* MZT.

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## 124 Maternal mRNA clearance via terminal uridylation during the maternal-to-zygotic transition

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During the maternal-to-zygotic transition (MZT), maternal transcriptome is degraded and replaced by de novo-synthesized zygotic transcriptome in a highly coordinated manner. However, it remains largely unknown how mRNA stability is temporally regulated during early development in vertebrates. Through genome-wide profiling of RNA dynamics and 3' terminal sequences, we found that induction of mRNA uridylation coincides with the onset of maternal mRNA degradation. Such induction of uridylation is consistently observed in mouse and *Xenopus* embryos but not in *Drosophila*, suggesting the conserved role of uridylation in vertebrate mRNA decay pathway. By using morpholino (MO)-mediated knockdown (KD) experiments and high-throughput mRNA sequencing, we revealed that mRNA uridylation plays a critical role in the progression of the MZT. When TUT4 (Zcchc11) and TUT7 (Zcchc6), previously identified as mRNA uridylation enzymes in human cells, are knocked down in zebrafish, maternal mRNA clearance is significantly delayed, leading to impaired zygotic transcription and developmental defects during gastrulation. Maternal mRNAs with short poly(A) are preferentially targeted by TUT4 and TUT7, whereas maternal transcripts with long poly(A) are less affected by uridylation activity. Our study demonstrates that mRNA uridylation is essential for timely degradation of maternal transcripts, thereby directing the progression of early development.

## 125 Identification of diverse target RNAs that are functionally regulated by human Pumilio proteins, PUM1 and PUM2

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Human PUM1 and PUM2 belong to the eukaryotic PUF family of sequence specific RNA-binding proteins. In this study, we address two key questions: First, what are the biological functions controlled by human PUMs? Second, how do human PUMs regulate target RNAs?

To dissect the mechanism of PUM regulation, we developed reporter assays that measure their impact on target mRNAs. We found that both PUMs potently repress protein expression and promote degradation of mRNAs that possess a high affinity binding site, the Pumilio Response Element (PRE). Our analysis revealed two modes of repression including antagonism of the translational activity of poly(A) binding protein, PABPC1, and recruitment of the CNOT deadenylase complex.

We sought to identify target RNAs that are functionally regulated by PUMs. To do so, PUMs were depleted from cells and differential expression of RNAs was measured using RNA-Seq. We identified nearly 1000 new RNAs that were significantly affected by PUM depletion, including both mRNAs and non-coding RNAs. Bioinformatic analysis of PUM-regulated RNAs identified the PRE as the most enriched motif in transcripts that increased in abundance, indicative of direct binding and repression by PUMs. We then developed a transcriptome wide computational model of PUM regulation that incorporates PRE position and frequency within an RNA relative to the magnitude of regulation. The model reveals significant correlation of PUM regulation with PREs in 3'UTRs, coding sequences and non-coding RNAs, but not 5'UTRs.

To define high confidence direct PUM targets, we cross-referenced our list of PUM-regulated RNAs with all PRE containing RNAs and with experimentally identified PUM-bound mRNAs. As a result, we define nearly 300 direct targets that include both PUM-repressed and, surprisingly, PUM-activated target RNAs. Both categories of PUM targets were validated by quantitative RT-PCR, western blotting, and reporter assays.

Annotation enrichment analysis further revealed that PUMs regulate genes from multiple human diseases including cancer and cardiovascular disease. Our findings show that PUMs control prominent signal transduction pathways and developmental and neurological processes, and pave the way for determining how the PUM-dependent regulatory network may change in other tissue types.

## 126 Reconstitution of mRNA substrate selection by the Ccr4-Not deadenylase complex

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Almost all eukaryotic mRNAs contain a stretch of adenosine nucleotides at the 3' end known as the poly(A) tail. The removal of the poly(A) tail from an mRNA, a process called deadenylation, is an important mechanism of gene expression regulation. It is the first step in the decay of the transcript, and is also linked to repression of translation. Deadenylation is predominantly catalysed by a conserved multi-protein complex called Ccr4-Not. While the poly(A) tail is a feature of almost all mRNAs, cells control the rate at which each undergoes decay by the targeting of Ccr4-Not in both a gene-dependent and a context-dependent fashion. Substrate-selective deadenylation is therefore a central biochemical process in the control of gene expression.

Despite its importance, intact Ccr4-Not had not previously been obtained in sufficient quantity and purity for rigorous biochemical analysis. We have recently reported the purification of recombinant Ccr4-Not complex [1]. By reconstituting the deadenylation reaction *in vitro*, we found that Ccr4-Not could readily differentiate between RNA substrates when an RNA-binding adaptor protein was present. More recently, we have characterised the biochemical properties of Ccr4-Not and adaptor proteins, and identified key features that permit Ccr4-Not to deadenylate RNAs containing destabilising motifs such as AU-rich elements (AREs) and Pumilio-response element (PREs). Notably, selectivity and processivity can both be achieved in this *in vitro* system. Interestingly, Ccr4-Not can be programmed to distinguish between RNAs of very similar sequence, and specifically deadenylate the target when it is present in an RNA mixture. These findings give insight into how cells control the decay of RNA in a gene-dependent manner.

[1] Stowell, J.A., Webster, M. W., ... Passmore, L. A. (2016) Reconstitution of Targeted Deadenylation by the Ccr4-Not Complex and the YTH Domain Protein Mmi1. *Cell Reports*.

## 127 Mpp6 incorporation into the nuclear exosome contributes to RNA channeling through the Mtr4 helicase

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The nuclear exosome mediates the processing and decay of a large variety of transcripts, including rRNAs, sn(o)RNAs, tRNAs and non-coding RNAs such as CUTs and PROMPTs. The exosome is formed by a 10-subunit core complex (Exo-10) that is present in both the nuclear and cytoplasmic compartments and that degrades RNAs in a processive manner. Genetic and biochemical data have shown that the exosome core associates with compartment specific cofactors. In the nucleus, Exo-10 associates with the distributive RNase Rrp6 and its interacting partner Rrp47, with the helicase Mtr4 and the small protein Mpp6. Biochemical and structural data have shed many insights on how the core complex and some of the cofactors function. The least understood cofactor is Mpp6.

Mpp6 is a 20 kDa RNA binding protein predicted to be intrinsically disordered. How Mpp6 binds Exo-10 and how it impacts on the activities of the nuclear holo-complex is currently unclear. We have mapped the domain of Mpp6 that binds to the exosome core and determined the crystal structure of this complex at 3.2 Å resolution. The structure shows how the conserved central domain of Mpp6 binds onto conserved surfaces of Rrp40. The binding site is consistent with previous crosslinking-mass spectrometry data and we also confirmed it in biochemical assays with structure-based mutations. Using RNase protection assays, we show that Mpp6 is required to effectively channel RNA through the Mtr4 helicase into the exosome core.

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## 128 Comparative analysis of Arabidopsis mutants revealed distinct impact of the core non-sense mediate RNA decay factors on transcriptome homeostasis

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Nonsense mediated RNA decay (NMD) is an evolutionary conserved mechanism attributed to not only degradation of aberrant transcripts but also to fine tuning cellular transcriptome. While the mechanism and regulation of NMD has been extensively studied, deeper understanding of its biological relevance is still limited, partially because NMD inactivation confers lethality in a majority of model organisms. We discovered that impaired growth or lethality of Arabidopsis NMD mutants can be subjugated by genetic abrogation of defense signaling, which appears to be the major physiological consequence of NMD inactivation in plants. This allowed us to perform comparative analysis of plants deficient in UPF3, UPF1, and SMG7, key proteins essential for different steps of NMD. UPF3 inactivation had the smallest effect on transcriptome, which is consistent with the previous observation in *Drosophila* suggesting peripheral role of UPF3 in NMD. Although SMG7 and UPF1 act on the consecutive steps of NMD and should give a similar phenotype, still we observed different impact of these NMD factors on transcriptome as well as plant growth and development. For example, *smg7* exhibit a meiotic defect that is not present in *upf1* plants, and vice versa, UPF1 inactivation causes a severe growth retardation that is not observed in *smg7*. Further, high resolution Alternate Splicing (AS) RT-PCRs on selected NMD targets revealed accumulation of alternate isoforms in all NMD mutants, but the effect was most robust in *upf1* plants. This observation was further strengthened by transcriptome analysis, where impact of UPF1 was in an order of magnitude higher than the ones of SMG7 and UPF3. Comparative phenotypic and transcriptional study on NMD mutants in Arabidopsis reveal not only significance of each NMD factors but also increase our understanding of biological relevance of NMD.



**129 ATP hydrolysis by UPF1 promotes translation termination at premature stop codons***Lucas Serdar, DaJuan Whiteside, Kristian Baker***Case Western Reserve University, Cleveland, OH, USA**

Premature termination of translation at nonsense codons present within mRNA protein coding regions yields C-terminally truncated polypeptides with potentially deleterious functions to the cell. Nonsense-mediated mRNA decay (NMD) represents a quality control pathway that limits production of these aberrant protein products by recognizing the nonsense-containing mRNA and targeting it to accelerated degradation. How the NMD machinery is able to monitor the relative position of the terminating ribosome to determine it is premature, and communicate this information to the mRNA decay machinery remains unclear. UPF1, a critical component of the NMD machinery, is an RNA helicase whose ATPase activity is essential for NMD, but whose role in this process is unresolved. We show here that in yeast, expression of ATPase-deficient UPF1 results in a defect in translation termination on NMD substrates, causing ribosomes to stall in the vicinity of the premature termination codon. Moreover, we demonstrate that the ability of UPF1 to impinge upon premature termination requires its RNA binding activity, and is promoted by NMD co-factors UPF2 and UPF3 by a mechanism that is independent of previously characterized roles for UPF2 and UPF3 in NMD. Our results reveal a functional interaction between the NMD machinery and the terminating ribosome that is modulated by the ATPase activity of UPF1 and is required for targeting substrates for rapid degradation.

**130 The Rapid tRNA Decay Pathway in Yeast Comprehensively Monitors the Structure of tRNAs as well as their Charging Status***Matthew Payea, Isreal Moreno, Eric Phizicky***Department of Biochemistry and Biophysics, Center for RNA Biology, University of Rochester, Rochester, New York, USA**

Mutations in tRNAs or in tRNA modifying enzymes frequently leads to reduced levels or function of the tRNAs, often resulting in mitochondrial diseases and neurological defects in humans. In the yeast *Saccharomyces cerevisiae*, the quality of mature tRNAs is monitored by the rapid tRNA decay (RTD) pathway, in which the 5'-3' exonucleases Rat1 and Xrn1 target non-optimal tRNAs for decay. The RTD pathway is known to degrade a subset of tRNAs lacking one or more specific modifications in the main body of the tRNA, fully modified tRNA<sup>Ser</sup> species with destabilizing mutations in the acceptor or T-stem, and unexpectedly, fully modified tRNA<sup>Tyr</sup> (*SUP4<sub>oc</sub>*) species with mutations in any region, including the anticodon stem. The degradation of *SUP4<sub>oc</sub>* anticodon stem mutants by the RTD pathway was surprising since this stem has limited interactions with the rest of the tRNA molecule and anticodon stem mutations were therefore not expected to increase 5'-3' exonucleolytic attack.

We provide five lines of evidence suggesting that the RTD pathway monitors the overall structural integrity of tRNAs and their charging status in yeast. First, we provide in vivo evidence that the RTD pathway also targets a fully modified Class II tRNA species with destabilizing anticodon stem mutations (tRNA<sup>Ser(CGA)</sup>), suggesting that the RTD pathway generally monitors the overall integrity of tRNAs. Second, our recent high throughput analysis shows that temperature sensitivity of tRNA variants with destabilizing mutations in different regions is strongly correlated with RTD. Third, we provide evidence that tRNAs can be stabilized against RTD by long range interactions, since stabilization of the acceptor stem prevents decay of anticodon stem RTD substrates in both tRNA<sup>Tyr</sup> and tRNA<sup>Ser(CGA)</sup>. Fourth, we provide evidence that tRNA charging status profoundly impacts RTD susceptibility, since poorly charged tRNAs are much more rapidly degraded than the corresponding charged species. Fifth, we provide in vitro evidence that anticodon stem RTD substrates in tRNA<sup>Tyr</sup> are more resistant to Xrn1 or Rat1 than an RTD substrate with a mutation in the acceptor stem. These results suggest that the RTD pathway monitors all aspects of tRNA function in yeast.

### 131 Uncovering the Origins of Cooperativity in a Linked Secondary and Tertiary RNA Folding Transition

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RNAs that fold between alternative structures can control cellular functions. Structured intermediates may undergo cooperative folding transitions where switches in RNA base pairing occur together with changes in tertiary structure. Understanding these complex rearrangements would greatly aid the engineering of novel structured RNAs. To test if such a complex folding transition could be understood from the properties of the pieces, we dissected folding of the P5abc RNA, a subdomain of the Tetrahymena thermophila group I intron ribozyme. In P5abc folding, a secondary structure change in the P5c helix is coupled to tertiary structure formation in a metal core (MC) connected by a three-way junction (3WJ). We conducted high-throughput quantitative SHAPE and DMS footprinting on rationally designed mutants and found: 1) the secondary structure switch in P5c and tertiary structure formation in the MC can occur independently; 2) the 3WJ forms bridging interactions that couples the P5c switch to the MC; and 3) in a separable step, P5c and the MC form long-range contacts that mutually reinforce their native structures. Cooperativity emerges between P5c and the MC first through contacts formed in the connecting 3WJ and then through the long-range contacts between P5c and the MC, with modest values of 1.3-1.8 and 0.9-2.7 kcal/mol, respectively. The modular behavior and incremental emergence of cooperativity may be general properties of structured RNAs, reflecting the relatively sparse nature of RNA tertiary connections. These properties may benefit the kinetics of folding by promoting transient accumulation of partially structured, on-pathway folding intermediates. The pliability of RNA structure endows RNAs with a dynamic conformational ensemble, and even single point mutations can drastically alter the folding landscape, a key for evolution in an RNA world.

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### 132 Molecular architecture and dynamics of an mRNA-transport complex while recognizing a *cis*-acting localization element of its target mRNA in yeast

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mRNA localization is an important mechanism of gene regulation that exists throughout eukaryotes. It is required for processes such as stem-cell division, embryogenesis and neuronal plasticity. Despite its identification in a wide range of organisms the molecular mechanisms underlying mRNA localization and localized translation have not been well understood. For instance, is not well understood how *cis*-acting mRNA localization elements (LEs) are specifically recognized by motor-containing transport complexes. To the best of our knowledge, no high-resolution structure is available for any LE in complex with its cognate protein complex.

Using X-ray crystallography, NMR, and complementary techniques, we carried out a detailed assessment of an LE of the *ASH1* mRNA from yeast. We structurally characterized the LE alone, when in complex with its co-transcriptionally interacting RNA-binding protein She2p, and when the cytoplasmic myosin adapter She3p joins the complex to form a highly specific, cytoplasmic transport particle. Although the RNA alone forms a flexible stem loop with a well-defined hinge region, She2p binding induces marked conformational changes and results in a co-complex where the RNA adopts an L-like shape. However, high specificity and affinity for this LE is only achieved by joining of the unstructured myosin adapter She3p. Surprisingly, this beneficial effect on binding is achieved by the introduction of additional steric hindrance and not by increasing the number of base-specific contacts. The notable RNA rearrangements and joint action of a globular and an unfolded RNA-binding protein offer unprecedented insights into the step-wise maturation of an mRNA-transport complex. It may also serve as example for how unstructured RNA-binding proteins can modulate RNA recognition by proteins with globular RNA-binding domains.

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### 133 Folding and splicing of group II intron ribozymes at the single molecule level

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Group II intron ribozymes are self-splicing introns that follow closely the splicing mechanism of the spliceosomal machinery. Our focus lies on the group II intron ribozyme Sc.ai5gamma from *S. cerevisiae* mitochondria. This large intronic RNA follows a linear folding pathway to a compact structure devoid of kinetic traps.[1]

Here, we extend the intronic sequence by the two flanking exons to investigate their influence on folding and directly observe splicing by single-molecule Förster Resonance Energy Transfer (smFRET) and classical activity assays. Fluorescent labels were attached by using peptide nucleic acids (PNA) carrying a fluorophore and hybridizing them to internal loops.[2] PNAs do not interfere with catalysis and much better labelling yields and lower background fluorescence were obtained compared to classical methods. smFRET studies were carried out on surface immobilized molecules,[3] but by a new method to encapsulate these large RNAs into phospholipid vesicles. This has the distinct advantage of bypassing direct immobilization and surface effects as well as keeping the splicing products in close proximity to each other. The presence of the two flanking exons strongly influences the intron folding; fewer FRET states and a shift towards higher FRET values are observed which is indicative of less dynamic molecules and a general higher compaction. Correlation with classical splicing activity assays of the same constructs revealed new insights into the cleavage kinetics supporting a model of folding and subsequent splicing activity. New folding intermediates and splicing of the 5'-exon were observed at the single molecule level.[4]

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### 134 Repuckering at the Nucleophilic Ribose of a Catalytic RNA Can Be Observed by NMR and Is Obligatory for Function

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Recent technical developments in NMR spectroscopy and biomolecular computation have increased our ability to analyze molecular disorder and conformational changes in RNA with atomic resolution. Unambiguously tying these observed or predicted dynamics to molecular function, however, remains challenging. In this work, we use metabolically-driven isotope labeling and NMR  $^{13}\text{C}$  relaxation dispersion to identify microsecond-timescale sampling of the minor C2'-endo sugar pucker of the nucleophilic ribose at the cleavage site of the lead-dependent ribozyme. To assess the functional significance of this conformational change, we substituted a bicyclo[3,1,0]hexane nucleotide, which prevents sampling of the C2'-endo pucker while leaving the 2'-hydroxyl group intact, at the same ribose. We found that this substitution reduces the self-cleavage rate by > 98%, suggesting that the conformational fluctuations identified by NMR are in fact important for catalytic function. This general methodology for deriving specific "dynamics-function" correlations along the RNA backbone may be of use in a wide variety of systems, including the formation of the tertiary structure interface between the two internal loops comprising the active site of the hairpin ribozyme.

**135 The structure and mechanism of the TS nucleolytic ribozyme***Timothy Wilson, Yijin Liu, David Lilley***University of Dundee, Dundee, UK**

We present a high resolution crystal structure of the TS (originally called twister-sister) nucleolytic ribozyme in a pre-reactive conformation. The structure comprises two co-axial helical stacks organized by a three-way junction and two tertiary contacts. Five divalent metal ions are directly coordinated to RNA ligands, making important contributions to the architecture of the ribozyme. The scissile phosphate lies in a quasi-helical loop region that is organized by a network of hydrogen bonding. All modifications to an invariant cytosine that lies close to the scissile phosphate markedly reduce or abolish ribozyme activity, and it is likely that this nucleobase directly participates in catalysis. A divalent metal ion is directly bound to the nucleobase 5' to the scissile phosphate, with an inner-sphere water molecule positioned to interact with the O2' nucleophile. The rate of ribozyme cleavage correlated in a log-linear manner with divalent metal ion  $pK_a$ , consistent with proton transfer in the transition state, and we propose the bound metal ion acts as a general base for the cleavage reaction. Our data indicate that the TS ribozyme functions predominantly as a metalloenzyme.

Y. Liu, T. J. Wilson and D. M. J. Lilley The structure of a nucleolytic ribozyme, with an important role for a catalytic metal ion. *Nature Chem. Biol* in the press. DOI: 10.1038/nchembio.2333

**136 Mechanism for activation of the 2'OH nucleophile in the *glmS* ribozyme revealed by inverse thio effects, active site mutants, and cofactor variants***Jamie Bingaman<sup>1</sup>, Daniel Seith<sup>1</sup>, Sixue Zhang<sup>2</sup>, David Stevens<sup>2</sup>, Neela Yennawar<sup>3</sup>, Sharon Hammes-Schiffer<sup>2</sup>, Philip Bevilacqua<sup>1,4</sup>*

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The first step of small ribozyme self-cleavage is activation of the nucleophilic 2'OH to attack the adjacent phosphate. Surprisingly little is understood about this process. Small ribozyme crystal structures generally involve covalent modification of the 2'OH and so do not offer direct insight. Most small ribozymes have a guanine poised to hydrogen bond with the nucleophilic 2'OH, but it is unclear whether this guanine acts as a prototypical general base, as its position might suggest. The *glmS* ribozyme contains an active-site guanine, G33, positioned to act as a general base, and is thought to use glucosamine-6-phosphate (GlcN6P) as a general acid to facilitate catalysis. We provided theoretical evidence for the possible role of the *protonated* form of G33, which hydrogen bonds with the nucleophilic 2'OH, in facilitating catalysis not by acting as a classical general base, but by facilitating proton abstraction from the 2'OH nucleophile by an external base followed by sharing of its N1H proton with the attacking 2'O.<sup>1</sup> Additionally, we recently described three new roles of the GlcN6P cofactor in the *glmS* ribozyme through thio effect and thiophilic metal ion rescue studies on the holo and aporibozyme: (1) alignment of the active site, (2) activation of the 2'OH nucleophile, and (3) charge stabilization during self-cleavage.<sup>2</sup> Specifically, a novel, stereospecific inverse thio effect at the *pro-R<sub>p</sub>* non-bridging oxygen (NBO) atom in the wild-type aporibozyme supports the idea that GlcN6P's competitive hydrogen bonding with this NBO serves to free the 2'OH nucleophile for attack in the holoribozyme. We are extending these studies to inverse thio effects of the *glmS* ribozyme in the presence of active site variants and cofactor modifications, as well as examining whether these effects extend to other small ribozymes. Collectively, these studies lead to a model in which a multitude of hydrogen bonding interactions with the *pro-R<sub>p</sub>* non-bridging oxygen atom of the scissile phosphate trigger activation of the 2'OH nucleophile.

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### 137 Building a ribozyme with tRNA synthetase activity

Ji Chen<sup>1</sup>, Gerald Manuel<sup>2</sup>, Robert Corn<sup>2</sup>, Andrej Luptak<sup>2</sup>, Barbara Golden<sup>1</sup>

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Expanding the genetic code by codon-specific incorporation of unnatural amino acids has generated significant interest in the fields of protein engineering and synthetic biology. Here, we have engineered an aminoacyl-tRNA synthetase-like ribozyme that could be used for recoding proteins by codon-specific incorporation of natural and unnatural amino acids into proteins. Our design is based on the structure of a T-box riboswitch, which can bind and discriminate tRNAs based on their anti-codons. We linked this riboswitch to an artificial ribozyme, dFx, that can charge any tRNA with chemically-activated, unnatural amino acid substrates. tRNA-specific aminoacylase activity required circular permutation of the dFx ribozyme, as a linear fusion did not appear to bind the anticodon and CCA-tail of the tRNA simultaneously. The resulting aminoacyl-tRNA synthetase ribozyme, which we have named STARzyme, demonstrates specificity for its cognate tRNA. We show that specificity determinants for tRNA recognition involve the anticodon of the tRNA, discriminator base that precedes the CCA tail and, likely, the elbow region of the tRNA. Notably, the specificity of this ribozyme for tRNA substrate can be programmed in a predictable manner.

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### 138 Widespread Riboswitch Classes Reveal the Importance of Free Guanidine in Bacterial Metabolism

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The *ykkC* motif RNA was discovered over a decade ago by using bioinformatics and was the longest standing orphan riboswitch candidate.<sup>1</sup> The cognate ligand of the *ykkC* riboswitch remained unsolved to this point mostly due to the seemingly disparate set of genes under its regulatory control. These genes commonly code for proteins annotated as urea carboxylases, multi-drug efflux transporters, sulfonate/nitrate/carbonate transporters, and arginases. Recently,<sup>2</sup> guanidine was implicated as this elusive ligand when it was found to induce expression of a riboswitch-*lacZ* reporter gene fusion in a large screen of various growth conditions. Furthermore, reporter gene expression is activated at a lower concentration of guanidine when the *ykkCD* operon is knocked out in *B. subtilis*. These gene products, which likely function as a selective guanidine transporter, are commonly associated with this riboswitch class. In-line probing was used as an *in vitro* biochemical approach to further evaluate guanidine as the cognate ligand. Using this technique, it was shown that *ykkC* motif RNAs from distantly related bacteria become more structured at specific sites in a dose-dependent manner upon the addition of guanidine. We also found that representatives of two other RNA motifs, called mini-*ykkC* and *ykkC*-III, also bind guanidine *in vitro*.<sup>3,4</sup> Similar genes are associated with all three guanidine-binding RNA motifs, and therefore these three distinct classes of RNA have been termed the guanidine-I, -II and -III riboswitches. Although little is known so far about the biological relevance of guanidine, the wide distribution of these riboswitches and the genes they control suggest that free guanidine is a greatly underappreciated metabolite. Additional findings suggest that these riboswitches regulate numerous genes responsible for detecting and responding to toxic levels of this nitrogen-rich metabolite.

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### 139 Improved RNA Mango aptamers and bimolecular, G-quadruplex-based fluorescent “turn-on” switches

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<sup>1</sup>Simon Fraser University, Burnaby, BC, Canada; <sup>2</sup>Université de Strasbourg, Strasbourg, France; <sup>3</sup>Imperial College, London, UK; <sup>4</sup>MRC London Institute of Medical Science, London, UK

As RNA is not intrinsically fluorescent, RNA aptamers, such as RNA Mango<sup>1</sup>, that bind to small fluorogenic compounds promise to facilitate RNA imaging. RNA Mango binds a thiazole orange-based fluorophore, termed TO1-Biotin, with a  $K_D$  of 3.4 nM. Using microfluidics and fluorescence-based sorting<sup>2</sup>, we isolated three new Mango constructs (Mango-II, III and IV) that have improved binding and up to ~4-fold higher quantum yield, yielding complexes brighter than eGFP. These variants have improved structural stability, respond differently to monovalent salts, and are much more tolerant to magnesium. Mutational analysis and structural probing revealed critical features of these aptamers, which differ significantly from the original Mango. Mango binds TO1-Biotin via a three-layer G-quadruplex core isolated from external duplex RNA sequence by a novel GAAA tetraloop-like junction structure<sup>3</sup>. Changes to the core of this motif provide significantly higher binding in Mango-II. The much brighter Mango-III and Mango-IV constructs are distinct from either Mango-I or Mango-II with each containing a unique core structure responsible for TO1-Biotin binding. Using structural and probing data we designed bimolecular RNA Mango-I and II based constructs that conditionally turn “on”. This was achieved by replacing the GAAA junction element found in these aptamers with sequence that substantially inhibits fluorescence until addition of a complementary “trigger” sequence. These constructs offer advantages over conventional molecular beacons and rapidly become more than two orders of magnitude brighter once triggered. These new Mango aptamers and bimolecular constructs have much potential for *in vitro* and *in vivo* applications and are demonstrably superior at *in vivo* imaging<sup>4</sup>.

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## End of Oral Abstracts

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## POSTER ABSTRACTS

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### 140 The analysis of the interaction between FUS and hnRNP UL1 in human cells

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In the S phase of the human cell cycle FUS plays a role as a positive regulator of replication- dependent histone gene expression in complex with U7 snRNP (1). Moreover, FUS was also found to interact with heterogeneous ribonucleoprotein (hnRNP) UL1. This interaction was predominant in G2 and G1 phase (1). Interestingly, the interaction between U7 snRNP and hnRNP UL1 has also been reported in cell cycle-arrested condition resulting in repression of histone gene expression (2). It suggests that FUS can mediate U7 snRNP/hnRNP UL1 complex assembly outside of S phase in order to inhibit histone synthesis that could be harmful to the cell.

To further analyze the mutual interaction between FUS and hnRNP UL1 we prepared molecular vectors encoding FLAG-tagged hnRNP UL1 and FLAG-tagged hnRNP UL1 fragments: i) containing SAP and SPRY domains (aminoacids 1-388); ii) containing only SAP domain (aminoacids 1-100); iii) without any known domain (aminoacids 389-856). All four vectors will be transiently transfected to HeLa cells followed by immunoprecipitation of FLAG-tagged full length hnRNP UL1 and its derivatives. Similar experiment will be done using vectors encoding FLAG-tagged FUS derivatives: i) without N-terminus; ii) without C-terminus; iii) without RRM motifs; iv) without Gly-rich motif. Western blot will be then performed in order to define which fragment of hnRNP UL1 and FUS is responsible for mutual interactions.

It has been detected by CLIP assay that FUS can bind to exon7 of hnRNP UL1 mRNA (3) and can influence the splicing profile of hnRNP UL1 transcripts. The exon7 exclusion leads to synthesis of hnRNP UL1 protein without SPRY domain. To analyze whether FUS can influence the expression of different hnRNP UL1 protein isoforms we will use qRT-PCR and quantify the level of mRNAs in cells with FUS depletion and FUS overexpression. The same experiment will be done using cells synchronized to S and G1 phases.

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## 141 Investigating the molecular mechanisms of stochastic gene expression

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Individual cells within genetically identical populations show high levels of variability or “noise” in their gene expression. This variability plays important roles in cellular processes such as adaptation. Noisy expression is suggested to be involved in cancer relapse, due to the variability in expression allowing a small fraction of cells to escape cancer therapy. A previous model suggests that the noise is due to random opening and closing of the chromatin at the promoters, which creates “on” and “off” states during the expression of genes. We aim to more clearly define the molecular mechanisms involved in noisy gene expression by specifically perturbing the chromatin at a particular gene locus.

We constitutively tethered the histone acetyl transferase domain of p300, a transcriptional co-activator, to the regulatory region of the Cox-2 gene. Tethering of this HAT domain will render the gene locus constitutively open, and if the model mentioned above is valid, it will lead to a decrease in the noise of the expression in this gene. This constitutive tethering was accomplished by attaching the p300-HAT domain to DNase deficient Cas9. A stable cell line was created expressing the Cas9-p300 fusion gene along with a guide RNA designed to target the fusion protein to the Cox-2 gene in HeLa cells.

We then measured the noise in expression of the Cox-2 gene in HeLa cells that express the construct and compared their noise levels to unmodified cells. In order to measure the noise in gene expression of the Cox-2 gene, we imaged Cox-2 mRNA by single molecule FISH and counted mRNA molecules present in single cells. Our results show that cells in which the Cox-2 promoter is constitutively open, have a marked decrease in the variability of mRNA transcripts per cell, signifying a decrease of noisy gene expression. These results indicate that promoter accessibility to the transcriptional machinery plays an important role in heterogeneous gene expression.

Through understanding the fundamental molecular mechanisms of gene expression, future studies can be performed to control and lessen the magnitude of gene expression variability within cell populations and then explore their phenotypic consequences.

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## 142 Insights into the formation of RNA polymerase II CTDsome

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RNA polymerase II (RNAPII) is a eukaryotic enzyme responsible for transcription of protein coding and non-coding genes. RNAPII uses its long and flexible C-terminal domain (CTD) to recruit specific protein/RNA-binding factors for regulation of transcription. CTD consists of tandem repeats of the heptapeptide consensus YSPTSPS. The CTD sequence is post-translationally modified in a dynamic manner, yielding specific patterns that are recognized by appropriate factors in coordination with the transcription cycle events.

Repetitive nature of the CTD, its phosphorylation patterns, and structural variability make the CTD not only a unique platform to regulate interaction networks but also represent a great challenge for structural biology. To visualize the structural assemblies of CTD we have developed a model system that allows mimicking the full-length CTD with the specific phosphorylation pattern in vitro. By combining this system with nuclear magnetic resonance spectroscopy (NMR), X-ray crystallography and small-angle X-ray scattering (SAXS) we follow changes in structural behavior of both, the CTD and respective binding factor, at the atomic level resolution.

Here, we report a hybrid structure of a long CTD fragment bound to Rtt103p, a protein that is related to the 3'-end processing and transcription termination processes. The reconstruction shows that the CTD retains its highly flexible character upon binding, forming a beads-on-a-string topology with no fixed contacts between the effectors molecules. However, Rtt103p dimerizes using a previously unknown dimerization domain. This dimerization event creates topological and mobility restraints, which in turn tunes its affinity towards the CTD by increase of local concentration and governs exposure of the CTD sequence to other protein factors. We propose that the CTD code readers, such as Rtt103p, and other CTD effector molecules may form a high order structure that is essential for the conception and interpretation the of CTD code.



### 143 Targeting Pol I through CX5461, a specific Pol I inhibitor, or Pol I siRNA, affects HCMV transcription, not virus production

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The nucleolus is a non-membrane-bound nuclear organelle found in all eukaryotes. Its assembly is triggered by activation of RNA polymerase I-mediated transcription and regulated in a cell cycle-dependent manner in mammalian cells. Many viruses including human cytomegalovirus (CMV) can specifically target the nucleolus to facilitate viral transcription and translation. CMV infection results in increased protein synthesis and an enlarged nucleoli. CX-5461 targets the SL1 transcription factor of the Pol I complex and induces autophagy and senescence in a number of solid tumor cell lines, but inhibits neither Pol II transcription of mRNA nor the synthesis of DNA or proteins. We examined the antiviral effect of CX5461 compared with the anti-CMV drug Ganciclovir. In order to confirm that CX-5461 is working as a Pol I inhibitor, we assessed its effects on FURd incorporation (a fluorine-conjugated uridine analogue, which reflects that rRNA comprises more than 80% of cellular RNA) and production of 47S transcripts. These parameters were both significantly reduced. Normal cells (fibroblasts) were pretreated with CX5461 and Ganciclovir and then infected with CMV strain VR1814. The potential antiviral effect of CX5461 was examined on immediate early (IE), pp65, gB transcripts and IE and pp65 protein levels as well as virus production. IE transcripts were quantified by qPCR and were significantly reduced by both drugs. Immunofluorescence and western blot analyses confirmed significantly reduced levels of IE and pp65 proteins in infected cells treated with CX5461. Experiments using siRNA to Pol I also demonstrated significantly reduced levels of viral transcripts and IE protein levels. However, production of infectious virus was unaffected at 7 days post infection. To conclude, we provide evidence to suggest a potentially important role of Pol I, direct or indirect, in the early transcription of HCMV, yet no essential for later virus production.

### 144 Erythroid Krüppel-like factor (EKLF) mutations at position E339 of the second zinc finger change the DNA binding specificity and affinity, providing an altered transcriptional outcome

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The Erythroid Krüppel-like Factor is specific transcription factor that plays an important role in globin gene switching, erythroid lineage commitment and red blood cell differentiation and maturation. There are two dominant missense mutations in EKLF that we are interested in: mouse E339D, which is associated with hemolytic neonatal anemia (Nan-EKLF) and human E325K, which is equivalent to mouse E339 and causes congenital dyserythropoietic anemia type IV (CDA-EKLF). Positions E339/E325 are involved in the recognition and binding of DNA by EKLF. There are three categories of EKLF binding site recognition specificities. The first category comprises sites that contain cytidine in the middle position of the EKLF binding motif, which is recognized and bound by WT and Nan-EKLF. The second category comprises of sites with thymidine in the middle position, which are recognized only by WT-EKLF. The third recognition category contains purine in this crucial position and is not recognized by WT and Nan-EKLF. We applied the electrophoretic mobility shift assay (EMSA) in order to analyze the binding specificity and affinity of Nan-EKLF, CDA-EKLF to the promoter sequences of the target genes for WT-EKLF. We found that CDA-EKLF recognizes and binds a sequence for the gene E2F2 (site -1) 5' TTG GGG TGG 3', which has guanidine in its the central position, unlike WT-EKLF and Nan-EKLF, which are not able to recognize and bind purine in this position. To determine the full length of the DNA-binding site for CDA-EKLF and Nan-EKLF we performed the cyclic amplification and selection of targets experiment (CASTing) using purified CDA-EKLF and Nan-EKLF zinc finger domains and a random oligonucleotide library. The newly selected sequences were verified by an in vitro experiment - using EMSA and in vivo using the Luciferase Reporter Assay. Our results show alterations in the DNA binding motif for both of the mutants in comparison to the WT-EKLF. Newly defined consensus recognition and binding sequences for EKLF mutants confer a new function to the EKLF protein and cause a pathological phenotype to appear.

## 145 Function of Prp19C subunits in TREX recruitment and transcription

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Processing and packaging of the mRNA largely takes place already during transcription of protein coding genes by RNA polymerase II (RNAPII). The tight coupling of these different steps is thought to provide efficiency and quality control. Consequently, numerous RNA-binding proteins work together in a highly coordinated and controlled manner. The conserved TREX complex couples transcription to nuclear mRNA export and packages the mRNA into a messenger ribonucleoprotein particle (mRNP). In addition, it recruits the mRNA exporter to the mRNP. Our lab previously showed that TREX occupancy at the transcribed gene is ensured by the Prp19 splicing complex (Prp19C). Furthermore, Prp19C is required for efficient transcription. However, the molecular mechanism of Prp19C function and the roles of the different Prp19C subunits in mRNP formation and transcription have remained unknown.

Here, we show that Prp19C interacts directly with TREX. The core Prp19C consists of the essential subunits Prp19, Cef1, Clf1, Syf1, Cwc2 and Prp46 and the non-essential subunits Ntc20, Snt309, Isy1, Syf2, Snt309 and Cwc15. Importantly, we determine the function of the non-essential Prp19C subunits in Prp19C stability, Prp19C-TREX interaction, Prp19C-RNAPII interaction, TREX occupancy and transcription. Taken together, our analyses show that specific Prp19C subunits play differential roles in gene expression.

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## 146 A genome-wide transcriptome and translome analysis of Arabidopsis transposons identifies a unique and conserved genome expression strategy for Ty1/Copia retroelements

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Retroelements, the prevalent class of plant transposons, have major impacts on host genome integrity and evolution. They produce multiple proteins from highly compact genomes and, similarly to viruses, must have evolved original strategies to optimize gene expression, although this aspect has been seldom investigated thus far. Here, we have established a high-resolution transcriptome/translatome map for the near-entirety of *Arabidopsis thaliana* transposons, using two distinct DNA methylation mutants in which transposon expression is broadly de-repressed. The value of this map to study potentially intact and transcriptionally active transposons in *Arabidopsis thaliana* is illustrated by our comprehensive analysis of the co-transcriptional and translational features of Ty1/Copia elements, a family of young and active retroelements in plant genomes, and how such features impact their biology. Genome-wide transcript profiling revealed a unique and widely conserved alternative splicing event coupled to premature termination that allows for the synthesis of a short subgenomic RNA solely dedicated to production of the Gag structural protein and that preferentially associates with polysomes for efficient translation. Mutations engineered in a transgenic version of the Arabidopsis *EVD* Ty1/Copia element further show how alternative splicing is crucial for the appropriate coordination of full length and subgenomic RNA transcription. We propose that this hitherto undescribed genome expression strategy, conserved amongst plant Ty1/Copia elements, enables an excess of structural versus catalytic components, mandatory for mobilization.

## 147 miR-5191 exerts tumor suppressor function by targeting RPS6KB1 in colorectal cancer cells

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MicroRNAs (miRNAs) are a class of small non coding RNAs that play pivotal roles in cancer physiology as an important epigenetic regulator of gene expression. We have previously discovered several miRNAs regulating the proliferation of colorectal cancer (CRC) cell HCT116. Among them, we here characterized miR-5191 as a tumor-suppressive miRNA in CRC cells. The expression of miR-5191 was lower in cancer cell lines than in counterpart normal cells. Transfection with miR-5191 led to significant decrease in cell viability, proliferation, invasiveness and colony-forming capability, while inducing apoptosis, in HCT116 and HT-29 cells. RPS6KB1 was identified as a direct target of miR-5191, downregulation of which is responsible for the tumor-suppressive function of miR-5191. Our results suggest a novel tumor suppressive function for miR-5191 and its potential applicability for tumor control.

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## 148 Identification of targets of tumor suppressor microRNA-34a using a reporter library system

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MicroRNAs (miRNAs) play critical roles in various biological processes by targeting specific mRNAs. Current approaches to identifying miRNA targets are insufficient for elucidation of an miRNA regulatory network. Here, we created a cell-based screening system using a luciferase reporter library composed of 4,891 full-length cDNAs, each of which was integrated into the 3'-untranslated region (3'-UTR) of a luciferase gene. Using this reporter library system, we conducted a screening for targets of miR-34a (a tumor suppressor miRNA). We identified both previously characterized and novel targets. MiR-34a overexpression in MDA-MB-231 breast cancer cells repressed expression of these novel targets. Among these targets, GFRA3 is crucial for MDA-MB-231 cell growth, and its expression correlated with the overall survival of patients with breast cancer. Furthermore, GFRA3 was found to be directly regulated by miR-34a via its coding region. These data show that this system is useful for elucidation of miRNA functions and networks.

## 149 snoRNA-derived small RNAs associate with *Saccharomyces cerevisiae* ribosomes in a stress-dependent manner

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In recent years there has been a growing number of studies demonstrating the existence of small RNAs derived from snoRNAs (sdRNAs) in multiple eukaryotic organisms. Such RNAs have initially been observed in high throughput sequencing studies and assumed to be processed by miRNA machinery. We have identified ribosome-associated sdRNAs in yeast *Saccharomyces cerevisiae*<sup>1</sup>, an organism lacking miRNAs. Although sdRNA were detectable in sequencing data, their low abundance hampered their detection by other methods, e.g. northern blot. Recently, we provided evidence that low-abundant yeast sdRNAs can be reliably detected with means of stem-loop pulsed reverse transcription followed by PCR (SL-RT-PCR), with as little as 50 ng of the input RNA<sup>2</sup>.

Based on our observations that the tRNA cleavage<sup>3</sup> as well as their association with the ribosomes<sup>4</sup> is regulated in a stress-dependent manner, we have decided to clarify whether small RNAs derived from snoRNAs are also produced specifically in association with various stress responses. In order to quantify an absolute number of snoRNA copies which serve as substrates for a defined processing to sdRNAs, we have employed SL-RT combined with a digital droplet PCR amplification. We have observed that the processing of small RNAs derived from snoRNA 128, snoRNA 67 and snoRNA 83 was up-regulated under heat stress and also during cold shock (snoRNA 67) or hypoosmotic conditions (snoRNA 128). This would suggest that the increased cleavage of snoRNAs might be a mechanism that developed in yeast to help them cope with adverse environmental conditions. On the other hand, we have measured number of sdRNA copies associated with the ribosomes derived from yeast subjected to differential growth conditions. Therefore, stress-related association of sdRNAs with the ribosomes might point into the mechanisms of translation regulation by sdRNAs in yeast.

<sup>1</sup>Zywicki M, Bakowska-Zywicka K, Polacek N (2012) Nucleic Acids Res. 40, 4013-24

<sup>2</sup>Walkowiak M, Mleczko AM, Bakowska-Zywicka K (2016) BioTechnologia 97, 19-26

<sup>3</sup>Bakowska-Zywicka K, Mleczko AM, et al. (2016) FEBS OpenBio 6, 1186-1200

<sup>4</sup>Bakowska-Zywicka K, Kasprzyk M, Twardowski T (2016) FEMS Yeast Res, 16: pii: fow077

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## 150 Inactivation of the Arabidopsis ribonuclease RNS2 causes depletion of Argonaute1 from membrane compartments

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Argonaute (AGO) proteins are the central components of the RNA-Induced-Silencing-Complex (RISC). They bind directly to small RNAs and they regulate complementary mRNAs by endonucleolytic cleavage or by inhibited translation and/or accelerated decay. In plants and animals a fraction of AGO associates with endo-membranes, in particular with the rough endoplasmic reticulum. Such association has been linked to RISC activity and turnover but the mechanisms controlling it and its functional relevance are still ill-defined. In this study, we identify an Arabidopsis mutant that shows a dramatic reduction of AGO1 levels specifically from membrane, and not soluble, compartments, and we investigate the cause and the consequences of such defect.



## 151 The role of Methyl Transferase (MTA70) and the implications of m6A modification in miRNA biogenesis in plants

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Among various mRNA modifications, methylation of adenosine at N6 position is the most abundant mRNA modification. It is present near the 3'UTR's and the stop codons within a specific motif-RRACH (R= G/A; H= A/C/U), and is present in almost 3-5 sites per mRNA molecule in plants as well as mammals. Discovery of RNA de-methylase like Fat Mass and Obesity associated enzyme (FTO) indicates toward spatial and temporal control of the modification.

The m6A modification has also been shown to be a mark for further processing of animal pri-miRNA. HNRNPA2B1 protein has been shown to read the m6A mark and recruit the micro-processor machinery. The knockdown of the METTL3 gene that is responsible for the m6A methylase enzyme has been shown to be lethal in both animals and plants.

In this study we aim to identify the role of m6A methylation in plant miRNA biogenesis. MTA70, is the ortholog of METTL3 in plants. Interaction of MTA70 with other proteins involved in miRNA biogenesis was checked using Yeast Two Hybrid system. These experiments showed interactions of MTA70 with proteins involved in very early stages of miRNA biogenesis, namely Cycling DOF factor2 (CDF2), Negative on TATA less 2b (NOT2b) and its isoform, and TOUGH1 (TGH1). Microscopy studies showed these proteins co-localise with MTA-GFP. Co-immunolocalization also showed MTA to co-localize with RNA pol II and MTA's presence in nuclear speckles with SC35 protein. Sequencing data from mutants expressing MTA70 at a very low level showed miRNA's to be mostly downregulated (as compared to WT), while some upregulated examples were also found. The pri-miRNA levels in the same mutant were mostly upregulated, with few down regulated examples. RNA dot blot analysis with m6A antibody confirmed the presence of m6A in small RNA's (15-30nt) along with its lower level in MTA mutant. Further experiments like ChIP, RIP, Co-IP and FLIM-FRET are to be done to understand the influence of MTA70 and m6A modification on plant miRNA biogenesis.

## 152 Understanding biogenesis and functional roles of a deeply conserved non-canonical miRNA harbored in the rDNA operon of Dipteran species

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MicroRNAs are an abundant class of small RNAs that are essential for various cellular processes. Recent advances in next-generation sequencing led to the discovery of a variety of non-canonical miRNA pathways often bypassing the Drosha-mediated cleavage. Our lab recently discovered a well-expressed and evolutionarily conserved non-canonical miRNA that is located in the internal transcribed spacer-1 (ITS-1) of the Ribosomal RNA operon (rRNA), named miR-ITS-1 (a.k.a. miR-10404). While miR-ITS-1 was known to be processed by a Drosha-independent mechanism, whether the miR-ITS-1 processing is coupled with rRNA biogenesis was unknown. We knocked down a panel of ribosomal proteins and rRNA processing factors in Kc167 cells and found that a subset of factors were essential for miR-ITS-1 biogenesis. In particular, the results suggested that processing of precursor rRNA at ITS-1, a step that separates small subunit processing pathway and the large subunit pathway is important for miR-ITS-1 biogenesis. On the other hand, disruption of the rRNA processing downstream of the 5.8S rRNA had no detectable effects on miR-ITS-1 biogenesis.

To understand biological roles of miR-ITS-1, we searched for its potential targets and identified dMYC, the sole homolog of mammalian Myc, as a candidate. Given the known roles of dMYC in promoting pre-rRNA transcription, we hypothesized that miR-ITS-1 may be involved in homeostatic regulation that bridges cell growth and ribosome biogenesis. By using a reprogrammed mir-ITS-1 in a canonical miRNA backbone, we demonstrated that dMYC 3'UTR harbors functional miR-ITS-1 target sites, and endogenous dMYC mRNA could be downregulated by overexpression of miR-ITS-1. Currently, transgenic flies overexpressing the ITS-1miRNA are being studied to understand the regulatory roles of miR-ITS-1 in fly development.

### 153 Human SNORD13 is required for the synthesis of Ac<sup>4</sup>C<sub>1842</sub> in 18S rRNA

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Most box C/D small nucleolar RNAs (SNORDs) play pivotal roles in ribosome biogenesis, mostly through their ability to guide cleavages and/or site-specific 2'-O-methylations of ribosomal RNA precursors (pre-rRNAs). Here, we focus on human SNORD13 discovered two decades ago and whose mode of action remains still poorly documented. This vertebrate-specific SNORD is outstanding since it contains two long, conserved complementarities matching the 3'-end region of 18S rRNA around the highly conserved acetylated cytosine Ac<sup>4</sup>C<sub>1842</sub>. Remarkably, recent studies revealed that knock-down of SNORD13 in human cells is associated with a ~ 50% decrease in Ac<sup>4</sup>C<sub>1842</sub> levels, strongly suggesting that SNORD13 base-pairing influences 18S rRNA folding which in turn may expose the target cytosine to the cytosine acetyltransferase NAT10 (Sharma et al, 2015, Nucleic Acids Research). In this study, we not only identify *cis*-acting elements required for faithful accumulation of SNORD13 but we also show, through CRISPR-mediated loss of function, that constitutive lack of SNORD13 results in the complete disappearance of N4-acetyl cytidine at C1842 in 18S rRNA, thus further demonstrating the prominent role of SNORD13 in 18S rRNA acetylation. Ongoing research aimed at deciphering the mode of action of SNORD13 as well as the function of Ac<sup>4</sup>C<sub>1842</sub> will be presented.

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### 154 A highly potent microRNA killing machine from *Caenorhabditis elegans*

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microRNAs (miRNAs) are known to be regulating expression of more than two third of all the eukaryotic genes by post-transcriptional means, and regulation of these tiny regulators play an important role in determining their functionality. Recent reports are indicating that differential turnover plays a crucial role in determining the presence and abundance of miRNAs. Although, biogenesis of miRNAs has been extensively studied, very little is known about the basic miRNA turnover pathway, let alone, its differential regulation to bring about different physiological outcomes. In *Caenorhabditis elegans* (*C. elegans*) XRN-2 has been shown as a 'miRNase', and later PAXT-1 as its co-factor, which contributes by exerting a stabilizing effect on the former. But, XRN-2 has been implicated in the processing and turnover of a wide variety of RNAs across evolution. Therefore, in order to understand the modus operandi of XRN-2-the- 'miRNase', in its actual niche and decipher its regulatability, employing a novel biochemical strategy, we purified this 'miRNase' from endogenous sources as a component of a macromolecular protein complex. Although, recombinant XRN-2 is active on a variety of different RNA molecules, the complex acts on specific miRNAs, and not other groups of RNAs. The RNA-binding receptor component of the complex was not only found to be crucial for worm development and physiology, but it also conferred *in vivo* substrate specificity to the complex which corroborated with that of its activity, *in vitro*. Assays with the purified complex in an isolated system revealed its receptive *modi operandi* and regulatability, which indicates to the possibility of playing a decisive role in the adaptation of *C. elegans* in different environmental conditions, and thus might have conferred selective advantage to the organism.

## 155 Evolution of MicroRNA827 Targeting in the Plant Kingdom

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MicroRNA827 (miR827), a conserved miRNA, emerged from the common ancestor of vascular plants. Unlike most ancient miRNAs, which conservatively target the same set of genes across species, miR827 shifted its target preference during evolution. Previous studies demonstrated that miR827 is induced by phosphate (Pi) starvation and regulates Pi transport and storage in *Arabidopsis thaliana* and rice (*Oryza sativa*) by post-transcriptional repression of *NITROGEN LIMITATION ADAPTATION (NLA)* and *PHOSPHATE TRANSPORTER 5 (PHT5)*, respectively, two genes that encode two different types of SPX (SYG1/PHO81/XPR1)-domain-containing proteins. Here, we identified a transition of miR827 target preference during plant evolution. Target prediction analysis revealed that in most angiosperms, miR827 conservatively targets *PHT5* homologs, but only in Brassicaceae and Cleomaceae does it target *NLA* homologs, suggesting that the transition occurred during the Brassicales evolution. This transition may not be associated with the selection pressure of *NLA* and *PHT5*. Intriguingly, we found a lineage-specific loss of the miR827-regulatory module in legumes. Further analysis of miR827-mediated cleavage efficiency and the expression of transcript variants of *PHT5* in *A. thaliana* indicated that accumulation of mutations in the target site and exclusion of the target site by alternative transcriptional initiation eliminated *PHT5* targeting by miR827. Our study reveals the uniqueness of miR827-mediated regulation among conserved plant miRNAs. Despite the change in its target preference, its upregulation by Pi starvation and its role in regulating cellular Pi homeostasis were retained.

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## 156 Hyper-phosphorylation of an unstructured loop of Argonaute proteins triggers dissociation from mRNAs

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Argonaute proteins associate with microRNAs, which guide them to complementary target mRNAs. With such a pivotal role in gene silencing, these proteins represent ideal targets for regulatory post-translational modifications. Using quantitative mass spectrometry, we find that a C-terminal Serine/Threonine cluster is phosphorylated at five different residues in human. This conserved hyper-phosphorylation does not affect microRNA binding, localization or catalytic activity of human Ago2. However, mRNA binding is strongly affected. Strikingly, on Ago2 mutants that cannot bind microRNAs or mRNAs, the cluster remains unphosphorylated indicating a role at late stages of gene silencing. Altogether, our data suggest that the phosphorylation state of the Serine/Threonine cluster contribute to Argonaute mRNA binding or release probably after an initial round of silencing.

## 157 Characterization of a chemically distinct tRNA ligase-related tRNA fragment

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Transcription results in RNA molecules that must undergo further processing to become functional. Transfer RNAs (tRNAs) are no exception; their processing entails removal of 5' and 3' sequences, a series of chemical modifications and the eventual removal of introns. Interestingly, both precursor tRNAs (pre-tRNAs) and mature tRNAs also suffer precise fragmentation. Beyond translation, tRNA fragments (tRFs) have been shown to participate in epigenetic modifications, oncogenesis, viral replication and apoptosis. Chemical modification of RNA bases is of particular interest for tRF biology, as tRNAs are amongst the most heavily altered RNA species. These modifications have been linked to changes in stability and structure, but the majority do not have a well described function.

Our work is focused on a tRF generated when the tRNA ligase complex is disrupted. This tRF is chemically distinct from previously described tRFs, as it contains a 5' tri-phosphate and a 2'-3' cyclic phosphate, and it also retains the 5' leader sequence not found in mature tRNAs. Intriguingly, as this tRF is generated during tRNA biogenesis, it is not clear what, if any, modifications are present on it. Previous work has suggested that this tRF may sensitize cells to p53-related apoptosis. To date, we have established an *in vivo* method to generate and purify this tRF, and are currently characterizing the tRNA ligase-related tRF, utilizing mass spectrometry in collaboration with Sebastian Leidel to determine the state of post-transcriptional modifications.

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## 158 Novel bodies containing miRNA precursors identified in the plant cell nucleus

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MicroRNAs (miRNAs) are short, non-coding RNAs that are involved in the regulation of gene expression in eukaryotes. Plant miRNA genes (MIRs) are mostly independent transcriptional units which are transcribed by RNA Polymerase II into long miRNA precursors (pri-miRNAs). These primary MIR transcripts are cleaved by the type III endoribonuclease Dicer-like 1 (DCL1) producing shorter versions of miRNA precursors (pre-miRNAs). Each pre-miRNA consists of a stem-loop structure in which the miRNA coding sequence is embedded. Pre-miRNAs are further processed by DCL1 that results in the accumulation of mature miRNAs. In contrast to animals, in plants both steps of miRNA biogenesis take place in the nucleus.

Using Fluorescence in situ Hybridization (FISH) we observe in plant cell nuclei novel bodies in which miRNA precursors are accumulated. These pri-miRNA-containing nuclear structures are different from dicing bodies that have been previously described in plants as a sites of miRNA biogenesis. The new bodies observed by us do not contain also any Cajal bodies markers. The FISH hybridizations were performed in isolated nuclei that derived from two-week old seedlings, four-week old leaves and six-week old leaves in order to check if the number and localization of novel nuclear bodies are developmental-dependent. The number of nuclear bodies containing pri-miRNAs ranged from one to three in each nucleus, and was similar in all the developmental stages and tissues tested. Interestingly, the new bodies contain miRNA precursors with and without introns. The presence of pri-miRNA-containing nuclear structures were confirmed for: pri-miRNA163, pri-miRNA156, pri-miRNA159 and pri-miRNA393a.



## 159 Biological functions of the paternally-expressed SNORD genes at the imprinted Prader-Willi syndrome (PWS) domain

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The imprinted Snurf-Snrpn chromosomal domain harbors numerous paternally-expressed SNORD genes most of which are organized as two large non-coding DNA arrays: the SNORD115 and SNORD116 gene clusters. These tandemly-repeated SNORD genes, only found in placental mammals and mostly expressed in the brain, have many features of the canonical SNORDs that guide 2'-O-methylations, yet they lack apparent complementarity with ribosomal RNA species. Their functions remain therefore highly elusive. Remarkably, human studies and mouse genetics support the notion that deficiencies in the expression of these peculiar SNORD genes, particularly SNORD116 genes, may contribute to the pathophysiology of the Prader-Willi syndrome (PWS), a rare disorder characterized by overeating and obesity. Through the use of novel SNORD-deficient mouse lines, we are now exploring the biological relevance of PWS-encoded SNORD genes, with particular attention to the potential involvement of SNORD115 in the regulation of the alternative RNA splicing and/or A-to-I RNA editing of the brain-specific serotonin 2C (5-HT<sub>2C</sub>) receptor pre-mRNA.

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## 160 High-throughput Small RNA Sequencing Profiling of Sacbrood Virus-infected Asian Honey Bees

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MicroRNAs (miRNAs) are key regulators of many biological processes including development, cell proliferation and metabolism. Recent studies of interactions between host miRNAs and virus genes indicate that the host miRNAs also influence the life cycles of viruses. In Asian honey bees (*Apis cerana*), Sacbrood virus (SBV) represents a serious viral disease that causes colony collapse disorder. Here, we performed high-throughput small RNA sequencing of *Apis cerana* in two different developmental stages (*i.e.*, adults and larvae), and aimed to analyze differential expression patterns of miRNAs between SBV-infected and non-infected wild-type samples. From this, we identified a total of 217 miRNAs that consist of 186 conserved miRNAs and 31 novel miRNAs. In addition, we found that many conserved miRNAs are differentially expressed upon SBV-virus induction. By identifying these signature miRNAs that are associated with symptoms of SBV infection, we computationally predicted miRNAs that regulate SBV-associated target genes. Collectively, we provide the first transcriptome analysis of miRNAs in *Apis cerana*, which could be useful for elucidating biological roles of miRNAs in the regulation of SBV viral disease.

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## 161 Genome-wide mapping of DROSHA cleavage sites on primary microRNAs and novel substrates

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MicroRNA (miRNA) maturation is initiated by DROSHA, a double-stranded RNA (dsRNA)-specific RNase III enzyme. By cleaving primary miRNAs (pri-miRNAs) at specific positions, DROSHA serves as a main determinant of miRNA sequences and a highly selective gate-keeper for the canonical miRNA pathway. However, the sites of DROSHA-mediated processing have not been annotated on a genomic scale, and it remains unclear to what extent DROSHA functions outside the miRNA pathway. Here we establish a protocol termed 'formaldehyde crosslinking and immunoprecipitation followed by sequencing (fCLIP-seq)' that allows identification of DROSHA cleavage sites at single nucleotide resolution. fCLIP identifies numerous processing sites that do not match the ends of annotated mature miRNAs, particularly at the 3' termini, suggesting widespread end modifications during miRNA maturation such as trimming and tailing. fCLIP also finds many pri-miRNAs that undergo alternative processing, yielding multiple miRNA isoforms. Moreover, we discover dozens of novel substrates that are bound and cleaved by DROSHA. These substrates are processed less efficiently than canonical pri-miRNAs, and produce only minute amounts of small RNAs. Depletion of DROSHA results in an increase of the hairpin-containing transcripts. Thus, these hairpins may serve mainly as cis-elements for DROSHA-mediated gene regulation rather than as miRNA precursor. fCLIP-seq not only accurately maps the cleavage sites of DROSHA and suggests noncanonical functions of DROSHA, but also could be a general tool for investigating interactions between dsRNA binding proteins and structured RNAs.

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## 162 Roles of microRNA in NK cell development and immunity

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MicroRNAs play key roles in immune cell differentiation and immunity. Here, we show typical specimens involving miRNA effects on natural killer (NK) cell development, activation, and its homeostasis. During natural killer (NK) cell development, miR-583 plays an inhibitory role in IL-15 signaling by repressing CD132 required for NK cell differentiation. Upon stimulation, human miR-27a\* and miR-150 are negative regulators of NK cell cytotoxicity by silencing perforin (Prf1) and granzyme B (GzmB) essential effector molecules for NK cell cytotoxicity. Thus, effector immune cells including NK cells are regulated by microRNAs, and modulating endogenous miRNA levels in effector immune cells represents a potential immunotherapeutic strategy for cancer.

## 163 Design and Use MicroRNA-specific CRISPR/Cas9 Library to Identify Growth-Regulating MicroRNAs

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The CRISPR-Cas9 gene-editing system, composed of the Cas9 nuclease in complex with a single guide RNA (sgRNA), allows researchers to direct DNA cleavage at a specific site and conduct genome-scale knockout screens. To facilitate human microRNA-specific screening, we designed 4-5 sgRNAs per microRNA gene and constructed a lentiviral library constituted of 7,382 sgRNAs targeting 85% of the 1,881 miRBase-annotated microRNA stem-loops plus 1,000 non-targeting control sgRNAs. To test the utility of this library, we transduced the lentiviral sgRNA library into HeLa cells with a tet-ON Cas9. The transduced cells were cultured and the frequency-change of each sgRNA in the population was measured by amplicon deep sequencing. MAGeCK and RSA analysis of commonly changed sgRNAs from two replicate experiments identified potential growth-regulating microRNAs. Together with miR-Seq of the cell lines we used, the screen successfully picked out miR-31, a cervical cancer overexpressing microRNA shown to promote HeLa cell proliferation. Additional microRNAs are also identified and will be presented. Since the cellular function of a microRNA is highly cell-type specific and context dependent-for example, miR-31 is oncogenic in colorectal but tumor-suppressive in ovarian cancer, our microRNA-specific CRISPR library would be useful for unbiased screening of microRNA function in relevant cell types and under appropriate conditions.

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## 164 The widespread occurrence of tRNA-derived fragments in *Saccharomyces cerevisiae*

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Recently, a number of ribosome-associated noncoding RNAs (rancRNAs) has been discovered to bind and regulate the ribosome in organisms representing all three domains of life. In our previous studies, we have described novel classes of rancRNAs in *Saccharomyces cerevisiae* [1]. We were able to detect and confirm by independent experimental methods multiple novel stable RNA molecules differentially processed from well-known RNAs, like rRNAs, mRNAs, tRNAs or snoRNAs. We further demonstrated that the mRNA-derived rancRNA is needed for rapid shutdown of global translation and efficient growth resumption under hyperosmotic conditions [2].

Currently our interest is focused on revealing the potential of tRNA-derived small RNAs as specific regulators of protein biosynthesis in *Saccharomyces cerevisiae*. Although similar tRNA fragments has been observed in number of organisms and shown to associate with AGO proteins, yeast, due to lack of RNAi/miRNA machinery, provide an unique opportunity to study the DICER-independent pathways of tRF action. We have performed the comprehensive analysis of tRNA fragments abundance in yeast *Saccharomyces cerevisiae* under 12 different growth conditions. In order to measure the abundance of highly modified tRNA-derived fragments, we have employed the northern blotting method, which is independent from the reverse transcription.

We have experimentally verified that the cleavage is not limited to specific tRNAs, although the relative efficiency of cleavage can differ. Moreover, we did not observed significant differences in the accumulation of total tRNA fragments pool depending on stress condition nor increase in the relation to the optimal conditions. However, our data strongly suggest that the tRNA cleavage is regulated in a stress-dependent manner by specific selection of tRNA species which serve as substrates for a defined processing. The resulting set of *S. cerevisiae* tRNA fragments provides a robust basis for further experimental studies on biological functions of tRFs.

[1] Zywicki M, Bakowska-Zywicka K, Polacek (2012) Nucleic Acids Res. 40, 4013-24;

[2] Pircher A, Bakowska-Zywicka K et al. (2014) Mol Cell. 54, 147-55.

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## 166 EndoRNAi and piRNA pathways in mouse oocytes

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RNA interference (RNAi) is an antiviral innate immunity and genome defense pathway, which is essentially absent in mammals. An exception are mouse oocytes, which express oocyte-specific truncated Dicer isoform (DicerO) and exhibit highly active endogenous RNAi. A rodent-specific retrotransposon insertion (denoted MT1) functions as an oocyte-specific promoter expressing a highly active N-terminally truncated Dicer isoform. DicerO is essential for oocyte maturation; deletion of MT1 insertion causes female sterility due to meiotic spindle defects.

Further characterisation of mice lacking the originally identified MT insertion (MT1) showed that a second MT element (MT2) can also be recruited as an alternative DicerO promoter. Expression of evolutionary younger MT2 partially rescues the sterile phenotype of MT1 KO females in an outbred background. Moreover, a test of RNAi capacity of MT1 KO oocytes showed no difference compared to WT controls. We thus generated mice lacking both MT elements in Dicer to completely eliminate DicerO and suppress endoRNAi. We currently study their phenotype and explore redundancy of RNAi and piRNA pathways by producing mice lacking both pathways in the female germline.



## 168 In vitro evolution of fluorogenic RNA aptamers by ultrahigh-throughput functional screening using droplet-based microfluidics

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Fluorogenic RNA aptamers such as Spinach1 or Mango2 are small RNA folds able to specifically interact with and trigger the fluorescence of fluorogenic dyes (respectively the 3,5-difluoro-4-hydroxybenzylidene imidazolinone and the Thiazole Orange). By analogy with the fluorescent proteins, these new genetically encodable fluorescent probes open exciting perspectives in RNA biology provided bright enough RNA/dye systems can be developed. Both Spinach and Mango aptamers were isolated by SELEX and were therefore primarily selected for their capacity to bind their cognate dye rather than for their capacity to activate its fluorescence. Consequently, both systems suffered from a moderate brightness and other limitations.

Using our recently introduced microfluidic-assisted In Vitro Compartmentalization ( $\mu$ IVC) ultrahigh-throughput screening technology, we succeeded in isolating new variants of each aptamer affording superior properties. In this approach, aptamer-coding genes contained in a mutant library are individually expressed into picoliter water-in-oil droplets, the fluorogenic capacity of each encoded aptamer is assayed in ultrahigh-throughput regime (several thousands per second) and the genes coding for the best variants are recovered.

On the one hand, combining several of the mutations identified by the  $\mu$ IVC process and further engineering the resulting aptamer allowed us to develop iSpinach, a molecule dedicated to in vitro applications affording a reduced salt-sensitivity, an improved brightness and a higher thermal stability<sup>3</sup>. On the other hand, rescreening an original Mango mutant library allowed us to isolate, three new Mango variants with improved brightness, affinity, thermal stability and dye selectivity. The superior properties of these variants should drastically improve RNA imaging in cells.

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## 169 Dissecting the role of RNA-Binding Proteins in Plant Immunity

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RNA-Binding Proteins (RBPs) have emerged as master regulators of gene expression and are essential for cellular RNA homeostasis since they are involved in all steps of RNA life including its biogenesis, stability, processing (splicing, editing and polyadenylation), post-transcriptional modification, subcellular localization, translation and turnover. Furthermore, RBPs can play diverse roles in RNA biology, including acting as RNA chaperones, nucleases, helicases, polymerases, RNA modifying enzymes, and regulators of RNA localisation, stability and translation. Even though the function of RBPs in several cellular processes has been extensively studied for metazoans, bacteria and viruses, plant RBPs have received little attention in the literature. Moreover, although recent studies have pinpointed the importance of RBPs in plant immunity, the evidence that RBPs play important roles in stress responses has been collected stepwise and comprehensive approaches are lacking.

Therefore, to gain a global perspective into the role of RNA-Binding Proteomes (RBPomes) in plant immunity, we are currently optimizing the cutting-edge technique ‘RNA interactome capture’ to our model system. By applying ‘RNA interactome capture’ to *Arabidopsis thaliana* plants infected with *Pseudomonas syringae*, we hope to identify the scope of RBPs involved in the plant defence response against pathogens, as well as the host RBPs targeted by pathogenic bacteria. Targeted functional analyses will be performed to further validate the identified RBPs and determine their specific role in RBP-mediated plant immunity.

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## 170 *In vitro* selection of cGMP aptamers from a human genomic library

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Aptamers are structurally complex single-stranded RNA sequences with high affinity and specificity to target ligands. Aptamers are typically isolated via *in vitro* selection to enrich for RNA sequences that bind a wide variety of targets. Selected RNAs are subsequently characterized by electrophoresis-based techniques such as hydroxyl radical probing, dimethyl sulfate, nucleotide analog interference mapping, or selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE). In recent years, next generation sequencing has enhanced the capabilities of *in vitro* selection by enabling rapid identification of aptamers within an enriched library, as well as structural determination by allowing massively parallel analysis of RNA structures.

We have isolated RNA sequences with affinity for 3',5'-cyclic guanosine monophosphate (cGMP) from a human genomic library, and developed a pipeline that couples next generation sequencing and SHAPE to provide total sequence and structure identity of aptamers isolated through *in vitro* selection. Using this pipeline, the cGMP-binding RNA sequences were readily characterized; and an aptamer was mapped onto a highly conserved region of the human chromosome 20. Biochemical characterizations of this first human cGMP aptamer show a remarkable selectivity against isomers of cGMP, and will be presented in further details.

## 171 miRiam - new trends in microRNA quantification

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MicroRNAs (miRNAs) are small non-coding RNA molecules playing an important regulatory role in gene translation through silencing or degradation of target mRNAs. They are involved in a wide range of biological processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis or inflammation, and also in pathophysiology of many diseases. Recently, numerous studies have suggested circulating miRNAs as promising diagnostic and prognostic biomarkers of cardiovascular diseases, neurological disorders, cancer, metabolic syndrome and many other diseases. Monitoring the level of specific miRNA together with protein-based biomarkers may represent more efficient tool for diagnosis of these diseases and prognosis estimation.

Current methods of miRNA determination are either low in specificity and sensitivity or very expensive and high-technology demanding like Next-generation sequencing (NGS) or qRT-PCR which is considered to be the gold standard for miRNA expression analysis.

We are introducing a novel, immunoassay-based method of miRNA quantification which involves hybridization of miRNA isolated from a patient sample to complementary biotinylated DNA oligonucleotide probe. The DNA/RNA hybrids are then transferred onto a stationary solid phase coated with monoclonal antibody specific to perfectly matched DNA/miRNA hybrids. After washing, solid phase is incubated with streptavidin-HRP conjugate and the resulting complexes are visualized (after another washing step) by a chromogenic substrate.

Here we present data from our immunoassay for miRNA-21-5p quantification named miREIA-21-5p. Assay exhibits great analytical specificity, limit of detection as low as 0,1 amol/μL miRNA, excellent analytical characteristics and strong correlation with qRT-PCR (Pearson correlation coefficient >0.90). The assay has been validated for miRNA isolated from serum, plasma and whole blood.

Moreover, the assay can be run on common immunoassay analyzers, is compatible with standard clinical workflow, does not require amplification steps and results are obtained in less than three hours including miRNA profiling. Our method enables to analyze miRNA using conventional immunoassay analyzers, thus, it can promote utilization of miRNA biomarkers in clinical and laboratory practice.

## 172 CoLoC-seq, a new genome-wide approach to profile organellar RNA importomes

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Mitochondria are essential organelles of eukaryotic cells involved in several key processes, including cellular respiration, apoptosis and iron-sulfur clusters formation. Despite the presence of their own small genome, mitochondria critically depend on the import of nuclear-encoded macromolecules to ensure their functions. Beside ~1,500 proteins, in apparently all eukaryotes, from protists to humans, some noncoding RNAs (tRNAs, 5S rRNA, miRNAs...) are partially redirected into mitochondria where they participate in key genetic processes. Therefore, the mitochondrial RNome represents an intricate mixture of transcriptome and importome. While studies of transcriptomes have now been facilitated by RNA-seq, robust identification of nuclear-encoded transcripts imported into organelles is still challenging since cytosolic contamination, which remains even after most thorough purification, is dramatically amplified during cDNA library generation. Our laboratory developed a conceptually novel methodology, Controlled Level of Contamination (CoLoC), which allows, by following the biochemical RNase-mediated depletion dynamics of cellular transcripts, to unequivocally distinguish between RNAs genuinely present inside mitochondria and mere contaminants. By coupling this methodology to deep sequencing (CoLoC-seq), we comprehensively landscaped the RNA importome of human HEK293T cells and not only confirmed the status of some known imported RNAs but also identified a few novel nuclear-encoded mitochondrial-resident transcripts. The number and identity of these RNAs suggests that the mitochondrial import is a highly selective process that may supplement the organellar RNome with new, unexpected functionalities. In conjunction with orthogonal techniques, the generic CoLoC-seq methodology will help to shed light on the composition of spatially confined subcellular RNA pools in a variety of models.

### 173 RNase H sequence preferences influence antisense oligonucleotide efficiency and have shaped the HIV-1 genome

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RNase H cleaves RNA hybridized to DNA. It is present in all domains of life, in multiple viruses and in some retroelements and is essential for mammalian development and survival, as well as for HIV-1 replication. In addition, RNase H is responsible for mediating the antisense activity of DNA-containing single-stranded oligonucleotides, which are a major class of nucleic acid therapeutics.

Although RNase H is known to exhibit cleavage site preferences, they are not well understood. We developed a sequencing-based method to measure the cleavage of thousands of different RNA-DNA duplexes and thereby comprehensively characterized the sequence preferences of HIV-1, human and *E. coli* RNase H enzymes. We found that the catalytic domains of *E. coli* and human RNase H1 have nearly identical sequence preferences, which is consistent with their very similar tertiary structures. Those preferences correlate with the efficiency of RNase H recruiting antisense oligonucleotides and explain the location of the crystalized RNase H enzyme on the RNA-DNA duplex.

The HIV-1 RNase H has different sequence preferences, which likely reflects the requirement of the RNA-DNA duplex to bend in order to fit the scissile phosphate in the nuclease active site. The preferences that we observe agree with those described previously, but thanks to much more comprehensive nature of our dataset, we were able to perform a HIV-genome-wide prediction of cleavability. We found that sequences matching the preferred HIV-1 RNase H motif are spread in the HIV-1 genome with distances ensuring efficient RNA cleavage during replication. Moreover, we have observed that the host tRNA-Lys3, which is almost universally utilized by lentiviruses to prime the replication, uniquely optimizes cleavability of its both strands, which probably facilitates viral replication.

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### 174 Non-canonical RNA-guided Argonautes: Tools for probing RNA biology

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The RNA expression profile is specific to cell type, tissue, developmental stage, and disease. Therefore, probing RNA biology *in vivo* will not only broaden our knowledge of developmental processes, and disease mechanisms, but it will also suggest new therapeutic targets. Current technology to track and manipulate specific single-stranded RNA (ssRNA) *in vivo* is based on oligonucleotide probes, on genetically encoded aptamers, or on RNA binding proteins. Even though these methods are widely applied to explore RNA biology, they are limited due to the low stability of the probes in live cells, the necessity of targeted genetic manipulations of the RNAs of interest, and the requirement to re-design the RNA binding protein for each new target, respectively. The discovery of a non-canonical 5'-hydroxyl RNA guided Argonaute (Ago) protein subfamily unfolds new opportunities to utilize it as an easily programmable tool to image and manipulate endogenous RNA *in vivo*. Here we present mechanistic insights into *Marinitoga phiezophila* Argonaute (MpAgo)-guide RNA (gRNA) and ssRNA substrate binding. We demonstrate that high temperature is necessary to assemble the stable MpAgo-gRNA complex (RNP). Our findings indicate that 5'-end nucleotide of the guide RNA is important for ssRNA substrate cleavage, binding efficiency, and binding specificity. The most efficient and specific ssRNA substrate binding resulted when the gRNA contained uracil at its 5'-end. Notably, we find that site-specific covalent crosslinking of the gRNA to the MpAgo significantly improves the specificity and affinity of MpAgo RNP to bind ssRNA substrate. Finally, efficient and specific MpAgo RNP-mediated endogenous mRNA isolation from mammalian cell lysate demonstrates that MpAgo RNPs hold promise as useful tools to image and manipulate endogenous RNAs in living cells.



## 175 Phenotypic plasticity of non-coding RNA mutants

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The recent completion of the non coding RNA (ncRNA) deletion collection in *Saccharomyces cerevisiae*, has provided a powerful tool for studying the function of ncRNAs in different environmental context. The mutants have been screened for observable phenotypes using the Singer ROTOR HDA on different carbon sources, nutrient limitations and stressors. Colony size was used as measure to assess fitness. To date the collection has been grown in 25 different conditions, to test the ability of individual strains to utilise different sugars as well as their ability to tolerate a variety of alcohols, acids and incubation temperatures. The results so far show high phenotypic plasticity of the ncRNAs with several mutants displaying different phenotypes according to the medium used.. The largest variation in fitness can be seen in medias containing alcohols. Scatter plots of the fitness profiling of the mutants grown on the same type media (i.e. different alcohols) show a similar 'fingerprints'. From these data, strains differing significantly from the wild type can be selected for further research and hypotheses made regarding their function.

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## 176 Structure-seq2: Sensitive and accurate genome-wide profiling of RNA structure *in vivo* in plants and bacteria

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RNA structure contributes to a variety of RNA functions.<sup>1</sup> To elucidate these structurally-correlated RNA functional trends, it is beneficial to determine the structure of the entire transcriptome. Additionally, as the physio-chemical environment can dramatically change RNA structure,<sup>2</sup> structural determination using *in vivo* analysis provides a more rigorous structure-prediction. Structure-seq2, a method developed by our lab,<sup>3</sup> uses DMS chemical probing followed by reverse transcription with a random hexamer to calculate reactivities that can be used to help predict RNA structure genome-wide and *in vivo*. Compared to the original Structure-seq,<sup>4</sup> two variations of Structure-seq2 are both more sensitive and accurate in that the sequencing results have a higher quality with four-fold less starting material. This was accomplished by reducing the presence of a deleterious by-product, lowering ligation bias, reducing mismatches, and improving read coverage. Applying Structure-seq2 to rice provides evidence of hidden breaks present in chloroplast 23S rRNA and a previously unreported N<sup>1</sup>-methyladenosine in 25S rRNA. Structure-seq2 is also being used to determine the RNA structurome of *Bacillus subtilis* to uncover novel regulatory functions attributed to RNA structure in bacteria.

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## 177 New chemistries for the analysis of RNA dynamics

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Cells dynamically modulate their RNA levels in response to stimuli and stresses. Although RNA-seq experiments offer a snapshot of RNA transcripts at a specific time, steady-state RNA levels can be slow to adjust, limiting the insight into the biological state of a sample achieved by standard RNA-seq studies. Here we present a set of chemical advances that provide increasingly sensitive analysis of changing RNA populations. We illustrate how these chemical developments can be integrated into other approaches to reveal the rich biology of RNA dynamics.

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## 178 Venturing into terra incognita of protein small molecule interactions using combination of novel and classical approaches

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Interactions between biological molecules enable life. The significance of a cell-wide understanding of molecular complexes is thus obvious. Protein-metabolite interactions (PMIs) have received little interest in the past; technological progress has been causing this to gradually change.

For us, the most interesting interactions are between ligand and receptor, the triggers of signalling events. While the number of small molecules with proven or proposed signalling roles is rapidly growing, the lion's share of their protein receptors remains unknown. Conversely, there are numerous signalling proteins with predicted ligand-binding domains, the identity of the small-molecule counterparts of which remains elusive. The identification of such signalling PMIs stands at the core of this project. In our research we use Arabidopsis cell cultures; the envisaged technological platform, however, is applicable to other systems.

Herein, we report a simple yet effective in vivo method for global detection of small molecules bound to proteins guided by co-fractionation (Veyel et al., 2017). Through combination of size filtration with size exclusion chromatography (SEC) our protocol facilitates the unbiased prediction of protein-metabolite interactions in a cell-wide manner without the need of protein or metabolite baits. A map of the protein-metabolite complexes obtained from SEC experiments assisted in the selection of novel PMIs, now being examined by targeted approaches, including affinity purification starting either from a protein or a small molecule of interest, DARTS, and microscale thermophoresis.

Among number of interesting small molecules, which we found bound to the protein fraction, our attention was drawn to the positional isomer of secondary messenger 3'5'cAMP, namely 2'3'cAMP, an RNA degradation product. Using an elegant strategy we identified and confirmed identity of the 2'3'cAMP receptor protein in plant and human cells, linking 2'3'cAMP to the RNA fate regulation (Kosmacz et al., submitted).

## 179 Direct nanopore sequencing of 16S ribosomal RNA

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Nanopores can directly interrogate biological RNA strands, offering a fundamentally different method for sequencing RNA. We describe a method for directly sequencing individual, full-length 16S ribosomal RNA molecules. A customized, 16S rRNA-specific oligonucleotide adapter facilitates ligation of Oxford Nanopore RNA sequencing adapters and subsequent sequencing on a MinION sequencer. Similar to the way DNA sequencing occurs on a MinION, a RNA-specific motor protein moves each RNA strand in single-nucleotide steps through a protein nanopore in the sequencer array. The unique pattern of ionic current impedance produced by each ribonucleotide in the strand passing through the pore can be deconvoluted into RNA sequence. Over 100,000 reads mapping to *E. coli* str. MRE600 are possible when a sequencing sample is prepared from a purified 16S rRNA sample. We demonstrate the modified nucleotides 7-methylguanosine and pseudouridine can be detected in *E. coli* 16S rRNA reads at known modification positions. The customized 16S rRNA-specific adapter can be selectively hybridized to 16S rRNA in *E. coli* total RNA and produces similar results to sequencing purified 16S rRNA. Using this selective sequencing strategy the presence of *E. coli* 16S ribosomal RNA can be detected in a total human RNA background. More broadly, our results demonstrate the potential for nanopore RNA strand sequencing to read long strands of RNA and identify modified nucleotides, which will simplify RNA-seq applications and provide a unique view of RNA sequence content in clinical and research setting.

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## 180 Repurposing microarrays for high-throughput genotype-phenotype mapping of fluorescent RNAs

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In the last few years, we have witnessed the development and utilisation of various RNA aptamers that bind fluorophores and induce their fluorescence. RNA mimics of the green fluorescent protein (GFP) have a great potential to facilitate research in a diverse variety of biological processes including RNA trafficking, processing, and regulation of gene expression. Fluorescent RNAs can also be adapted to serve as molecular imaging probes exhibiting changes in fluorescence upon binding of small molecules or ions *in vivo*. To facilitate the study of genotype-phenotype relationships in fluorescent RNA aptamers, we repurposed commercially available gene expression microarrays for high-throughput screening of fluorescent RNAs. We designed a library of 50,000 mutants of the RNA aptamers Broccoli and Spinach, with each variant fused to a different sequence complementary to a specific probe on the microarray. Our library allows us to measure fluorescent properties of all possible single and double mutants of Broccoli on a single microarray chip, which greatly simplifies the process of screening for variants that exhibit fluorescence alterations in changing conditions. The applications include the development of fluorescent sensors of ions and small molecules, and global mapping of epistasis in RNAs, which could give insight into the structure and mechanism of activity of those molecules.

## 181 Characterization of small proteins in *E. coli* and *Salmonella Typhimurium*

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Small proteins in bacteria have been underrepresented since the beginning of the whole-genome annotation era, as their size makes both their prediction and their investigation challenging. Information regarding their presence has been slowly building up in the recent years, and functional characterization demonstrated that they play important roles in diverse cellular processes.

The Gram-negative bacteria *E. coli* and *Salmonella* are two well-established model organisms. While the first is probably the best characterized non-pathogenic bacterium, the other is a model for host-pathogen interaction as it can establish infection in a variety of hosts. In both cases, small proteins shorter than 100 aa account for approximately 10 % of the currently annotated proteome.

Here we inspected dual RNA-seq data (Westermann et al., 2016) to identify *Salmonella* small proteins that are expressed and regulated during host infection, transposon-directed insertion sequencing (TraDIS) (Barquist et al., 2013) to reveal the consequence of their inhibition for infection, and gradient profiling (Smirnov et al., 2016), that informs about the involvement of small proteins in larger complexes, both in *Salmonella* and *E. coli*. We complemented these experimental data with results from a recently developed computational pipeline for the prediction of small proteins, called sPepfinder. In doing so, we will identify novel bacterial small proteins and we may obtain first insights into their cellular functions, likely providing new information about bacterial physiology and pathogenesis.

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## 182 Characterising gene-environment interactions in the fitness landscape of a yeast snoRNA

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In a previous study, we systematically surveyed the fitness landscape of yeast U3 snoRNA using a library of random mutants and a high-throughput fitness assay. The results correlated with functional expectations, with the largest negative fitness effects residing in the box and hinge regions of the U3 snoRNA, regions important to protein and rRNA interactions respectively. These experiments also revealed the presence of epistatic interactions across the RNA, correlating with key structural and functional features. In order to now examine gene-environment interactions, we performed the fitness assay at a range of high and low temperatures. Completing the assay at 37°C revealed destabilising mutations consistent with the effect of temperature upon RNA structure. We are testing the hypothesis that destabilising mutations will become more deleterious at higher temperatures, whereas mutations which increase stability may have adverse fitness effects at low temperatures. The results will expose temperature dependent mutations and environmental interactions. Additionally by creating strains hypomorphic for proteins that interact with U3 snoRNA, we also study the role of gene-gene interactions in shaping fitness landscapes.



### 183 A Highly Efficient, Low-Bias and Directional RNA-Seq Library Preparation Method for Transcriptome Profiling

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RNA-seq (RNA sequencing) has undoubtedly become the most popular method for transcriptome analysis. It is widely used, both for gene expression analysis and for other applications, including detection of mutations, gene fusions, alternative splicing, and post-transcriptional modifications. Recent improvements in next generation sequencing technologies (NGS) and sample barcoding strategies allow analysis of multiple samples in parallel in a cost effective manner. As RNA-seq is increasingly adopted for molecular diagnostics, the quality and reproducibility of library preparation methods become more important. In addition, demand for library preparation methods that produce successful NGS libraries from ultra low input RNA or precious clinical samples is increasing.

To overcome these challenges, we have developed a streamlined RNA-seq library preparation method that retains information about which strand of DNA is transcribed (directional). Strand specificity is important for the correct annotation of genes, identification of antisense transcripts with potential regulatory roles, and correct determination of gene expression levels in the presence of antisense transcripts. Our method can be used across a wide range of input RNA (1-1000 ng total RNA) without any modifications to the protocol, making it a convenient method for RNA-seq library preparation. More importantly, GC content analysis, gene body coverage and gene expression correlation show that these important parameters remain consistent across varying inputs, even though input amounts vary by three orders of magnitude. As a result, our method has increased sensitivity and specificity for low-abundance transcripts, and reduced PCR duplicates and sequence bias, delivering high quality strand-specific data. Our method is compatible with poly A-tail enriched and ribosomal RNA depleted samples as well as RNA extracted from FFPE (formaldehyde fixed paraffin embedded) tissue samples, and is amenable to large-scale library construction and automation.

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### 184 Read distribution bias in transcriptome-wide RNA secondary structure probing data

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A transcriptome-wide chemical probing of RNA secondary structure relies on the employment of dimethyl sulfate (DMS) or SHAPE reagents to induce structure-dependent modification of RNA, either *in vitro* or *in vivo* followed by deep sequencing of cDNA libraries constructed from premature reverse transcription termination products. One of the most critical parts of those methods is the *in silico* estimation of the reactivity of individual nucleotides which reflects their structural accessibility. This process is hampered by substantial background of "natural" RT termination events, which are probing-independent. The current methodology is based on comparison of RT drop-off rates between the modified and control samples, using either simple enrichment analysis or replicate-based statistical modelling. One of the steps which is common for all of the above methods is normalization of the transcript abundance, which is fulfilled by inclusion of the transcriptome, transcript or nucleotide coverage in the reactivity calculation.

In our work, we present evidence for biases in read distribution between control and modified sample, which severely influence the reactivity estimation. We analyzed publicly available data derived from Mod-seq and SHAPE-seq 2.0 protocols, which are highly enriched in premature RT drop-off products. We have noticed that the introduction of DMS or SHAPE modifications causes a redistribution of part of the signal from "natural" RT termination positions to the modification-dependent positions. This results in overestimation of the RT drop-off in control sample and leads to underestimation of reactivity and loss of the signal. We have also noticed that by the appearance of an additional RT stops, both, total and local read coverage of transcripts is disturbed. Based on those observations we have developed a novel method for normalization and calculation of the structural reactivity, which efficiently compensates all of the above biases, without the need for replicates. In comparison to other existing methods, the resulting reactivities are in very good agreement with the model RNA structures, providing good discrimination between single and double-stranded nucleotides and are highly preserved between the replicates.

This work was supported by the National Science Centre [2011/03/D/NZ2/03304 to M.Ż.] and Ministry of Science and Higher Education [KNOW].

## 185 Circulating microRNAs as biomarkers of drug-induced tissue injury

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Currently available drug-induced tissue injury biomarkers have limited sensitivity and specificity. Additional biomarkers are needed to augment or supplant the current repertoire. Due to their tissue specificity which is generally conserved across species and presence in most biofluids, microRNAs have emerged as biomarker candidates. For example, miR-122 is used in preclinical and clinical studies as a biomarker of drug-induced liver injury. MicroRNAs have also important functions in cardiac physiology and pathologies and have also been implicated in mechanisms of drug-induced cardiac tissue injury. We have profiled microRNAs from 8 different heart structures across species by using a miRNA-seq approach to build a microRNA heart atlas. The resultant microRNA signatures were used to discriminate between different heart tissue substructures. Potential microRNA-mRNA interactions were explored using anticorrelation expression analysis and microRNA target prediction algorithms. A subset of microRNA/mRNA interactions were further characterized and validated using *in vitro* reporter gene assays. We used a rat model of acute cardiac injury induced by short term isoproterenol treatment and also a rat model of drug-induced cardiac-valves injury. We have explored the potential correlation between drugs known to trigger heart liabilities and elevated levels of heart-enriched microRNAs in plasma, serum and urine. Our data show that the profiling of miRNAs in the context of drug safety assessment can add value by providing novel safety biomarkers, especially when measured in body fluids such as plasma.

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## 186 Extracellular-enriched tRNA halves assemble into nuclease-resistant dimers and high molecular weight aggregates with potent immunostimulatory activity

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Transfer RNAs can serve as substrates for endonucleolytic cleavage to yield small RNA fragments (comprising tRNA halves and tRFs) involved in gene regulation processes. Over the past few years a plethora of mechanisms involving tRNA halves have been reported, including inhibition of translation initiation, promotion of stress-granule formation and sequestering of mRNA-stabilizing proteins. Recently, tRNA halves were even shown to mediate epigenetic inheritance during fertilization. Consistent with a role in intercellular communication, we have surveyed the extracellular small RNA profile of different cell lines and found that tRNA halves showed the highest degree of extracellular enrichment (Tosar et al. 2015; NAR). These RNAs were predominantly not associated with extracellular vesicles but were readily recovered in ultracentrifugation supernatants and eluted in size-exclusion chromatography (SEC) with apparent weights of 44 and 20 kDa. The latter of these peaks showing remarkable nuclease resistance. After failed attempts to identify the protein counterpart in these complexes, we came to the notion that the P1 peak was actually composed of tRNA-Gly homodimers. The capacity of these fragments to stably dimerize in solution was demonstrated with synthetic wild-type and mutant RNA oligos. Transfection of MCF-7 cells with a biotinylated version of these RNAs showed that oligomerization ability is essential for intracellular stability. We then focused in the nuclease-resistant peak and found it is actually a dsRNA by-product of a nuclease-sensitive high molecular weight aggregate, only detectable upon addition of nuclease inhibitor to cell conditioned medium. Sequencing of selected SEC peaks confirmed tRNA halves as building blocks in these aggregates, although rRNA fragments and certain snoRNAs were also detected. Considering these aggregates could form partially dsRNA particles, we studied their recognition by non-primed murine dendritic cells. Interestingly, the high MW aggregates were able to induce dendritic cell maturation and interleukin-1beta secretion at 100-fold lower concentration than the dsRNA synthetic analogue poly (I:C), an effect totally ablated by RNase treatment. Current effort in our lab is aimed to address the role of extracellular tRNA halves in the interplay between cancer and immune cells, while better characterizing the different supramolecular assemblies involving ncRNA fragments in the extracellular space.

## 187 PPR polyadenylation factor defines mitochondrial mRNA identity and stability in trypanosomes

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In *Trypanosoma brucei*, most mitochondrial mRNAs undergo internal changes by RNA editing and 3' end modifications. The temporally separated and functionally distinct modifications are manifested by adenylation prior to editing, and by post-editing extension of a short A-tail into a long A/U-heteropolymer. The A-tail stabilizes partially- and fully-edited mRNAs while the A/U-tail enables mRNA binding to the ribosome. Here, we identify an essential pentatricopeptide repeat-containing RNA binding protein, kinetoplast polyadenylation factor 3 (KPAF3), and demonstrate its role in protecting pre-mRNA against degradation by the processome. We show that KPAF3 recruits KPAP1 poly(A) polymerase to the 3' terminus, thus leading to pre-mRNA stabilization, or decay depending on the occurrence and extent of editing. In vitro, KPAF3 stimulates KPAP1 activity and inhibits mRNA uridylation by RET1 TUTase. Our findings indicate that KPAF3 selectively directs pre-mRNA toward adenylation rather than uridylation, which is a default post-processing modification characteristic of ribosomal and guide RNAs. As a quality control mechanism, KPAF3 binding ensures that mRNAs entering the editing pathway are adenylated and, therefore, competent for post-editing A/U-tailing and translational activation.

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## 188 The Nonstop decay and the RNA silencing systems operate cooperatively in plants

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Nonstop decay (NSD) is a translation-coupled, cytoplasmic mRNA quality control system, which targets two types of stop codon free mRNAs: nonstop mRNAs, which contain polyA sequences at the 3' end, and stop codon-less mRNAs, which lack the polyA tail. Stop codon-less mRNAs could be generated by an endonucleolytic cleavage in the coding region. NSD has two essential protein components: Pelota and Hbs1.

RNA silencing is a gene inactivation mechanism based on sequence homology between a small RNA (e.g. a micro RNA, miRNA) and a target gene. The matured small RNAs are loaded into an RNA-Induced Silencing Complex (RISC) and guide the complex to the homologous mRNAs for degradation. Target sites of plant miRNAs are mostly located within the coding region, thus RISC-cleavage generates 5' non-polyadenylated and 3' uncapped mRNA fragments, which could be either degraded by exonucleases or amplified by the RNA-dependent RNA polymerase (RDR) pathway. It is not known, how the balance between degradation-amplification of silencing cleavage products is regulated. As the NSD system eliminates the stop codon-less mRNAs in animals, and the 5' cleavage fragments of miRISC are stop codon-less mRNAs in plants, we hypothesized that the NSD machinery is involved in the efficient decay of RISC generated 5' cleavage products in plants.

Using various transient reporter gene assays we demonstrated that NSD system operates in plants and that Pelota, Hbs1 and the SKI complex are required for plant NSD. We showed that the 5' miRISC cleavage products can accumulate in the absence of the NSD system. Comparative transcriptome analysis of Pelota-silenced and control plants revealed the impact of the NSD system on the elimination of 5' cleavage products of the endogenous miRNA target transcripts.

Based on these findings, we concluded that NSD is an evolutionarily conserved eukaryotic system, and that NSD and RNA silencing systems act cooperatively in plants. As NSD is required for the degradation of the 5' cleavage products of several endogenous miRNA target mRNAs, we propose that NSD plays an important role to keep the balance between degradation and silencing amplification.

## 189 Splicing influences Set2 recruitment and H3K36me3 at intron-containing genes in *Saccharomyces cerevisiae*

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Several studies suggest that chromatin can influence pre-mRNA splicing through changes at specific histone modifications and vice versa. Tri-methylation occurring at lysine residue 36 of histone H3 (H3K36me3) has been proposed as a mark of the boundary between introns and exons in metazoa [1]. In human cells, splicing inhibition impaired recruitment of the H3K36 methyltransferase, SETD2, reducing H3K36me3 on intron-containing genes [2] and/or shifting the distribution of H3K36me3 towards the 3' end of genes [3]. However, the mechanism by which splicing affects histone modification is unknown.

To investigate how splicing affects H3K36 methylation in budding yeast, we used reporter genes with mutations that cause different defects in splicing and analysed changes in Set2 (yeast H3K36 methyltransferase) recruitment and H3K36 methylation using ChIP-qPCR [4]. Our results show that Set2 recruitment and H3K36 tri-methylation at the reporter gene increased after induction, and cis-acting mutations that block splicing reduced the level of both H3K36me3 and Set2. This reduction in methylation was also observed by inhibition of splicing in trans, using yeast strains in which individual splicing factors were conditionally depleted. ChIP-qPCR analysis in these strains showed a reduction of both Set2 recruitment and H3K36me3 over the exons of several endogenous intron-containing genes. These findings reinforce the idea that the process of splicing stimulates the deposition of the epigenetic mark at specific gene locations during transcription. By disrupting splicing at different stages of the spliceosome cycle we seek to determine which aspect(s) of splicing affect(s) recruitment of the Set2 methyltransferase.

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## 190 Altered transcriptional elongation landscapes modulate alternative splicing in cancer

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Changes in alternative splicing patterns are an emerging hallmark of cancer. For example, the histone variant macroH2A1 has two functionally distinct alternative splice isoforms: macroH2A1.1, which can regulate the gene expression of tumor suppression pathways and is often lost in cancer, and macroH2A1.2, which does not regulate these processes. Our data show that, in addition to being regulated by specific splicing factors, macroH2A1 splicing is regulated by the rate of RNA Polymerase II (Pol II) elongation along the macroH2A1 gene. We have shown that pharmacological and genetic changes to Pol II elongation rate alter macroH2A1 splicing and that the Pol II elongation rate along the macroH2A1 gene is different between normal and cancer cells. Several studies have highlighted the role that transcription elongation rate plays in regulating various splicing events, but it remains unknown if cancer cells use transcription elongation rate as a mechanism to modulate the alternative splicing patterns of key cancer genes. Our analysis of published RNA-seq data and publicly available data from The Cancer Genome Atlas identified a gene expression signature that predicts that altered elongation rates change alternative splicing in cancer. Based on these data, we hypothesize that loci-specific alterations in Pol II elongation rate play a critical role in the changes in alternative splicing that occur in cancer.

To address this hypothesis, we developed a genome-wide assay that is capable of measuring Pol II elongation rates across each gene as well as the rate of each individual splicing event in human cells (Splicing Kinetics and Transcript Elongation Rate by sequencing, SKaTER-seq). We have also developed a novel analysis pipeline utilizing probabilistic modeling to estimate rates of elongation and splicing from these data. We will present our findings on the relationship between local elongation rate and splicing rates genome-wide, and across normal and cancer cell lines. Understanding these rates and how they change during oncogenesis is the crucial first step to understanding the mechanisms behind splicing dysregulation in cancer and ways to mitigate it.



## 191 Experimental Evolution Uncovers Two Key Steps of Transcription-Splicing Coordination in Yeast

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Gene loss is one of the critical driving forces for adaptive evolution. Exactly how cells may respond and adapt to the functional loss of essential genes, which in aggregate constitute the core genome, remains largely an unresolved question. To experimentally test this issue, we exploited a unique splicing system, in which Prp28, an essential splicing factor, is purposely made dispensable or bypassed by mutations functionally linked to U1 snRNP, for experimental evolution. Through whole genome sequencing and extensive genetic analyses, we showed that there are two evolutionary routes toward the restoration of the fitness of the ancestral Prp28-less cells. The first route progresses through novel adaptive alterations within the U1 snRNP proper alone. The second route, however, involves in altering both the U1 snRNP and SAGA, a chromatin-remodeling transcriptional co-activator complex. Molecular engineering to slow down the elongation rate of RNA polymerase II in the ancestral Prp28-less cells rescues the splicing and fitness defects to a large extent, indicating that fine-tuning the interconnected transcription and splicing systems is evolutionarily selected. Sequencing other evolved clones identified various mutations in genes involved in transcriptional machinery, further suggesting a tight coupling of the evolutionary paths of transcription and splicing systems. To further explore how cells compensate the loss of essential genes, we expand our essentiality-bypassed concept to another essential splicing factor Sub2, which functions in another stages in splicing. Likewise, we found both of chemical and genetic perturbations of the transcriptional machinery allowed bypassing Sub2. Our findings thus pinpoint the transcription-splicing coordination at two key steps of splicing, i.e., the steps in which U1 snRNP and U2 snRNP interact with the 5' splice site and branch site, respectively. Taken together, our results demonstrate that the exquisite synchronization between cellular complexes is a critical strategy for compensating the loss of essential genes in evolution and thus underscores a key consideration for system optimization.

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## 192 Identification and characterization of novel group of intragenic enhancers in *Arabidopsis*

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Enhancer RNAs (eRNAs) and their transcription is believed to be linked to enhancers' activity and spatial organization of the genome. Some data also suggest that processing and/or degradation of mammalian eRNAs by the exosome may help recruit transcription factors to enhancers. However, information about plant enhancers remains scant and plant eRNAs have not yet been reported. To examine enhancers in *Arabidopsis thaliana*, we analyzed the chromatin landscape, RNA Pol II occupancy, and the polyadenylated and non-polyadenylated *Arabidopsis* transcriptomes, using exosome-deficient lines to capture stable and unstable transcripts. This analysis identified a unique set of >1900 regions in *Arabidopsis* that carry classic chromatin signatures of metazoan transcriptional enhancers. Most of these regions, which we termed Putative Enhancers (PEs) are intragenic and bidirectionally transcribed.

Analysis of *Arabidopsis* Hi-C data at high resolution indicated that 24% of identified PEs interact with genomic regions located elsewhere on the same chromosome. Most PEs interact with protein-coding genes and PE regions carry the same sets of transcription factor binding motifs carried by their target genes, strongly suggesting that these genes could be the targets of PEs and supporting the idea of co-regulation of expression between PEs and their targets. We also confirmed the conservation of the 3' end PE regions in the 1035 *Arabidopsis* accessions that were recently sequenced.

### **193 Identification of Dlk1-Dio3 imprinted gene cluster noncoding RNAs as novel candidate biomarkers for liver tumor promotion**

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The potential roles of microRNAs in cellular responses to xenobiotic stress, the development of pathophysiological changes and other toxicological phenomenon such as susceptibility/resistance to xenobiotics are currently the focus of active research. In particular, we have investigated the temporal sequence of molecular and pathological perturbations at early stages of phenobarbital (PB) mediated liver tumor promotion in vivo. Molecular profiling (mRNA, microRNA, Histone modification, DNA methylation, and proteins) of mouse liver during 13 weeks of PB treatment revealed progressive increases in hepatic expression of long noncoding RNAs and microRNAs originating from the Dlk1-Dio3 imprinted gene cluster, a locus that has recently been associated with stem cell pluripotency in mice and various neoplasms in humans. PB induction of the Dlk1-Dio3 cluster noncoding RNA (ncRNA) Meg3 was localized to glutamine synthetase-positive hypertrophic perivenous hepatocytes, suggesting a role for  $\beta$ -catenin signaling in the dysregulation of Dlk1-Dio3 ncRNAs. Early and late transcriptomic responses to sustained PB exposure were investigated in liver tissue from double knock-out CAR and PXR (CAR(KO)-PXR(KO)), double humanized CAR and PXR (CAR(h)-PXR(h)), and wild-type C57BL/6 mice. Our data identify Dlk1-Dio3 ncRNAs as novel candidate early biomarkers for mouse liver tumor promotion and provide new opportunities for assessing the carcinogenic potential of novel compounds.

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### **194 Physiological relevance of the nucleocytoplasmic shuttling of the SR protein SRSF1**

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The serine/arginine-rich (SR-) family proteins constitute a diverse group of pre-mRNA splicing factors that are essential for viability. They can be characterised based on the presence of one or two RRM domains and an RS domain. A subset, of which SRSF1 is the prototype, is capable of nucleocytoplasmic shuttling; a process governed by continual cyclic phosphorylation of the RS domain. In contrast, SRSF2 is unable to shuttle due to the presence of a nuclear retention sequence (NRS) at the C-terminus of its RS domain (1). When this NRS is fused to SRSF1, it prevents nucleocytoplasmic shuttling of the SRSF1-NRS fusion protein. We previously identified a role for SRSF1 in promoting translation of specific mRNA transcripts, particularly those encoding RNA processing factors and cell-cycle proteins (2). Here, we aim to study the physiological relevance of SRSF1 cytoplasmic functions. We have used CRISPR/Cas9 editing to knock-in the NRS naturally present in SRSF2 at the SRSF1 genomic locus, creating an SRSF1-NRS fusion protein. We have been unable to successfully generate viable homozygous clones in mouse ES cells, despite being able to easily tag the genomic SRSF1 locus. This strongly suggests that the ability of SRSF1 to shuttle to the cytoplasm is essential for viability in ES cells. Importantly, undifferentiated ES cells have a distinct cell cycle profile from terminally differentiated cells, thus, we are currently investigating a putative role for SRSF1 in enhancing the translation of mRNAs encoding cell cycle proteins that are essential in mouse ES cells. Finally, we are using proteomics to identify interactors of endogenous cytoplasmic SRSF1 and those that bind the NRS of SRSF2 to gain insights into the mechanism of nuclear retention for non-shuttling SR proteins. In summary, we will present our ongoing work to determine the physiological significance and mechanisms of cytoplasmic SRSF1 function, including those, which may dictate cellular viability.

## 195 Decreased expression of *sucA* gene by inactivation of RNase III activity through YmdB inhibits the biofilm formation and induces apramycin susceptibility

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Antibiotic resistance associated with biofilm formation is a major concern when treating bacterial infections with drugs. The genes and pathways involved in biofilm formation have been extensively studied and are also involved in antibiotic resistance. Recent studies show that overexpression of *E. coli* YmdB protein alters gene expression profiles and inhibits biofilm formation. Therefore, it is expected that YmdB and its regulated genes play a key role in development of biofilm and antibiotic resistance phenotypes. The present study screened antibiotics to identify those whose susceptibility profiles were regulated by YmdB levels. This protocol identified apramycin. Additional screening for genes negatively regulated by inactivation of RNase III activity via YmdB overexpression revealed that a gene associated with the tricarboxylic acid cycle gene, *sucA*, was necessary for the YmdB-like phenotype. Taken together, these data suggest that regulation of RNase III activity by trans-acting factors may be the key to identifying genes or pathways connecting biofilm and antibiotic resistance phenotypes. This information could be used to reduce the emergence of biofilm-associated multidrug-resistant bacteria.

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## 196 *In vivo* model of kinetic regulation of alternative splicing

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Virtually all mammalian RNA polymerase II (Pol II) genes undergo alternative splicing (AS) and hence express multiple mRNA isoforms. Importantly, changes in alternative isoform expression play an essential role in cellular differentiation and organism development. Interestingly, RNA processing, including AS occurs co-transcriptionally and depends on transcription elongation dynamics. The kinetic model of AS regulation proposes that the rate of Pol II elongation governs AS selection. In agreement, the use of a mutant form of Pol II with a lower elongation rate leads to increased inclusion of alternative cassette exons. This mutant is equivalent to the C4 point mutation, which was identified in the *Drosophila* Pol II largest subunit and resulted in non-lethal developmental defects in the heterozygous state, suggesting that the rate of transcriptional elongation could play a role in regulating development.

We have generated mouse embryonic stem cells (ESC) knocked-in for this slow Pol II mutant allele (R749H). Using RNA-seq we have identified transcriptome and splicing changes in these cells. The presence of a slow RNA Pol II resulted in gross changes in transcription and influenced AS, affecting both exon inclusion and intron retention. Mouse chimaeras derived from slow/slow ESCs fail to transmit through the male germ line suggesting that inappropriate processivity of RNA pol II could affect spermatogenesis. In addition introducing the R749H mutation into mouse embryos using the CRISPR/Cas9 system caused defects around the time of gastrulation.

To dissect how the slow Pol II mutation might be affecting transcription and AS during differentiation we have focused on neuronal development. Neural stem cells (NSCs) can be generated from ESCs in culture and have the ability to self-renew and to differentiate into all three neural cell types. We found that slow/slow ESC-derived NSCs lost self-renewal capacity and showed signs of premature terminal differentiation instead. Importantly, this was correlated with significant changes in the expression and splicing of genes important for NSC, including components of the Notch and the EGF pathway, among others. We will discuss possible scenarios linking the observed transcriptional and phenotypic changes in the light of the kinetic model of AS regulation.

## 197 Histone gene replacement reveals a post-transcriptional role for H3K36 in maintaining metazoan transcriptome fidelity

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Histone H3 lysine 36 methylation (H3K36me) is thought to participate in a host of co-transcriptional regulatory events. To study the function of this residue independent from the enzymes that modify it, we used a “histone replacement” system in *Drosophila* to generate a non-modifiable H3K36 lysine-to-arginine (H3K36R) mutant. We observed global dysregulation of mRNA levels in H3K36R animals that correlates with the incidence of H3K36me3. Similar to previous studies, we found that mutation of H3K36 also resulted in H4 hyperacetylation. However, neither cryptic transcription initiation, nor alternative pre-mRNA splicing, contributed to the observed changes in expression, challenging previously reported roles for H3K36me. Interestingly, knockdown of the RNA surveillance nuclease, Xrn1, and the deadenylase CCR4, restored mRNA levels for a class of downregulated, H3K36me3-rich genes. We propose a post-transcriptional role for modification of replication-dependent H3K36 in the control of metazoan gene expression.

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## 198 Toxic transcripts: synonymous mutations affect the fitness of *E. coli* through mRNA toxicity

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Evolutionary analyses suggest that synonymous mutations are under selection, but the fitness effects of such mutations have been difficult to measure experimentally. We used a library of synonymous coding-sequence variants of GFP to analyse the effects of synonymous mutations on the fitness of *Escherichia coli*. When over-expressed in the BL21-Gold(DE3)plysS *E. coli* strain, the GFP variants had a surprisingly broad range of toxic effects, ranging from a slight reduction in growth rate to massive degradation of ribosomes and cell death. These effects on growth were consistent in other *E. coli* strains that we tested. For one of the toxic GFP variants, we mapped the toxic phenotype to a 70bp region near the 3' end of the gene and established that a single synonymous change at some of the positions within this region was enough to rescue the toxic effect. The toxic effect on fitness was translation-independent and caused by mRNA itself. To understand the mechanism, we evolved suppressor strains that could tolerate expression of toxic GFP variants, and performed genome sequencing of 22 such strains. We found a cluster of mutations common to all suppressors within the promoter of T7 RNA polymerase gene which reduce mRNA levels of the toxic GFP constructs, thereby reducing toxicity. We reason that high amounts of toxic transcripts cannot be tolerated by the cells as they may be binding to or interacting with some essential proteins or regulatory RNAs, thereby inhibiting normal cell growth. Thus, we conclude that expression levels and the nucleotide sequence of the transcript jointly contribute to fitness.



## 199 Antagonistic regulation of alternative polyadenylation (APA) by SRSF3 and SRSF7 controls nuclear mRNA export and retention

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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. Using iCLIP and FRAC-Seq we discovered that SRSF3 and SRSF7 link alternative polyadenylation (APA) to mRNA export. SRSF3 recruits the nuclear export factor NXF1 preferentially to the 3' end of transcripts, where both proteins form a heterotrimeric complex with bound mRNA, and orchestrates splicing, 3' end formation and efficient export of transcripts with long 3'UTRs. When SRSF3 is limiting, either alternative terminal exons are included in the final transcripts via splicing, or the 3'UTRs are shortened by proximal APA. In both cases NXF1 binding sites disappear and the transcripts are retained in the nucleus. Interestingly, this regulation is antagonized by SRSF7, which binds preferentially around proximal poly(A)-sites and its depletion leads to the retention of mRNAs with extended 3'UTRs. We are currently investigating the underlying mechanisms and consequences of this regulation and we will discuss our findings at this meeting.

## 200 Dual RNA processing roles of Pat1b via cytoplasmic Lsm1-7 and nuclear Lsm2-8 complexes

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The conserved Pat1 family of RNA-binding proteins activate decapping of mRNA in complex with the Lsm1-7 heptamer ring, are key players in the 5' to 3' mRNA decay pathway and residents of P-bodies at steady-state, though recently shown to shuttle through nuclei<sup>1,2</sup>. The Lsm2-8 heptamer binds U6 snRNA, a di/tri-snRNP component, and functions in splicing<sup>3</sup>. Using mass spectrometry, co-immunoprecipitation and immunofluorescence approaches coupled with RNAi in HEK293T and HeLa cells, we provide evidence for a nuclear Pat1b-Lsm2-8 complex enriched in Cajal bodies, the site of snRNP biogenesis, in addition to the well-characterized cytoplasmic Pat1b-Lsm1-7 complex in P-bodies. Furthermore, we establish the novel set of interactions connecting Pat1b-Lsm2-8-U6 snRNA-SART3 and additional tri-snRNP components. RNAseq following Pat1b depletion, which disassembles P-bodies, revealed the preferential up-regulation of mRNAs encoding proteins involved in RNA metabolism, and containing 3' UTR AU-rich elements. Interestingly too, these Pat1b-sensitive transcripts are normally found concentrated in P-bodies. Evidence was also obtained for changes in >180 alternative splicing events, indicating that Pat1b promotes inclusion of cassette exons with weak donor sites and flanked by short and GC-rich introns. Our data demonstrate the unsuspected dual role of a decapping enhancer in pre-mRNA processing as well as in mRNA decay, via distinct nuclear and cytoplasmic Lsm heptamer rings.

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## 201 Mud2, a component of the U1 snRNP complex, functions in transcription

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Different steps in gene expression are intimately linked to coordinate and regulate this complex process. Specifically, already during transcription numerous RNA-binding proteins are loaded onto the mRNA and package the mRNA into a messenger ribonucleoprotein (mRNP). Interestingly, these RNA-binding proteins are often involved in more than one step. For example, TREX functions in transcription, mRNP packaging and nuclear mRNA export. Previously, we showed that the Prp19 splicing complex (Prp19C) is needed for efficient transcription as well as TREX complex occupancy at transcribed genes.

Here, we show that Mud2, which has a defined role in splicing, interacts with Prp19C and is needed for Prp19C occupancy in *S. cerevisiae*. Interestingly, Mud2 is not only recruited to intron-containing but also intronless genes indicating a role in transcription. Indeed, Mud2 is necessary for full transcriptional activity *in vivo* and *in vitro*. Taken together, we classify Mud2 as a novel transcription factor that is also necessary for the recruitment of mRNA-binding proteins to the transcription machinery. Thus, Mud2 is a multifunctional protein important for transcription, splicing and mRNP packaging.

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## 202 The 5' splice site-dependent suppression of pre-mature polyadenylation in plants

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Eukaryotic messenger RNAs are produced from primary transcripts as a result of extensive processing, including addition of the cap structure at the 5' end, splicing, and cleavage and polyadenylation at the 3' end of transcripts. The poly(A) tail protects mRNA, stimulates its export from the nucleus to the cytoplasm, and in the cytoplasm is important for translation initiation. Selection of alternative polyadenylation signals can lead to protein variants which may differ in their stability, translation properties, transport and in consequence subcellular localization. Interestingly, the interplay between sequences recognized by U1 snRNP and 3' end processing factors protects newly synthesized precursor mRNAs from premature termination caused by cleavage and polyadenylation at so called cryptic polyadenylation sites.

We show that in plants 5' splice sites protect miRNA gene primary transcripts (pri-miRNAs) from premature polyadenylation. This has been proved for both miRNA gene types: independent transcriptional units, as well those in which miRNAs are encoded within introns of protein-coding genes. Our observations indicate that in plants, similar to mammals, the presence of 5' splice sites that are recognized by U1 snRNP controls selection of the proper polyadenylation signal. In order to decipher the molecular mechanism of the involvement of U1 snRNP in selection of polyadenylation sites we carried out extensive studies on molecular interactions between U1 snRNP proteins and factors involved in polyadenylation. We discovered that AtPRP40, an Arabidopsis homolog of yeast auxiliary U1 snRNP protein PRP40, interacts with proteins of the plant polyadenylation machinery. Interestingly, AtPRP40 interacts also with the CTD domain of RNA polymerase II. Our results suggest that AtPRP40 can be involved in communication between U1 snRNP, the miRNA biogenesis machinery and probably also factors taking part in selection of poly(A) sites.

### 203 Distinct pairs of forkhead transcription factors control processing of pre-mRNAs from different genes during meiotic differentiation in fission yeast

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We are investigating mid-meiotic gene regulation in the fission yeast *Schizosaccharomyces pombe*. Whereas other laboratories showed that mRNA accumulation during differentiation parallels increased pre-mRNA splicing (Moldon *et al.*, *Nature*, 2008), our work revealed that the polyadenylation decision is more critical for ensuring that expression peaks at the appropriate time (Cremona *et al.*, *Mol. Biol. Cell.* 2011; Potter *et al.*, *RNA*, 2012). The *trans*-acting factors known to regulate mid-meiotic gene expression in *S. pombe* belong to the forkhead family, whose members also regulate countless developmental and metabolic pathways in metazoans. The constitutively expressed Fkh2 inhibits expression of some mid-meiotic genes during vegetative growth, while the meiosis-specific family member, Mei4, exerts positive control (e.g., Moldon *et al.*, 2005); however, the underlying mechanism(s) have remained elusive. We seek to understand the transition from negative to positive regulation in relation to RNA processing. Our analysis of recently published ChIP-Seq data (Alves-Rodriguez *et al.*, *Cell Reports*, 2016) led to the unexpected finding that many mid-meiotic genes bound by Mei4 during meiosis do not associate with Fkh2 in proliferating cells, counter to a model in which the positive regulator simply displaces the negative regulator. To determine the reason for this discrepancy, we analyzed processing of transcripts from two genes previously proposed to follow the accepted paradigm: *spo4* (encoding a protein kinase) and *rem1* (encoding a cyclin). Strikingly, their splicing and polyadenylation patterns over a meiotic time course in cells harboring a deletion of either the *fkh2* or *mei4* gene were quite distinct. As neither the arrangement of *cis*-acting signals nor the production of anti-sense transcripts could explain the discrepancy, we tested the possibility that other forkhead factors might participate. The results indicated that, whereas the mid-meiotic pattern of *rem1* splicing, polyadenylation and RNA accumulation could be recapitulated in proliferating cells by simultaneously eliminating Fkh2 and overexpressing Mei4, this was not true for *spo4*. For this gene, recreating meiotic regulation in non-meiotic cells required elimination of a different forkhead family member, Fhl1. As forkhead factors were not previously known to control RNA processing decisions, our work may provide insight into their regulatory mechanisms in metazoans.

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### 204 Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by CircPABPN1

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HuR influences gene expression programs and hence cellular phenotypes by binding to hundreds of coding and noncoding linear RNAs. However, whether HuR binds to circular RNAs (circRNAs) and impacts on their function is unknown. Here, we have identified en masse circRNAs binding HuR in human cervical carcinoma HeLa cells. One of the most prominent HuR target circRNAs was hsa\_circ\_0031288, renamed CircPABPN1 as it arises from the PABPN1 pre-mRNA. Further analyses revealed that HuR did not influence CircPABPN1 abundance; interestingly, however, high levels of CircPABPN1 suppressed HuR binding to PABPN1 mRNA. Evaluation of PABPN1 mRNA polysomes revealed that PABPN1 translation was modulated positively by HuR and hence negatively by CircPABPN1. We propose that the extensive binding of CircPABPN1 to HuR prevents HuR binding to PABPN1 mRNA and lowers PABPN1 translation, providing the first example of competition between a circRNA and its cognate mRNA for an RBP that affects translation.

## 205 Targeting long non-coding MALAT1 triple helix with small molecules

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Human metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a long noncoding RNA (lncRNA) overexpressed in many cancers and whose upregulation promotes increased cell migration and metastasis. The enhancement of oncogenic processes by MALAT1 in cancers has been associated with its unusual 3'-end sequence. MALAT1 terminates with a highly-conserved sequence comprising of two U-rich motifs (ENE-like structure) and a 3'-genomically-encoded A-rich tract. A recent crystal structure of MALAT1 revealed that the ENE restricts accessibility of the 3'-A-rich tract by triple helix formation. This triplex in turn protects MALAT1 from deadenylation-dependent decay leading to its accumulation in the nucleus. More recently, it was shown that METTL16, an RNA methyltransferase complex, recognizes MALAT1 triplex and binds to it. In an effort to identify small molecules able to recognize MALAT1 triplex, we utilized a fluorescently-labeled 59-nt MALAT1 construct and screened it with about 26,000 small molecules using our small molecule microarray platform. After statistical analysis and visual evaluation, 30 hits were identified and purchased. Herein we report our studies toward characterization of these hits and their mode of interaction using a suite of biochemical and biophysical techniques.

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## 206 A collection of inducible non-coding RNA overexpression plasmids for non-coding RNA phenotypic and functional analysis in yeast

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A number of non-coding RNAs (ncRNAs) have been ascribed a function but there are still thousands of ncRNAs whose functions are unknown. We are interested in elucidating the function of two novel classes of ncRNAs in the yeast *S. cerevisiae*: Cryptic Unstable Transcripts (CUTs) and Stable Unannotated Transcripts (SUTs). As many CUTs and SUTs overlap protein coding genes a method is required to ascertain their function without perturbing the overlapping gene. Therefore, the sequence for all CUTs and SUTs (approximately 1800) have been cloned downstream of the inducible GAL1/10 promotor on a plasmid. These plasmids have been transformed into the reference MATa yeast strain BY4741 to produce a resource of approximately 1800 yeast strains, each overexpressing an individual ncRNA. These strains have been arrayed in plates to allow robotic printing onto galactose plates under different conditions to identify growth defects by colony size over time by computerised imaging. In addition, we will also assess this resource by combining individual ncRNA overexpression plasmid strains with one viable query protein deletion strain to search for novel gene networks by synthetic dosage lethal (SDL) analysis (Douglas et al., 2012; Sopko et al., 2006). Overexpression fitness phenotypes may be revealed in combination with viable deletion mutations when no phenotype is present in the wild-type background. The query mutation in a MAT $\alpha$  strain with the mating type specific reporter (MFA1pr-HIS3) is crossed to the array of ncRNA overexpression plasmids in the MATa strain. Diploids are selected and sporulated. MATa haploids with the query mutation, overexpression plasmid and mating type specific marker are then selected. We hope that this resource will be a valuable tool for the large scale analysis of ncRNA function.

Douglas et al. (2012). Functional analysis with a barcoder yeast gene overexpression system. *G3* 2, 1279-1289.

Sopko et al. (2006). Mapping pathways and phenotypes by systematic gene overexpression. *Molecular Cell* 21, 319-330.



## 207 Circular RNAs in cancer

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One of the best-characterised circRNAs, discovered in 1993, is produced from the Sex-determining gene (*SRY*) in mouse. The function of this circRNA is not clear, but, it is localised in the cytoplasm and is not associated with ribosomes. Here, we have shown that *SRY*-box2 (*SOX2*) mRNA forms circRNA in melanoma and breast cancer cells. *CircSOX2* is derived from the 3' UTR of *SOX2* and is localised in the nucleus. We have also shown that *SOX2OT*, an overlapping transcript to *SOX2*, forms circRNA, that it includes two exons and that, similar to linear *SOX2OT*, it is localised in the nucleus. Expression analysis of more than 45 melanoma and breast cancer cell lines confirmed that expression of circular and linear transcripts is not correlated. However, there are strong positive correlations between linear *SOX2* and *SOX2OT* expression in all cell types. We also found both linear and circular *SOX2OT* are upregulated in breast cancer cell lines upon drug treatment.

In breast cancer cell lines, we have shown that the lncRNA *ZFAS1* forms novel circular RNA. Interestingly we have identified different isoforms of *circZFAS1* bound to monosome, light and heavy polysomes in the cytoplasm similar to their linear counterparts. We have also found different species of *circZFAS1* in leukemia cell lines compared to breast cancer cells. This suggests different functions for the various isoforms of *circZFAS1* in the two cancer types.

In melanoma cell lines, we have identified several known and novel isoforms of the circRNAs processed from *ANRIL*. We have found the expression of more than fifty different isoforms of *circANRIL* in melanoma cell lines. Examination of the subcellular localisation of linear and circular forms of *ANRIL* has revealed that linear forms are found exclusively in the nuclear fraction, whereas *circANRIL* is found in the cytoplasm, suggesting distinct functions for these two RNA species.

In summary, we have identified novel forms of circRNAs in several different cancer types and are now investigating their function.

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## 208 Influence of RNA Secondary Structure on the Function of the lncRNA SAMMSON

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SAMMSON, also known as survival associated mitochondrial melanoma-specific oncogenic noncoding RNA, is a long-noncoding RNA expressed only in malignant melanoma cell lines and metastatic melanoma tissue samples. In Leucci et al (2016), it was found that upon depletion of SAMMSON, melanoma cells undergo apoptosis, suggesting that this lncRNA is essential for melanoma cell survival. It is crucial to find a cure for this cancer as the incidence rate of melanoma has doubled in the past few decades. Because melanoma cells are addicted to SAMMSON, this lncRNA is an attractive avenue for the pursuit of small molecule melanoma therapies. Very little is known about SAMMSON, though studies have shown that SAMMSON interacts with the mitochondrial protein p32, which is necessary for proper mitochondrial maintenance. The emerging field of lncRNA biology has revealed that lncRNAs are implicated in a multitude of cellular processes, but the structures of very few lncRNAs are known. Among known structures, those found within the lncRNA are necessary for the RNA to carry out its function. Using in vivo structure probing, we will solve the secondary structure of SAMMSON and elucidate necessary structural features for SAMMSON interaction with p32.

## 209 The long non-coding RNA lnc-31 controls Rock1 expression coordinating cell cycle exit and differentiation

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Myogenesis is a complex process regulated by protein factors acting in concert with non coding RNAs such as microRNAs (miRNAs) and long non coding RNAs (lncRNAs). Transcriptome analysis performed during in vitro murine myoblast differentiation allowed the identification of new lncRNAs differentially expressed along myogenesis. Among them, we focused on lnc-31 that contains, in the third exon, the precursor of miR-31. lnc-31 is highly expressed in proliferating myoblasts, it is localised in the cytoplasm and its expression has been recently shown to sustain myoblasts proliferation. Transcriptome analysis, upon lnc-31 knock-down, shows a down-regulation of cell cycle promoters and, at the same time, the up-regulation of genes promoting differentiation. This finding reinforces the indication of lnc-31 promoting myoblasts proliferation and add new clues on the role of this lncRNA in coordinating cell cycle exit and differentiation. In order to molecularly dissect the mode of action of lnc-31, we characterized its protein and mRNA interactors. We found that lnc-31 interacts with the RNA/DNA binding protein Ybx1 and Rock1 mRNA. UV-CLIP using antibody against Ybx1 confirmed the binding to lnc-31. Furthermore, bioinformatic analysis reveals the presence of a putative binding region between the third exon of lnc-31 and the 5'UTR of Rock1 mRNA. Luciferase assay, using the 5'UTR of Rock1 fused with Renilla Luciferase, showed that the overexpression of lnc-31 caused an upregulation of luciferase activity and this effect was abolished when the putative binding region was deleted from the UTR. These results confirm the bioinformatics prediction and demonstrate that lnc-31 is able to positively control Rock1 expression. Notably, knock-down experiments showed that, in proliferating myoblasts, both lnc-31 and Ybx1 are required for controlling Rock1 expression at post-transcriptional level, possibly facilitating its translation. Since Rock1 has been described as negative regulator of myogenesis by preventing the exit of myoblasts from the cell cycle, our data allow us to suggest that the lnc-31-Ybx1 complex may promote myoblast proliferation favouring the translation of Rock1 mRNA; upon differentiation, when lnc-31 is down-regulated, Rock1 protein levels decrease allowing myoblasts to exit from the cell cycle and enter into the myogenic program.

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## 210 The diverse roles of long noncoding RNAs in the p53 tumor suppressor pathway

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We investigate how long noncoding RNAs (lncRNAs), transcriptionally induced by the key tumor suppressor factor p53, modulate the p53-dependent responses to stress. By combining p53 ChIP-seq and RNA-seq analyses in the context of a p53 pathway activated by oncogenic stress, we identified a set of p53-regulated lncRNAs. Functional and mechanistic studies revealed that these lncRNAs act through diverse mechanisms to add important layers of regulation to the p53 tumor suppressor network. On the one hand, we found that a subset of the newly identified lncRNAs appear to act *in cis* to transcriptionally modulate the expression of neighboring genes, many of which are themselves key components of the p53 transcriptional network, including Cdkn1a/p21, Gadd45g, and Zmat3. On the other hand, we found that a lncRNA, which we have named lncRNA-RAPT1 (RNA Activator of p53 Translation 1), acts *in trans*. lncRNA-RAPT1 is a direct target of p53 and is preferentially induced under conditions of senescence. We have generated a conditional knockout of lncRNA-RAPT1 in the mouse and found that its absence impairs the induction of senescence. Moreover, by gene expression profiling, we observed that the absence of lncRNA-RAPT1 leads to a downregulation of a subset of p53 target genes, suggesting a global role in the p53 pathway. Surprisingly, we found that lncRNA-RAPT1 is exported to the cytoplasm, where it promotes the translational of p53 itself. We are currently investigating the significance of this lncRNA-mediated translational input.

**211 Epigenetic switch of a macrosatellite repeat in colorectal cancer**

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Colorectal cancer is a heterogeneous disease characterized by a complex interplay between genetic and epigenetic alterations. Global DNA hypomethylation has been associated to genomic instability, a hallmark of cancer, however the mechanisms linking DNA hypomethylation and chromosomal aberrations are not completely clear. We have identified a macrosatellite repeat frequently demethylated in colorectal tumors; this demethylation is accompanied by changes in histone marks and transcriptional upregulation, leading to accumulation non-coding transcripts. We are studying whether these ncRNAs could play a role in the oncogenic process, and we also aim to identify contributors to maintain these macrosatellite repeats silenced in normal conditions. Epigenetic modifications in other macrosatellites have been associated to different diseases, however the molecular underpinnings remain poorly understood. Our studies could shed new light on how a particular macrosatellite may contribute to colorectal and other cancers, and increase our knowledge on the epigenetic control of an enigmatic part of the human genome.

**212 Functional profiling and genetic interaction map of non-coding RNAs in yeast**

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**Background:** An open reading frame (ORF) deletion collection in the yeast *Saccharomyces cerevisiae* has been proven to be useful for functional genomics analyses. There is now the need to uncover the molecular function of non-coding RNAs (ncRNAs) and understand the genetic interactions between them. Recently we have created a collection of ~450 ncRNA knock-out (KO) mutants in the MAT $\alpha$  background that were used for analysis in this study.

**Aims:** The aim of this project is to create a genetic interaction map of ncRNAs in *S. cerevisiae* by (i) fitness analysis of ncRNAs double KO mutants generated using Synthetic Genetic Array (SGA) and (ii) screening the double KO library in different nutritional context and stress conditions.

**Methods:** Double KO mutants were generated by crossing a MAT $\alpha$  query strain (carrying single gene deletion) with the MAT $\alpha$  ncRNA KO collection using SGA. After SGA sizes of the single and double KO mutants were recorded. Analysis of double vs single KOs was performed using the 'R' package. Generated negative and positive scores that represented gain or loss of fitness were used to generate scatter-plots and genetic network. Fitness analysis is being performed in various conditions.

**Results:** To date, ~100 query strains have been generated carrying deletions in snRNAs, SUTs, CUTs and tRNAs. Twenty seven have been used in SGA to generate double mutations. Several gene interactions have been discovered showing either loss or gain in fitness, such as for example  $\Delta$ SUT193- $\Delta$ SUT055 or  $\Delta$ SNR13- $\Delta$ SUT347. Genetic networks for discovered interactions will be presented as well as fitness results of double KOs.

**Conclusions:** The SGA analysis on a small number of query strains (27) showed a number of positive and negative genetic interactions, with a number being lethal. Phenotypic analysis of double mutants will help us determine the function of ncRNAs.

### 213 A senescence-specific lncRNA modulates the pro-inflammatory response of senescent cells

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Senescence is a condition of permanent growth arrest caused by different potentially oncogenic insults. Beside the effect on cell proliferation, during senescence many pro-inflammatory factors, collectively known as senescence-associated secretory phenotype (SASP), are produced to activate the immune response and reinforce senescence in a paracrine and autocrine manner. While the role of many protein factors in senescence is well established, only recently the involvement of non-coding transcripts (ncRNAs) in this process is emerging.

In the last years, genome-wide analysis demonstrated that the eukaryotic genome is pervasively transcribed and that the majority of the transcripts do not encode proteins. Among them, long non-coding RNAs (lncRNAs) refer to non-coding transcripts generally longer than 200 nucleotides with a crucial role in several biological processes being able to regulate almost every level of gene expression.

To functionally characterize novel lncRNAs able to modulate cellular senescence, we took advantage of an in vitro cellular system resembling senescence response through the activation of H-RAS, a process called oncogene-induced senescence (OIS). We evaluated the expression profile of primary human fibroblasts during OIS and we identified a robust set of 30 lncRNAs significantly induced in senescence. Among them, we found that an intronic, antisense lncRNA, thereafter named senescence-induced lncRNA (sin-lncRNA), is upregulated when senescence is induced in different ways. Indeed, sin-lncRNA expression is highly specific of senescent cells and it's driven by one of the master regulators of senescence, C/EBP $\beta$ , that directly activates sin-lncRNA transcription. Interestingly, despite its strong induction in senescence, sin-lncRNA functions as negative regulator of senescence response since its depletion increases growth arrest and promotes senescence features without affecting apoptosis. Genome-wide analysis of senescent cells depleted of sin-lncRNA revealed that the absence of this transcript increases the expression of several genes involved in matrix remodelling and recruitment of immune cells, suggesting that sin-lncRNA may have a role in controlling the pro-inflammatory response in senescence.

Sin-lncRNA is mainly enriched in the cytoplasm and our current focus is the elucidation of the molecular mechanisms by which this transcript exerts its function, in order to provide new insights on the regulatory network linking lncRNAs and senescence response.

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### 214 Withdrawn



## 215 Lobe-less, a long noncoding RNA that regulates *Drosophila* mushroom body morphogenesis

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In eukaryotes, thousands of long noncoding RNAs (lncRNAs) are transcribed from almost every part of the genome and are involved in multiple steps of gene regulation, including epigenetic transcriptional control. Although many of lncRNAs are predominantly expressed in the brain and other neural tissue, their physiological roles are largely unknown. Here we show that *Drosophila* Lobe-less (LOL) RNA is essential for mushroom body (MB) development, which is essential for learning and memory in insects. In *lol* mutants, mushroom body lacks their vertical lobes, an essential part of conditioned memory formation. To reveal LOL RNA functions in MB development, we performed mosaic analysis with a repressible cell marker (MARCM) that illuminates single cell morphology of mutant neurons. We found that vertical axon branches of all three types of MB neurons were misdirected to the horizontal extension in *lol* mutants, suggesting that LOL RNA is essential for axon guidance. RNA-seq analyses showed that expression of huge number of genes were affected by the *lol* mutation during embryogenesis and larval stage and adult heads in *lol* mutants. Genetic analysis indicates that *lol* strongly interacts with *Polycomb Group* genes encoding PRC1 chromatin modifier components. These results demonstrate an essential role of a long ncRNA in neural circuit formation in the *Drosophila* brain.

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## 216 Long non coding RNAs as New Paradigm for Lung Cancer Pathogenesis

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Worldwide lung cancer is the most commonly diagnosed cancer, and in India, it is the most common cause of cancer related death in males. The disease is frequently diagnosed at advanced stages, resulting in an overall 5-year survival rate of less than 15%. Conversely, if diagnosed in the early stages of the disease and thereafter, receiving effective treatments, the 5-year survival rate could possibly be increased to 85%. Moreover, the current diagnostic measures such as the chest X-ray and sputum cytology for early detection of lung cancer are very low. Therefore, taking advantage of recent developments in molecular genetics for the diagnosis and prognosis of lung cancer is clinically important. Currently, numerous tumor-specific molecular alterations have been identified in plasma and shown their potential as biomarkers in patients with lung cancers. However, none of the tested markers thus far had sufficiently achieved the required characteristics for the diagnosis of lung cancer.

In this regard, we are investigating the role and expression of Long non coding RNAs in lung cancer patients. Long non-coding RNA represents stable and reproducible markers for numerous solid tumors, including lung cancer, and has been hypothesized as non-invasive diagnostic markers for the several cancers. We have observed significant change in expression level of some long non-coding in lung cancer patients compared to healthy controls. We have also observed the altered expression of long non-coding RNAs in patients undergoing chemo and radiotherapy treatments. Detail results will be discussed at the time of presentation.

## 217 High-throughput annotation of full-length long noncoding RNAs with Capture Long-Read Sequencing (CLS)

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Accurate annotations of genes and their transcripts is a foundation of genomics, but no annotation technique presently combines throughput and accuracy. As a result, current reference gene collections remain far from complete: many genes models are fragmentary, while thousands more remain uncatalogued-particularly for long non coding RNAs (lncRNAs). To accelerate lncRNA annotation, the GENCODE consortium has developed RNA Capture Long Seq (CLS), combining targeted RNA capture with third generation long-read sequencing. We present an experimental re-annotation of the entire GENCODE intergenic lncRNA population in matched human and mouse tissues. CLS approximately doubles the annotated complexity of targeted loci, in terms of validated splice junctions and transcript models. The full-length transcript models produced by CLS enable us to definitively characterize the genomic features of lncRNAs, including promoter- and gene-structure, and protein-coding potential. Thus CLS removes a longstanding bottleneck of transcriptome annotation, generating manual-quality full-length transcript models at high-throughput scales.

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## 218 Roles of Introns in Long Non-Coding RNAs

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In the last years, long non-coding RNAs (lncRNAs) have become one of the leading topics of RNA research because of versatile cellular functions. Despite their low protein-coding potential, many lncRNAs undergo the same maturation pathway as protein-coding mRNAs including capping, splicing and/or polyadenylation. This project is focused on the activating type of long non-coding RNAs (lncRNA-a) which were shown to regulate expression of neighboring protein-coding target genes. However, the exact molecular mechanism how the lncRNA-a modulates the expression of target genes is unclear. Bioinformatic studies using transcriptomic data showed that, in general, lncRNAs are less efficiently spliced than mRNAs of protein-coding genes. Therefore, we hypothesize that lncRNA splicing can play an important role in lncRNA-a function. We have examined splicing efficiencies of two lncRNA-as and showed that these lncRNA-as are less efficiently spliced than pre-mRNAs transcribed from protein-coding genes. In addition, our data show that chromatin fraction contains preferentially unspliced lncRNA-as, which suggests that unspliced lncRNA-as are the active form in transcription regulation. First, we analyzed *cis*-factors that inhibit lncRNA-a2 splicing and discovered that intronic sequences are major determinants of inefficient splicing. To better understand splicing regulation of lncRNA-as we prepared several intron deletion mutants. Surprisingly, though lncRNA-a2 is spliced inefficiently, we have found intron splicing enhancers rather than silencers. Insertion of artificial intron splicing enhancers into intron sequence of lncRNA-a2 further enhanced splicing efficiency. Using bioinformatic analysis, we observed a correlation between number of thymidines in the polypyrimidine tract and splicing efficiency of lncRNAs. Direct experimental validation of this correlation confirmed that increased number of thymidines in polypyrimidine tract significantly improves splicing efficiency of lncRNA-a2. Together, our results suggest that inefficient splicing of lncRNAs is combined effect of lack of splicing enhancers and weak polypyrimidine tract. Finally, we showed that the deletion of the lncRNA-a2 intron at genomic level did not affect the expression of target genes regulated by lncRNA-a2, suggesting that introns are not essential for regulatory function.

## 219 Developmental programming by conserved lncRNA-TF pairs during the induction of the embryonic Heart

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Embryonic organogenesis requires precise timing, patterning and coordinated activity of core transcriptional networks that drive developmental progression. Understanding the mechanisms by which these transcriptional networks program developmental decisions is pivotal in devising ways to engineer specialized cell types or to re-engineer them for regenerative repair. Yet, our knowledge on the mechanisms that synchronize developmental progression of a tissue/organ is far from complete. Using a near synchronous human pluripotent stem cell based differentiation system recapitulating embryonic cardiac development, we discovered that a significant proportion of the key transcription factors (TF) governing cardiac developmental cell-fate decisions are accompanied by lncRNA. This phenomenon is evolutionarily conserved in mammals. Importantly, they are chromatin associated, poly adenylated and robustly transcribed. Interestingly, these lncRNAs associate with the promoter regions of key cardiac TFs and regulate their expression. These lncRNAs regulate the promoters of developmental TFs by forming specific RNA:DNA triple helical structures. Comprehensive loss of function analysis revealed that these lncRNA transcripts are essential for the developmental transition at which they are expressed. In addition we identified protein interacting partners of these lncRNAs, revealing further insights on the molecular mechanism by which this specific class of lncRNAs operate. Based on our data, we present a model where proximal lncRNAs enable the timely expression of developmental genes by facilitating the essential chromatin environment allowing for precision in embryonic cell-fate decisions. Together, we describe a regulatory layer in embryogenesis where lncRNA shape the developmental transcriptional code that programs cell-fate decisions.

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## 220 Structural analyses of human and mouse NEAT1 lncRNAs suggest long-range RNA interactions contribute to paraspeckle architecture

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In the past decade, lncRNAs have been increasingly recognized as important regulators of gene expression. Because they don't encode proteins, lncRNA structures and protein interactions are believed to be important for their functions. One relatively abundant lncRNA, NEAT1, functions in the formation of paraspeckles - nuclear bodies that have been implicated in multiple stress responses and diseases. NEAT1 has two isoforms produced by alternative 3' end processing. The short isoform (NEAT1\_v1) is 3.7 kb and contains a polyA tail, while the long isoform (NEAT1\_v2) is 23 kb in length and has a 3' end produced by RNase P cleavage. Though the NEAT1 primary sequence is not well conserved, the two isoforms and their function in paraspeckle formation were observed in both human and mouse cells. NEAT1 has a highly ordered spatial organization within the paraspeckle, in which NEAT1\_v1 and both the 5' and 3' ends of NEAT1\_v2 are localized to the periphery. The central sequences of NEAT1\_v2 are found within the core<sup>1</sup>. The structural features that maintain this spatial organization remain unknown, however it was suggested that interactions among proteins bound to NEAT1 may help maintain paraspeckle structure<sup>1</sup>.

We combined experimental RNA secondary structure probing (Mod-seq<sup>2</sup>) and computational structural analyses to investigate the structural features of NEAT1. The full length human and mouse NEAT1\_v1 structures were probed using SHAPE and Mod-seq. By comparing the structures of human and mouse NEAT1, we identified conserved structural features. Our results indicate NEAT1\_v1 is highly folded, and several regions have specific local secondary structural signatures conserved between human and mouse. Further computational analyses suggest that the 5' end of NEAT1\_v2 (or NEAT1\_v1) and the 3' end of NEAT1\_v2 form long-range base pairs with each other, which may contribute to the co-localization of NEAT1 5' and 3' ends. This interaction was verified *in vitro* using an RNA intermolecular gel-shift assay. Our results suggest that the secondary structure of NEAT1 and the long-range interactions among NEAT1 transcripts may have important architectural functions in paraspeckle formation.

## 221 Long Noncoding RNA MALAT1 Promotes Hepatocellular Carcinoma Development by SRSF1 Upregulation and mTOR Activation

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Transcriptome analysis of human genome has identified numerous RNAs that do not code for proteins. These non-coding RNAs are either small (sncRNA) or long (lncRNA). Substantial amount of information is available about SncRNA but very little is known about lncRNA.

Several long noncoding RNAs (lncRNA) are abrogated in cancer but their precise contributions to oncogenesis are still emerging. Here we report that the lncRNA MALAT1 is upregulated in hepatocellular carcinoma and acts as a proto-oncogene through Wnt pathway activation and induction of the oncogenic splicing factor SRSF1. RNA-seq analysis on PHM-1 cells overexpressing MALAT1 revealed that MALAT1 induced upstream activators of the Wnt pathway (e.g., both ligands Wnt2b and Wnt10a, as well as the receptor, Frizzled, which activates the pathway). In addition, several suppressors of the pathway were downregulated (e.g., DKK, TGFb, TGFbR). MALAT1 activates a transcriptional program, resulting in activation of the Wnt and ERBB3-4 signaling pathways and increased expression of c-MYC and cyclin D1. Induction of SRSF1 by MALAT1 modulates SRSF1 splicing targets, enhancing the production of antiapoptotic splicing isoforms and activating the mTOR pathway by modulating the alternative splicing of S6K1. Inhibition of SRSF1 expression or mTOR activity abolishes the oncogenic properties of MALAT1, suggesting that SRSF1 induction and mTOR activation are essential for MALAT1-induced transformation. Our results reveal a mechanism by which lncRNA MALAT1 acts as a proto-oncogene in hepatocellular carcinoma, modulating oncogenic alternative splicing through SRSF1 upregulation.

## 222 A cytoplasmic long non-coding RNA can regulate skeletal muscle differentiation through mRNA translation modulation

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Skeletal muscle differentiation is a highly regulated developmental process in which gene expression is finely modulated through different species of non-coding RNAs. Among them, a novel cytoplasmic long non-coding RNA has a relevant role in promoting murine C2C12 myoblast *in vitro* differentiation. The characterization of its molecular interactome is unveiling a complex network of mRNA, microRNA and protein interactors that suggests an interesting function in regulation of translation, through the recruitment of a RNA helicase. In particular, experimental evidences suggest that this long non-coding RNA is able to directly interact with the mRNA of a myogenesis-regulating transcription factor, thus modulating its translation efficiency.

Moreover, the long non-coding RNA contains a small open reading frame, and polysome profiling indicates its association with the ribosomal machinery, which could have a role in its molecular function. A high-throughput approach based on Ribosome Profiling has been set up in order to investigate the impact of this long non-coding RNA on translation of specific mRNAs. Furthermore, Ribosome Profiling suggests that the small open reading frame embedded in this transcript is actively translated. Taken together, these evidences show that this long non-coding RNA could be a key factor in post-transcriptional gene regulation during the early phases of myogenesis.



## 223 Alterations in the expression levels of two antisense long non-coding RNAs overlapping the *UGT73C6* gene of *Arabidopsis thaliana* result in changes in leaf area

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Long non-coding RNAs (lncRNAs) have been shown to be important modulators of gene expression in eukaryotes. In plants, lncRNAs are involved in a wide range of biological processes including flowering time regulation, root development, and hormone and stress responses. Our research focuses on a particular sub-type of lncRNAs that are transcribed from the opposite DNA strand of protein-coding genes, called natural antisense long non-coding RNAs (NAT-lncRNAs). Using the model organism *Arabidopsis thaliana*, we investigate the regulatory potential of two NAT-lncRNAs which overlap an UDP-glycosyltransferase gene (*UGT73C6*), referred as *NAT1*- and *NAT2-UGT73C6*. It has been previously described that *UGT73C6* has a key role in plant development by inactivating the plant hormone brassinosteroid (BR). Reporter gene lines fusing the promoter region of *NAT1*- and *NAT2-UGT73C6* indicate independent promoter activity in roots and shoots, respectively. Analysis of *NAT1*- and *NAT2-UGT73C6* transcripts showed that they are stable and spatiotemporally co-expressed with *UGT73C6* and other members of the *UGT73C* subfamily. Overexpression or down-regulation of each NAT-lncRNA significantly affects the overall leaf area, whereas other developmental processes, including flowering time and seed yield are not affected. However, the observed phenotypes do not seem to correlate with transcript levels of the overlapping protein-coding gene. Additionally, *NAT1*- and *NAT2-UGT73C6*, like *UGT73C6* and its closest homologue *UGT73C5*, remain un-responsive to BR treatment. Transient expression assays in *N. benthamiana* revealed translation of small open reading frames present in the *NAT2-UGT73C6* sequence, and that one of the produced peptides exhibits nuclear localization. In order to understand the molecular processes underlining changes in the leaf area due to variations in *NAT1*- and *NAT2-UGT73C6* abundance, we intend to investigate, in addition to the function of the RNA molecules, the potential role of short peptides encoded by the NAT-lncRNAs.

## 224 A resource for functional profiling of noncoding RNA in the yeast *Saccharomyces cerevisiae*

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Eukaryotic genomes are extensively transcribed generating many different RNAs with no known function. We have constructed 1779 molecular barcoded ncRNA gene deletion strains in the yeast *Saccharomyces cerevisiae* as tools for ncRNA functional analysis. This resource includes deletions of annotated ncRNAs as well as the stable unannotated transcripts (SUTs) and cryptic unstable transcripts (CUTs) whose functions are largely unknown. Fitness profiling of these deletion strains in different growth conditions and phases revealed many environmental-specific haploinsufficient and haploproficient phenotypes providing novel information on the importance of specific ncRNAs in each condition. Co-fitness analysis identified two ncRNA groups required for growth during heat stress and nutrient deprivation. Four new essential ncRNAs were identified with their deletion either repressing or inducing adjacent gene expression. Specifically, we discovered a function for SUT527 in the expression, 3' end formation and localisation of *SEC4*, an essential protein coding mRNA. Overall, our findings provide new insights into the function of ncRNAs and constitute a valuable resource for large-scale phenotypic analysis and future ncRNA research.

## 225 Role of antisense long non-coding RNAs in breast cancer invasion and metastasis

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Despite the significant improvements in cancer therapies over the past decades, metastatic solid cancers remain largely incurable. In this way, finding alternative strategies to prevent the invasion and tissue colonization of cancer cells are a future requirement in cancer treatment and patient welfare.

Recent large-scale transcriptome analyses have revealed that the human genome contains more than just protein-coding genes. Indeed, a large number of transcripts, including long non-coding RNAs (lncRNAs), lack protein-coding capacity. Increasing evidence suggests that lncRNAs could have a critical role in the regulation of diverse cellular processes, such as stem cell pluripotency, development, cell growth and apoptosis, and cancer invasion and/or metastasis. Antisense lncRNAs (lncRNAs transcribed from the opposite strand of coding or non-coding genes) have been recognized to regulate the expression of their corresponding genes at the transcription and/or post-transcriptional levels, therefore potentially participating in carcinogenesis by regulating oncogenes or tumor-suppressor genes.

To investigate the impact of antisense lncRNAs in the metastatic potential of breast cancer cells, we depleted specific sense and/or antisense transcripts through the use of strand-specific antisense oligonucleotides (LNAs Gapmers, Exiqon) in breast cell lines with different characteristics – MCF7 (luminal A ER<sup>+</sup>/PR<sup>+</sup>), MDA-MB-231 (triple negative mesenchymal-like cells) and the non-tumorigenic MCF10a cell line. Further *in vitro* assays were conducted to determine the role of the identified lncRNAs in the growth rate (resazurin-based assay) and capacity of these cells to migrate (wound healing assay). Gene expression analysis from total RNA after inhibition of the specific lncRNAs were also carried to evaluate alterations in the metastatic phenotype of the breast cancer cells. Finally, the capacity of these cells to migrate and colonize the lung of an immunodeficient mouse model (NSG) was assessed *in vivo*.

Our data confirmed that the downregulation of specific antisense lncRNAs led to a decrease of the growth rate and metastatic capacity of aggressive breast cancer subtypes. Altogether, our findings reveal antisense lncRNAs as novel therapeutic targets for breast tumors of different origins.

## 226 Function of the long noncoding RNA, WNT1-inducible signaling pathway protein 1-overlapping transcript 2 (WISP1-OT2) in human renal cells

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**Background:** The alternative transcript WISP1-OT2 (WNT1 inducible signaling pathway protein 1-overlapping transcript 2) was discovered upon treatment of primary culture of renal proximal tubule epithelial cells (RPTEC) with the ubiquitous nephrotoxic mycotoxin, ochratoxin A (OTA). WISP1-OT2 is a long noncoding RNA, 2922 nucleotides long, and transcribed from the end of WISP1 gene. We were able to detect WISP1-OT2 in healthy renal tissue and we observed an upregulation in clear cell renal cell carcinoma. WISP1-OT2 is located predominantly in the nucleus; however, its function is unknown.

**Aims:** (i) Determine the orientation of WISP1-OT2; (ii) investigate possible functional link between long noncoding RNA and mRNA of WISP1 gene; (iii) evaluate the influence of WISP1-OT2 knockdown on the cell function.

**Methods:** Orientation of WISP1-OT2 was investigated by gene-specific reverse transcription and subsequent PCR. To test an inhibitory effect of WISP1-OT2 on WISP1 mRNA, HEK293T cells were treated with PMA (Phorbol myristate acetate) or OTA alone, or in combination, in the presence or absence of antisense LNA<sup>TM</sup> GapmeRs (hybrid DNA:RNA oligonucleotides) that enter the nucleus and degrade target RNA, i.e. WISP1-OT2. Subsequently, WISP1-OT2 and WISP1 mRNA expression was detected by RT-PCR. To investigate the importance of WISP1-OT2 for gene expression, RNA-seq data were generated in the absence and presence of antisense oligonucleotides against WISP1-OT2. To obtain cellular glycolytic flux, we measured glucose consumption and lactate production. The impact on cell death was determined by caspase-III activation and LDH release.

**Results:** WISP1-OT2 is antisense-oriented long noncoding RNA. PMA induces specifically WISP1 mRNA, whereas OTA induces specifically WISP1-OT2. In the presence of PMA and OTA, WISP1 mRNA is significantly reduced when compared to PMA effect alone. This inhibitory effect of OTA on the WISP1 mRNA is not mediated by WISP1-OT2. WISP1-OT2 modulates cellular energy metabolism; in its absence cells shift from the TCA cycle to aerobic glycolysis. WISP1-OT2 acts anti-apoptotic, possibly via mitochondrial protection. Additionally, it upregulates growth-arrest specific-6 (Gas6) gene, implicated in cell survival.

**Conclusions:** (i) WISP1-OT2 is the long noncoding RNA, transcribed in antisense orientation relative to WISP1 gene; (ii) WISP1-OT2 does not influence the expression of WISP1 mRNA; (iii) WISP1-OT2 acts anti-apoptotic.

## 227 Analysis of the evolutionally preserved function of retrotransposon, SINE, and its therapeutic application

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Short interspersed nuclear elements (SINEs), one of retro transposable elements, are broadly distributed in the genomes of animals and plants. SINEs sequences and RNA secondary structures are ancestrally related to tRNA and 7SLRNA. Few years ago, we discovered a novel class of antisense long non-coding RNAs (named SINEUPs) that can UP-regulate the translation of a target coding mRNA by means of an embedded inverted SINEs, SINE-B2 from mouse genome and FRAM from human genome, without any change in its mRNA expression level. Although both SINEs up-regulated the translation of same target mRNA, their sequence and RNA structure are not completely matched.

To understand how SINEs up-regulate target mRNA translation, we cloned 20 eukaryotic tRNA SINE consensus sequences and compared their effect of protein translations for the target mRNA. In order to screen SINE sequences, we developed an effective high-throughput screening (HTS) system with Celigo S, which can rapidly detect living cells with an automated high-resolution imaging. As a result of HTS, working SINEs as translational enhancer were categorized into two groups of phylogenetic clade of SINE structures, suggesting that the categories of RNA structure and length are important to enhance the target translation.

We further developed an application method of SINEUPs targeting hepatocyte nuclear factor 4 alpha (Hnf4-alpha), which is known to be associated with the liver development and maturity onset diabetes of the young 1 (MODY1), in mouse liver with adeno- associated virus vector (AAV). The results of the in vivo experiment indicated that the AAV-SINEUP-Hnf4-alpha is efficient within physiological level, and selected SINEUPs are potential molecules for the translation enhancement as the therapeutic application.

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## 228 A computational approach for identification of protein-RNA interactions uncovers direct binders of Xist lncRNA

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Many RNAs work through their interactions with various regulatory protein forming different types of assemblies<sup>1,2</sup>. Yet, identifying these proteins using experimental methods remains challenging, making it feasible for a small number of transcripts and proteins. Accordingly, computational methods for predicting protein-RNA interactions would provide an important source of information to study the regulatory role of long non-coding RNAs (lncRNAs). We developed the Global Score algorithm to calculate interactions of proteins and lncRNAs at nucleotide and amino acid resolution<sup>3</sup>. We used the Global Score algorithm to investigate protein binding to the Xist lncRNA, which orchestrates X Chromosome inactivation. We validated our calculations by means of enhanced individual nucleotide CLIP method (eCLIP) and tested our method against a number of nucleotide-binding proteins. We show that Global Score is a robust computational framework to study RNA-protein interactions and an efficient tool to prioritize bona fide lncRNAs direct interactors. The structural properties of Xist lncRNA, investigated with the CROSS method to identify double- and single-stranded regions of long RNAs<sup>4</sup>, will be presented and discussed.

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## 229 Molecular Characterization And Inhibition Of The lncRNA LINP1, A Key Regulator Of DNA Repair In Triple Negative Breast Cancer

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Non-Homologous End Joining (NHEJ) is a major DNA repair mechanism conserved in eukaryotes and prokaryotes, and is used to repair double-strand DNA breaks that arise due to V(D)J recombination, ionizing radiation, and reactive oxygen species. Loss of NHEJ can result in chromosomal instability and promotes tumorigenesis. The first step in NHEJ involves the binding of Ku70–Ku80 heterodimers to both ends of a DNA break, followed by the recruitment of the catalytic subunits of DNA dependent protein kinase (DNA-PKcs). DNA end processing involves nucleases such as Artemis, and DNA polymerases to repair the damaged bases; followed by ligation of the blunt DNA ends by the XFL–XRCC4–DNA ligase IV complex. LINP1 is thought to form an RNA scaffold that interacts with both Ku70–Ku80 and DNA-PKcs to promote end joining. LINP1 is a 917 nt long ncRNA that is overexpressed in triple negative breast cancer (TNBC) tumors, in the TNBC cell lines MDA-MB-231 and MDA-MB-468, and in triple-negative immortalized mammary cells MCF10A. LINP1 is not detected in ER-positive MCF7 cells. In addition, activation of EGFR signaling via MEK and JNK kinases up regulates LINP1 whereas the tumor suppressor TP53 represses LINP1 via the microRNA miR-29. Intriguingly, knockdown of LINP1 sensitizes mice to ionizing radiation due to defects in DNA repair via the NHEJ pathway. Our ongoing studies are aimed at structurally characterizing LINP1 and developing chemical knockdowns suitable for cellular and preclinical examination of this target for synthetic lethality and therapeutic interventions.

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## 230 Splicing inhibition causes abnormal translocation of a neuron specific lncRNA Gomafu

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Gomafu, a neuron specific long non-coding RNA (lncRNA), is associated with splicing factors and regulates alternative splicing of schizophrenia-related genes. Despite being spliced and polyadenylated, Gomafu is never transported to cytoplasm and stays in nucleus. The mechanism of nuclear retention of Gomafu is unknown. It was reported that a splicing inhibitor Spliceostatin A (SSA) inhibits the nuclear retention of pre-mRNA, so we asked whether SSA also inhibits nuclear retention of Gomafu. HeLa cells expressing Gomafu were treated with SSA and the localization of Gomafu was analyzed by fluorescent in situ hybridization. We found that Gomafu was exported to cytoplasm by SSA treatment. Splicing inhibition by knocking down U4 snRNA also inhibited nuclear retention of Gomafu. Since inhibition of splicing causes abnormal accumulation of unspliced pre-mRNA in nucleus, we asked whether the accumulation of pre-mRNA occurred earlier than translocation of Gomafu. We found that Gomafu was exported to cytoplasm after an enlargement of nuclear speckle, which is marked by SC35 and may contain abnormal pre-mRNA. Therefore, abnormal accumulation of pre-mRNA by SSA might affect the transport of Gomafu to cytoplasm. These results provides insights into how nuclear long non-coding RNAs retain in nucleus.



## 231 Emerging role of long non-coding RNA NEAT1 as an epigenetic regulator

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Nuclear paraspeckle assembly transcript 1 (NEAT1) is the crucial structural platform of paraspeckles, which is one type of nuclear bodies. Here, we demonstrate that HSV-1 infection increases NEAT1 expression and paraspeckle formation in a STAT3-dependent manner. NEAT1 can associate with HSV-1 genomic DNA. By binding with STAT3, NEAT1 is required for the recruitment of STAT3 to viral gene promoters and facilitates the transcriptional activity of STAT3 in viral genes expression. We also found that NEAT1 could influence methylation, crotonylation and butyrylation of histone and other proteins in microglia and showed its powerful ability to mediate the expression of multiple genes involved in beta-amyloid peptide accumulation and Tau phosphorylation through histone modifications, suggesting NEAT1 have a key role in in progress of Alzheimer disease. Overall, our results provide insight into the roles of NEAT1 in the epigenetic control of genes expression.

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## 232 Long non-coding RNA Lnc268 inhibited tumorigenesis as well as lung metastasis of breast cancer

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Breast cancer is the most common malignant tumor in women. Metastasis is the main reason of breast cancer patient death. Long non-coding RNA (lncRNA) is a class of non-coding RNA that are greater than 200nt in length, which play important roles in tumorigenesis and development of cancers. Our study aimed to identify lncRNAs associated with breast cancer metastasis through transcriptome sequencing and investigating the underlying mechanism. In our previous work, we have identified a lncRNA, Lnc268, through the preliminary sequencing screening in two breast cancer cell lines which has different metastasis ability. We found that Lnc268 reduced cell migration and invasion in vitro and inhibited tumorigenesis as well as lung metastasis in vivo. Besides the functional study of Lnc268, the expression of Lnc268 is investigated in the clinical breast cancer patients. We propose that our work would decipher the relationship of Lnc268 expression with the metastasis and the prognosis of breast cancer patients and would provide new evidence for the diagnosis and treatment of breast cancer.

### 233 Characterisation of Alternatively Spliced Isoforms of Ago2

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miRNA-mediated gene regulation is a key regulator of many biological pathways and is essential for human development. miRNAs are predicted to fine-tune the expression of the majority of human transcripts and have diagnostic, prognostic and therapeutic potential in a variety of human diseases. Therefore it is critical to understand how the pathway itself is regulated.

Alternative splicing is a post-transcriptional process that allows for a single gene to code for multiple proteins, called protein isoforms. Alternative splicing is a pivotal regulatory mechanism that regulates gene expression in mammalian cells and controls many key processes including cell and tissue-specific differentiation. As the miRNA pathway is dependent on a series of multi-domain proteins that are critical for miRNA processing and function we hypothesized that alternatively spliced isoforms of these proteins will have unique functions with potential regulatory roles. To investigate this we performed a targeted RNA sequencing experiment to identify alternatively spliced isoforms of proteins in the miRNA pathway that are expressed in healthy human tissues and immortalised human cell lines.

We identified two alternatively spliced isoforms of Argonaute 2, the key protein of the miRNA-induced silencing complex (miRISC), which is essential for miRNA function. These isoforms, generated by exon skipping, have deletions in the domains of Ago2 that are essential for cleaving target RNA or unwinding the miRNA duplex. Compared to full length Ago2, these isoforms have unique, tissue-specific expression patterns and display altered binding affinity to miRNAs. These data suggest that these Ago2 isoforms have unique functions in the miRNA pathway, specific to particular human tissues or developmental stages.

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### 234 A nuclear export factor (Nxf) variant is required for small RNA-guided transcriptional silencing of transposable elements in *Drosophila melanogaster*

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Transposable elements are repressed by a nuclear small RNA-based system in most animal gonads in order to maintain the fitness of the host. In *Drosophila melanogaster*, Piwi—a nuclear Argonaute protein—is complexed with small guide RNAs (piRNAs) and targets the nascent transcripts of transposable elements. This induces heterochromatin formation and transcriptional silencing by unknown mechanisms.

Panoramix/Silencio was proposed as a linker between the Piwi/piRNA complex and the cellular heterochromatin machinery. To identify interactors of Panoramix/Silencio, we performed co-immunoprecipitation followed by quantitative mass spectrometry. We found that the key interacting protein is Nxf2 which belongs to the highly conserved nuclear export factor (Nxf) family. Here we show that—unlike the well-characterized Nxf proteins which are vital for general mRNA export—*Drosophila* Nxf2 is required for Piwi-mediated transcriptional silencing of transposable elements.

## 235 Importance of cellular organelles in controlling the miRNA-mediated gene expression in mammalian cells

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miRNA-mediated repression control expression of more than half of protein coding genes in metazoan animals. Translation repression is associated with target mRNA degradation initiated by decapping and deadenylation of the repressed mRNAs. Earlier evidence suggest Endoplasmic Reticulum (ER) as the site where miRNPs interact with their targets before the translation repression sets in but the subcellular location of subsequent degradation of miRNA-repressed messages was unidentified. We explore the subcellular distribution of essential components of degradation machineries of miRNA-target mRNAs. We have noted that interaction of target mRNAs with AGO2 protein on ER precedes the relocalization of repressed messages to Multivesicular Bodies (MVBs). The repressed messages subsequently get deadenylated, lose their interaction with AGO2 and also become decapped. Blocking maturation of endosomes to late endosome and MVBs by targeting the endosomal protein HRS, uncouples miRNA-mediated translation repression from target RNA degradation. HRS is also targeted by the intracellular parasite *Leishmania donovani* (*Ld*) that curtails HRS level in infected cells to prevent uncoupling of mRNA-AGO2 interaction, prevent degradation of translationally repressed messages and thus stop recycling of miRNPs pre-engaged in repression. Importance of other players in this process will be discussed.

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## 236 Withdrawn

## 237 HRP-2/hnRNP Q impedes *let-7*-mediated repression of *lin-41/TRIM71* in *C. elegans* and humans

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The evolutionarily conserved regulation of the RBCC-NHL protein LIN-41/TRIM71 by the *let-7* miRNA controls cell proliferation and differentiation during development in species from nematodes to humans. Mis-regulation of *lin-41/TRIM71* leads to developmental abnormalities and cancers. Recently, a body of evidence suggests that heterogeneous nuclear ribonucleoproteins (hnRNPs) and small noncoding RNAs function together during gene silencing, including translational control by miRNAs. So far, it remains elusive whether hnRNPs contribute to the *let-7*-mediated regulation of *lin-41/TRIM71*. Here, we show that RNAi knockdown of *C. elegans* HRP-2, the homolog of mammalian hnRNP Q, relieved heterochronic phenotypes in *let-7(n2853)* mutant animals, indicating the involvement of HRP-2 in *let-7-lin-41* regulation. In addition, we detected an RNA-dependent interaction between HRP-2 and the Argonaute ALG-1, the core effector protein of the miRNA-mediated silencing complex (miRISC). We also identified an HRP-2 response element in the *lin-41* 3'UTR at a position downstream of the two *let-7* complementary sites (LCSs) and close to the poly(A)-tail. Deletion of this response element caused further down-regulation of a GFP reporter carrying the *lin-41* 3'UTR, in a *let-7*-dependent manner. Thus, we propose that HRP-2 impedes *let-7*/miRISC activity when binding to the *lin-41* 3'UTR. Interestingly, we found that depletion of human hnRNP Q also enhanced *let-7*-mediated down-regulation of *TRIM71* in Huh7 or HEK293 cells. Similar to the case in *C. elegans*, hnRNP Q binds to a response element upstream of the poly(A)-tail in the *TRIM71* 3'UTR. Deleting this element from the 3'UTR significantly enhanced *let-7* repression. Taken together, our findings uncover a novel evolutionarily conserved function for HRP-2/hnRNP Q in inhibiting *let-7*/miRISC activity by binding to specific response elements in the *lin-41/TRIM71* 3'UTRs.

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## 238 The cap-binding protein 4EHP effects translation silencing by microRNAs

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microRNAs (miRNAs) are important components of gene regulatory networks and affect all aspects of cell biology by controlling the stability and translation efficiency of their target mRNAs. The CCR4-NOT complex effects miRNA-mediated silencing at least in part through interactions with DDX6 and 4E-T (eIF4E-Transporter) proteins, but the precise mechanism is unknown. Through screening for protein interactions in cells via the BioID method, we identified the cap-binding eIF4E-Homologous Protein 4EHP as a component of the CCR4-NOT/DDX6/4E-T axis. We demonstrate that the cap-binding activity of 4EHP contributes to the translational silencing by miRNAs through the CCR4-NOT complex. Direct interaction between 4E-T and 4EHP increases the latter's cap-binding affinity, suggesting that this interaction potentiates its competition with the eIF4F complex for binding to the mRNA cap. We propose a model wherein the 4E-T/4EHP interaction engenders a closed loop mRNA conformation that blocks translational initiation of miRNA targets.



**239 Biochemical and structural insights into the small RNA methyltransferase Hen1 in animal**Ling Peng<sup>1</sup>, Renfu Shang<sup>1</sup>, Fengjuan Zhang<sup>1</sup>, Jiayi Chen<sup>2</sup>, Jinbiao Ma<sup>2</sup>, Ligang Wu<sup>1</sup>, Ying Huang<sup>1</sup><sup>1</sup>Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China; <sup>2</sup>Fudan University, Shanghai, China

HUA ENHANCER 1 (Hen1) is a RNA 2'-O-methyltransferase that modifies the small non-coding RNAs (sncRNA) by adding a methyl group to the 2'-OH of 3'-terminal nucleotide, thus protecting them from other modifications and degradation. The molecular mechanism of the methylation of small RNA duplexes has been illuminated by the crystal structure of Hen1 in *Arabidopsis* (AtHen1). However, the molecular basis of single-stranded RNA methylation by mammalian Hen1 homologues, especially Piwi-interacting RNAs (piRNAs), remains elusive. Here we showed that mouse Hen1 (mHen1) is essential to maintain the length and abundance of piRNAs in mouse spermatogonial stem cells (SSCs). Moreover, we identified a class of tRNA-derived sncRNAs as the new substrates of mHen1, which was referred as Hen1-methylated tRNA-derived small RNA (hmRNA). We further solved the crystal structure of the catalytic domain of human Hen1 (HsHen1) with its cofactor AdoMet at 1.8 angstrom resolution. Comparisons with the structure of AtHen1 showed that they share a similar active site in binding the divalent cation and the cofactor AdoMet. *In vitro* methyltransferase assay indicated that the full catalytic domain of HsHen1 is sufficient to methylate a specific length of single-stranded RNAs in a manganese-dependent manner. In conclusion, our functional and structural findings provide important insights into the methylation details of sncRNAs in mouse SSCs and catalytic mechanism of mammalian Hen1.

**240 Plant ARGONAUTE4 family proteins prefer to bind to DNA targets in vitro**Hiro-oki Iwakawa<sup>1,2</sup>, Yukihide Tomari<sup>1,2</sup><sup>1</sup>Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; <sup>2</sup>Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan

In plants, the RNA-induced silencing complex (RISC) consisting of a 24-nt small non-coding RNA and an ARGONAUTE4 (AGO4) family protein (AGO4, AGO6, or AGO9) directs DNA methylation by recruiting the DNA methyltransferase DRM2 to the target loci. This pathway is called RNA-directed DNA methylation (RdDM), one of the major epigenetic pathways in plants. So far, AGO4-RISC has been believed to bind to the nascent transcripts of the plant-specific RNA polymerase Pol V. In contrast, a recent UV laser mediated-ChIP experiment showed that AGO4-RISC contacts DNA. However, it is still unclear if the interaction is mediated by the base pairing between the small RNA and the genomic DNA or if the genomic DNA interacts with AGO4 protein independently of base pairing. Here, in order to check if small RNAs in plant AGOs can interact with DNAs, we measured the binding affinity of purified plant RISCs to target RNAs or DNAs *in vitro*. AGO1 and AGO2, which play a major role in the microRNA pathway and antiviral RNAi pathway, respectively, interact more tightly with RNAs than with DNAs. Conversely, AGO4 family proteins bind to DNAs with higher affinity than to RNAs through base pairing with small RNAs, implying their direct DNA recognition.

**241 miRNA pathway functionality in the mouse female germline***Shubhangini Kataruka<sup>1</sup>, Matyas Flemr<sup>1,3</sup>, Jun Ma<sup>2</sup>, Richard Schultz<sup>2</sup>, Petr Svoboda<sup>1</sup>*<sup>1</sup>Institute of Molecular Genetics, Prague, Czech Republic; <sup>2</sup>University of Pennsylvania, Philadelphia, USA;<sup>3</sup>Friedrich Meischer Institute, Basel, Switzerland

During oocyte-to-embryo transition, transcription ceases prior to initiation of meiotic maturation and is not renewed until the zygotic genome activation. Thus, post-transcriptional control is essential for transcriptome regulation during this time. Post-transcriptional control mechanisms include RNA silencing pathways, which utilize small noncoding RNAs guiding repressive ribonucleoprotein complexes to mRNAs and other transcripts. Three different classes of small RNAs have been found in mouse oocytes: piRNAs, miRNAs, and endosiRNAs. Of them, however, only endosiRNAs acting in the RNA interference pathway are essential. This is supported by sterile knock-out phenotypes of Dicer and Ago2, which are essential for the RNAi pathway. Deletion of Dicer and Ago2 leads to severe defects in chromosomal alignment and spindle organization in the mouse oocytes. Although Dicer and Ago2 also participate in the miRNA pathway, fertility of females carrying oocyte-specific knock-out of Dgcr8, an essential miRNA biogenesis specific protein, suggests that miRNA pathway is dispensable for the oocyte-to-embryo transition. Reduced miRNA pathway activity in the female germline also correlates with disappearance of P-bodies in the growing oocytes and inefficient repression of miRNA-targeted reporters in fully-grown oocytes. Remarkably, while miRNAs are non-essential and inactive in the oocyte, their biogenesis and ability to mediate RNAi-like cleavage appears intact. In order to understand why is the miRNA pathway non-essential and non-functional in mouse oocytes, we study assembly of miRNA-containing ribonucleoprotein effector complexes and their ability to mediate translational repression and RNA degradation. We examine whether inefficient assembly of the miRNA effector complex (miRISC) and stoichiometry between miRNAs and cognate mRNAs could explain the observed effects.

**242 Evolutionary patterns of metazoan microRNAs reveal targeting principles in the let-7 and miR-10 families***Justin Wolter<sup>1,2</sup>, Victoria Godlove<sup>2</sup>, Nguyen Thuy-Duyen<sup>3,4</sup>, Kotagama Kasuen<sup>1,2</sup>, Alyssa Lynch<sup>1,3</sup>, Alan Rawls<sup>3</sup>, Marco Mangone<sup>1</sup>*
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MicroRNAs (miRNAs) regulate gene output by targeting degenerate elements in mRNAs and have undergone drastic expansions in higher metazoan genomes. The evolutionary advantage of maintaining copies of highly similar miRNAs is not well understood, nor is it clear what unique functions, if any, miRNA family members possess. We have studied the evolutionary patterns of metazoan miRNAs, focusing on the targeting preferences of the let-7 and miR-10 families. These studies reveal hotspots for sequence evolution with implications for targeting and secondary structure. High-throughput screening for functional targets reveals that each miRNA represses sites with distinct features and regulates a large number of genes with cooperative function in regulatory networks. Unexpectedly, given the high degree of similarity, single-nucleotide changes grant miRNA family members with distinct targeting preferences. Together, our data suggest complex functional relationships among miRNA duplications, novel expression patterns, sequence change, and the acquisition of new targets.

**243 Identification of factors important for miRISC silencing in *C. elegans****Pascale Michaud, Rachel Rioux, Martin J Simard***St-Patrick Research Group in Basic Oncology, Centre Hospitalier Universitaire de Québec-Université Laval (Hotel-Dieu de Québec), Laval University Cancer Research Centre, Québec City, Québec, Canada**

In order to repress their targets, microRNAs are loaded onto the *microRNA-induced silencing complex*, or miRISC. Repression is then induced by mRNA degradation and/or translational repression of target mRNA. The Argonaute proteins form the core of the miRISC and recruit the scaffold protein GW182 to induce mRNA deadenylation. However, it is still unclear if other components or modulators of the miRISC are necessary for its activity at the target.

To identify new factors important for miRISC activity, we designed a novel genetic screen using a transgenic animal containing the *gfp* gene fused to a 3' UTR in which the natural miRNA binding sites were replaced by box B RNA hairpins. This transgenic animal also expresses a functional *alg-1* gene fused to a  $\lambda$ N peptide. This peptide specifically binds to the box B sequences and this tethering of ALG-1 to the 3' UTR is sufficient to repress GFP expression. The repression of this reporter is therefore independent of miRNAs but still requires miRNA-specific Argonaute complex to fully block protein synthesis.

A forward genetic screen was conducted to find mutants which misregulate this GFP transgenic reporter, suggesting an alteration in a gene important for miRISC silencing. We isolated several candidates showing a derepression in GFP, while having no alteration in the Argonaute gene. High-throughput sequencing coupled to SNP mapping has allowed us to identify a mutation in the coding sequence of a Rab GTPase activator (RABGAP) in one of our candidates. We are currently working on further characterization of the importance of this RABGAP for microRNA-mediated silencing using several genetic and molecular approaches. Here, we present our progress at characterizing the importance of this new player of the microRNA pathway.

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**244 Withdrawn**

## 245 Characterization of B2 SINEs in the Mammalian Innate Immune Response

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Approximately 12% of the mammalian genome is composed of retrotransposable elements termed short interspersed nuclear elements or SINEs. These elements have classically been recognized mainly for their pathogenic effects due to their ability to replicate and re-integrate across the genome, occasionally with deleterious effects to host gene function. However, a growing collection of work suggests that these elements play unique roles in several host pathways, including those involved in host immunity. We observe robust induction of the murine B2 SINE family following infection of several cell types with the gammaherpesvirus MHV68. We observe a similar induction following stimulation of toll-like receptors (TLRs), an important family of pattern recognition receptors (PRRs) involved in innate immune signaling.

Recent work in the lab has uncovered a link between B2 SINEs and MHV68 gene expression. In this context, B2 expression stimulates phosphorylation of the major viral lytic cycle transactivator protein RTA in an IKK $\beta$ -dependent manner. These findings suggest that B2 SINEs may participate in immune signaling in other ways, or may act as a pathogen response mechanism so far undescribed. Here, we probe in more detail the precise molecular events that lead to B2 SINE induction following viral infection, TLR stimulation, and other forms of cellular stress. We examine the involvement of RNA polymerase III (pol III), Maf1, MAP kinases, MyD88, and other signaling complexes. We further examine the effects of B2 SINE over-expression and knockdown on the immune response following pathogen-induced stress. By describing these pathways, we anticipate arriving at a more whole understanding of how these elements participate in the mammalian immune response.

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## 246 Genetic and mechanistic diversity of piRNA 3' end formation

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Small regulatory RNAs guide Argonaute proteins in a sequence specific manner to their targets and thereby play important roles in eukaryotic gene silencing. Of the three small RNA classes, microRNAs and siRNAs are processed from double-stranded RNA precursors into highly defined 21-23mers by Dicer, an endo-ribonuclease with intrinsic ruler function. piRNAs instead—the 22-30nt long guides for PIWI-clade Argonaute proteins that silence transposons in animal gonads—are generated Dicer-independently from single stranded precursors. piRNA 5' ends are defined either by Zucchini, a mitochondria-anchored endonuclease, or by piRNA-guided target cleavage. Formation of piRNA 3' ends and hence piRNA length definition is only poorly understood. Here, we show that two genetically and mechanistically distinct pathways generate piRNA 3' ends in *Drosophila*. The initiating nucleases are Zucchini and the PIWI-clade proteins Aubergine/Ago3, respectively. While Zucchini-mediated cleavage directly defines mature piRNA 3' ends, Aubergine/Ago3-mediated cleavage liberates pre-piRNAs that require extensive resection by the 3'-to-5' exo-ribonuclease Nibbler/Mut-7, previously shown to trim the 3' ends of some miRNAs. Furthermore, we show that the phosphorylation of a single serine in Nibbler is required for the trimming of piRNAs but not for the 3' end trimming of miRNAs. Together, our data establish a coherent blueprint for piRNA biogenesis, and set the stage for the mechanistic dissection of the processes and players that govern piRNA 3' end formation.

**247 MicroRNAs are destabilized by interactions with seedless, non-natural targets**June Hyun Park<sup>1</sup>, Sang-Yoon Shin<sup>1,2</sup>, Soyoung Kim<sup>1</sup>, Taewook Kim<sup>1</sup>, Chanseok Shin<sup>1,2</sup><sup>1</sup>Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea;<sup>2</sup>Interdisciplinary Program in Agricultural Genomics, Seoul National University, Seoul, Republic of Korea

MicroRNAs (miRNAs) regulate gene expression by guiding the Argonaute (Ago)-containing RNA-induced silencing complex (RISC) to specific target mRNA molecules. It is well established that miRNAs are stabilized by Ago proteins, but the molecular features that trigger miRNA destabilization from Ago proteins remain largely unknown. To explore the molecular mechanisms of how targets affect the stability of miRNAs in human Ago (hAgo) proteins, we employed an in vitro system that consisted of a minimal hAgo2-RISC in HEK293T cell lysates. Strikingly, we showed that miRNA destabilization is dramatically enhanced by an interaction with seedless, non-canonical targets. We then showed that this process entails not only unloading of miRNAs from Ago, but also 3' end destabilization of miRNAs occurred within Ago. Furthermore, our mutation analysis suggests that conformational changes in the hinge region of the Ago PAZ domain are likely to be the main driving force of the miRNA destabilization. Our collective results suggest that non-canonical targets may provide a stability control mechanism in the regulation of miRNAs in humans.

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**248 Nucleoplasmic Sfpq Controls microRNA-mediated Silencing**Silvia Bottini, Emanuela Repetto, Michele Trabucchi

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There is a growing body of evidence about the presence and the activity of the miRISC (Ago2/microRNA complex) in the nucleus of mammalian cells. Here, we showed by quantitative proteomic analysis that Ago2 interacts with 133 RNA-independent and 166 RNA-dependent proteins. Many of the Ago2 interactors found here were previously independently identified as being associated to Ago2. To study the biological and mechanistic relevance of RNA as mediator of the interaction between Ago2 and other proteins for miRNA function, we decided to focus on Sfpq, Pspc1 and NonO, which form a protein complex. These three proteins were among the most abundant RNA-dependent Ago2 interactors. Our data indicate that Sfpq mediates the interaction of both Pspc1 and NonO with the nucleoplasmic miRISC. Therefore, Sfpq mediates the sequence specificity of a possible role of this complex in modulating nucleoplasmic miRISC activity.

By HITS-CLIP coupled with transcriptomic analysis in P19 stem cells, we demonstrated that Sfpq directly controls the miRNA targeting of a subset of miRNA-target mRNAs when it binds locally within a distance of 500 nucleotides (for both ectopic let-7a and endogenously expressed miRNAs). These data were validated in both mouse P19 and human NTERA-2 stem cells. Although Sfpq interacts with miRISC only in the nucleoplasm, it modulates miRNA targeting in both nucleoplasm and cytoplasm, indicating a nucleoplasmic imprinting of Sfpq-target mRNAs. Mechanistically, Sfpq binds to a set of long 3'UTR recognizing two specific binding motifs with a core sequence composed by UGU sequence, as we determined by de novo motif analysis from Sfpq HITS-CLIP analysis. Sfpq uses these two binding motifs to form long aggregates to optimize miRNA position/recruitment to selected binding sites, as we showed by HITS-CLIP analysis and on Lin28A 3'UTR using gene reporter assay and atomic force microscope. Finally, Sfpq regulates the let-7-dependent gene expression program to ultimately elicit the exit from stem cell status and to promote differentiation towards a neuron-like phenotype.

In conclusion, these data extend the miRNA-mediated post-transcriptional gene silencing into the nucleoplasm and indicate that a unique Sfpq-dependent post-transcriptional strategy takes place in cells for controlling miRNA-targeting activity on mRNA containing long 3'UTRs.



**249 The modular features that define efficient and specific RNA silencing guides**

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MicroRNAs (miRNAs) are single-stranded RNA molecules of about 22 nucleotides that regulate gene expression post-transcriptionally. In human, they act via sequence complementarity, where seven nucleotides located in their 5' moiety (referred to as the "seed") prompt the recognition of their target messenger RNAs (mRNAs)<sup>1</sup>. It was shown that the remaining nucleotides play a critical role in interference efficiency, although this involvement was not precisely characterized<sup>2,3</sup>. Here, we quantify the precise function of all miRNA portions. Using reporter assays, we deciphered a pattern revealing how base pairing from non-seed nucleotides contributes to gene silencing efficiency. We built a molecular model showing the corresponding sequence of motions of the miRNA-induced silencing complex (miRISC) that mediates post-seed base pairing. This model allows us to: compute the levels of downregulation from sequence data with high accuracy ( $r^2 > 0.5$ ,  $p\text{-value} < 10^{-12}$ ); and, enrich the design of efficacious guide sequences that simultaneously downregulate the expression of multiple target genes. This finding represents a major improvement to predict miRNA targets and silencing efficiencies compared to previous models based on sequence complementarity or energy. It enables the decoding of miRNA targets at the genome level with much increased accuracy, as well as the design of specific and effective RNA silencing guides.

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**250 Sensing self and foreign circular RNAs by intron identity**

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Circular RNAs (circRNAs) have been found in all domains of life, but their functions are still mostly unknown. We show that transfection of purified *in vitro* self-spliced circular RNA potently induce innate immunity genes and confers protection against viral infection in mammalian cells. The nucleic acid sensor RIG-I detects foreign circRNA, and RIG-I and foreign circRNA co-aggregate in cytoplasm. CircRNA activation of innate immunity genes does not depend on 5' triphosphate, double-stranded RNA structure, or the primary sequence of the foreign circRNA. Instead, the innate immune response is based on the introns that program the circRNA. A human intron expressing a foreign circRNA abrogates immune activation, and mature human circRNA is associated with diverse RNA binding proteins reflecting its endogenous splicing and biogenesis. These results reveal innate immune sensing of circRNA, a prevalent class of host and pathogen RNAs, and highlight introns as arbiters of self-nonsel identity.

## 251 Use of the yeast ncRNA knockout collection for the mode of action and target identification of orphan drugs

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In this study, we analysed drug-induced haploinsufficiency in the non-coding RNA (ncRNA) haploid and diploid deletion collections recently constructed by the Delneri-O'Keefe laboratories.

The main objective of this study was to systematically test the ncRNA mutants in the presence of two orphan drugs (Lithium citrate and Riluzole) in order to unravel biological information on the drug mode of action in yeast and help discover any possible ncRNA drug targets. Given that most of the basic biological processes are conserved within eukaryotes, data from the yeast screening essay could help to predict the drug mode of action and targets in human cells.

The sub-lethal concentration for each drug was determined by performing growth curves for the wild-type reference strain under increasing concentrations of each drug. The ncRNA deletion collection was then arranged in a 384-well format and used to inoculate YPD agar plates containing the previously determined sub-lethal concentrations of each drug. The images were captured after 72 hours of incubation at 30 °C and analysed using colony-size recognition software.

The colony size for each deletion strain was digitally scored, averaged for all technical replicates, and used as proxy for cellular fitness when compared to the wild-type reference strain. All ncRNA deletion mutants showing a statistically significant difference in colony size were considered as potential drug targets and designated for further analysis.

In overall, this study allowed us to identify ncRNA deletion mutants showing a specific response to either Lithium Citrate or Riluzole and hypothesize the possible underlying mode of action that will serve as foundation for our future work.

## 252 Circular RNA ZNF609 promotes the development of colorectal cancer

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Non-coding RNAs can function as potent gene regulators and consequently, such RNA molecules have been implicated in many diseases including all types of cancer. Using an intrasplenic tumor model for colorectal cancer, we have identified several long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs) differently expressed in primary tumors and metastases suggesting a role of these RNAs in tumor progression. Interestingly, we found that several of the lncRNAs and circRNAs encode for small peptides, which are excluded from annotation pipelines and have thus not been functionally analyzed so far. Of the circRNA candidates, we have identified circular RNA ZNF609 (ciZNF609), which is up-regulation in primary tumors as well as liver metastases from our mouse model. We developed a method for knocking down and overexpression of ciZNF609 and established inducible stable cell lines for in vivo analyses. In a xenograft mouse model, overexpression of ciZNF609 promotes colorectal cancer development. Currently, we are developing a method for rescuing the phenotype of ciZNF609 as well as studying the mechanisms ciZNF609.

## 253 Circular RNA expression analysis of Neuro-2A cells during Retinoic acid-induced differentiation

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Recently, several functional circular RNAs (circRNAs) have been reported. Comparison of circRNA expression profiles between several tissues shows that circRNAs are enriched in mammalian brain and are up-regulated during neural differentiation. Since circRNAs are physiologically stabler than other linear RNAs in the intracellular environment, it is hypothesized that neural circRNAs play functional roles in neural maturation and/or regulate gene expression in the matured neural cells. However, the function of the predicted neural circRNAs are poorly understood. In this study, we investigated the circRNA expression profile in Neuro-2A cells, which originate from Mus musculus neuroblastoma cell line, during *all-trans*-retinoic acid (RA)-induced differentiation. We, then, identified over 5000 species of circRNAs by using the UROBOROS program in the total RNA-seq data of the non-differentiated Neuro-2A deposited in the Gene Expression Omnibus (GEO). Also, RT-PCR analysis revealed that the expression of some species of the circRNAs disappeared after RA-induction. Furthermore, miRNA binding sites were found in these circRNAs, and therefore, we estimate that these circRNAs may act as microRNA sponges that regulate RA-induced cell differentiation.

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## 254 Coordinated circRNA biogenesis and function with NF90/NF110 in viral infection

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Circular RNAs (circRNAs) generated via back-splicing are enhanced by flanking complementary sequences. Expression levels of circRNAs vary under different conditions, suggesting participation of protein factors in their biogenesis. Using genome-wide siRNA screening that targets all human unique genes and an efficient circRNA expression reporter, we identify doublestranded RNA binding domain containing immune factors NF90/NF110 as key regulators in circRNA biogenesis. NF90/NF110 promote circRNA production in the nucleus by associating with intronic RNA pairs juxtaposing the circRNA-forming exon(s); they also interact with mature circRNAs in the cytoplasm. Upon viral infection, circRNA expression is decreased, largely owing to the nuclear export of NF90/NF110 to the cytoplasm. Meanwhile, NF90/NF110 released from circRNP complexes bind to viral mRNAs as part of their functions in antiviral immune response. Our results therefore implicate a coordinated regulation of circRNA biogenesis and function by NF90/NF110 in viral infection.

## 255 A noncoding function of *TYRP1* mRNA promotes melanoma growth

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Competition between RNAs to bind miRNA has been proposed to influence biological systems. However, the role of this RNAs competition in diseases onset is unclear. Here, we report that *TYRP1* mRNA, in addition to encoding tyrosinase-related protein 1 (*TYRP1*), indirectly governs cell proliferation by sequestering miR-16 on non-canonical miRNA response elements (MREs). Consequently, sequestered miR-16 is no longer able to repress its mRNA targets such as *RAB17*, which we show is involved in melanoma cell proliferation and tumor growth. Restoration of miR-16 tumor suppressor function can be achieved *in vitro* by silencing *TYRP1* or increasing miR-16 expression levels. Importantly, *TYRP1*-dependent miR-16 sequestration can also be overcome *in vivo* using small oligonucleotides that mask miR-16 binding sites on *TYRP1* mRNA. Together, our findings assign a pathogenic noncoding function to *TYRP1* mRNA and underscore miRNA displacement as a new antimelanoma targeted therapeutic approach.

## 256 MEF2C as an efficient microRNA modulator in skeletal muscle cells

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miRNAs are initially transcribed as long primary transcripts (pri-miRNAs) that undergo sequential processing by the RNase III endonucleases Drosha and Dicer to the mature 20–23 nucleotide species. Mature miRNA associate with the RNA-induced silencing complex (RISC) and functions as a guide by base pairing with its target mRNAs. The ability of individual miRNAs to regulate hundreds of transcripts allows these RNAs to coordinate complex programs of gene expression and thereby control nearly all developmental processes, including myogenesis. Myocyte enhancer factor 2C (MEF2C) protein function as key transcriptional regulator of skeletal muscle development. It was demonstrated also that MEF2C regulate expression of some miRNAs in cardiac cells. We have shown that knock-down of MEF2C in human skeletal muscle cell line (HskM) results not only in deregulation of miRNA expression level but also in 3' end modification of many microRNA.

In order to characterize the miRNA transcriptome, small RNA libraries of HskM with decreased MEF2C level were deep sequenced. Over 500 mature miRNAs were expressed in our samples and we identified 142 miRNAs that were differentially expressed ( $p < 0.05$ ) between controls and MEF2C knock-down cell culture model, among them, muscle-specific microRNAs (e.g. miR-206, -1, -133a) and miRNAs implicated in skeletal muscle differentiation (e.g. miR-27b, -181, -125b). Moreover, analysis of 5' and 3' miRNA modifications in Next Generation Sequencing small RNA data revealed nucleotide trimming at 3' end of mature miRNAs in samples with lowered level of MEF2C. Furthermore, we observed much higher number of reads with 3' - terminal oligo(U) stretches in controls than in MEF2C deficient cells, whereas homomeric stretches of A nucleotides occurred with similar frequencies at the end of the reads both in control and in MEF2C knock-down cells. Since, miRNA 3'-end processing or modification plays an important role in determining their biological fate, we speculate that MEF2C, in addition to transcriptional regulation of pri-miRNAs, can have an impact on microRNA turnover and modulate miRNAs specificity.

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## 257 Circular RNAs: noncoding RNAs with coding potential?

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Circular RNAs (circRNAs) are a novel class of noncoding RNAs, which are generated from pre-mRNAs by alternative splicing of one or several adjacent exons. Although present in all eukaryotes investigated so far, circRNAs are functionally still largely undefined. To address the open question whether circRNAs may code for proteins, we describe here the initial and transcriptome-wide characterization of ribosome-associated and therefore potentially translated circRNAs in lysates from HeLa cells and the neuronal cell line U373. By combining polysome fractionation, controlled by cycloheximide versus puromycin treatment, with RNA-seq analysis across the gradient, we identified a small set of potentially translated circRNAs, based on the puromycin-dependent changes of circRNA distribution. A subset of these candidates was validated, first, by RT-PCR in combination with RNase R treatment to confirm their circular conformation. Second, the circRNA association with ribosomes and the puromycin-dependent release was analyzed by RT-PCR across the gradient fractions. Third, screening for protein products, potentially encoded by circular transcript isoforms, was performed by Western blotting, using antibodies that detect both products derived from the linear mRNA and the circRNA. Fourth, to conclusively prove that the detected protein product is generated from a circRNA, we used siRNA knockdown specifically directed against the circRNA junction in combination with Western blotting.

In sum, our data demonstrate that in HeLa and U373 cells, the major fraction of circRNAs is not associated with ribosomes. Nevertheless there are at least a few cases of potentially translated circRNAs that are currently further pursued in their validation. In addition, the described method provides a general approach for the transcriptome-wide identification of translated circRNAs and other noncoding RNAs in cell lines or tissues.

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## 258 Genome-wide analysis of novel back-spliced exons

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Tens of thousands of circRNAs have been identified among different cell lines/species, and their biogenesis is enhanced by RNA pairs forming from orientation-opposite complementary sequences in flanking introns. We have recently shown that multiple circRNAs can be generated from single gene loci through alternative back-splice site selection and/or alternative splice site selection. Interestingly, by the upgraded CIRCexplorer pipeline, we annotate thousands of novel exons that are back-spliced or spliced in circRNAs. Among those back-spliced novel exons, they are preferentially flanked by orientation-opposite complementary sequences. Genome-wide characterization of these novel exons suggests that in general they are less conserved than those annotated ones. As back-splicing is suggested to be unfavorably processed by the spliceosome, it is yet clear how the spliceosome could specifically recognize these novel exons by back-splicing. Surprisingly, some less conserved exons with weak splice site strength are predominantly included in circRNAs through canonical splicing. We hypothesize that these exons might be first restrained and then further spliced in the circles after back-splicing. We have archived these novel circRNA-predominant exons in the CIRCpedia database (<http://www.picb.ac.cn/rnomics/circpedia>).



## 259 Investigation of RNA-based regulation of gene expression in proteobacterial energy metabolism

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The TCA (tricarboxylic acid) cycle is of major importance in bacterial energy and carbon metabolism. The diverse life styles of bacteria make it necessary to orchestrate the presence and activity of many enzymes at the same time. For example, the TCA cycle needs to be controlled in a highly flexible manner in response to environmental changes such as stress conditions or nutrient availability. Regulation is mainly facilitated on the level of transcription initiation and enzymatic turnover. Moreover, bioinformatics studies have identified several conserved RNA motifs in intergenic regions of TCA cycle genes in certain proteobacteria that implicate potential post-transcriptional regulation of gene expression.<sup>[1]</sup> We are characterizing a set of RNA motifs that are exclusively associated with genes that are involved in the TCA cycle. We have tested whether these motifs bind to small molecular metabolites, thereby acting as riboswitches. However, we found no specific interactions of the investigated motifs with cellular metabolites. Next, we focused on the possibility that these RNAs bind to regulatory proteins. We performed an RNA/protein pulldown assay in conditions that up- or down-regulate the expression of the genes of interest. The analysis of the proteins was performed by LC-MS/MS. Results of this approach will be presented. Our aim is to identify unknown proteins which are involved in post-transcriptional gene regulation of TCA cycle enzymes.

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## 260 The competition among small RNAs for binding to the Hfq protein affects their annealing to target mRNAs

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The chaperone protein Hfq is involved in the control of translation by numerous small regulatory RNAs in bacteria. The binding of sRNAs to Hfq is necessary for their pairing to partly complementary sequences in regulated mRNAs. As the concentration of Hfq in the cells is limiting for translation regulation by sRNAs, it leads to competition among them for access to Hfq. Moreover, it was showed that there is a hierarchy among sRNAs in their efficiency of competition with other sRNAs for binding to Hfq *in vivo*. Similar differences in competition for binding to Hfq were also observed *in vitro*, and it was proposed that sRNAs contained sequence and structure elements determining their competition performance.

To better understand how the competition among sRNAs affects their pairing to mRNAs the properties of nine *Escherichia coli* sRNAs in competition for Hfq were analyzed. Moreover, the effect of these competitor sRNAs on the kinetics of annealing of three different sRNAs to their target mRNAs was compared. Finally, the stability of the ternary complexes of sRNA, mRNA and Hfq in the presence of different competitor sRNAs was studied. The results suggested that sRNAs which interact with the opposite faces of the Hfq ring are more efficient competitors than those that interact with only one site. The data also showed that the competition for access to Hfq affected both the sRNA annealing and the stability of the resulting ternary complexes. Moreover, the results provided insights about the interactions of sRNA-mRNA pairs with Hfq after the complex formation.

## 261 tRNA<sup>Gly</sup> tune the response of *Escherichia coli* to oxidative stress

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*Escherichia coli* must adapt to constant environmental changes, including sudden generation of oxidative stress. The response to oxidative stress is known to be controlled mainly by OxyR and SoxR together with other transcriptional factors, but there is very little knowledge about a potential role of the translation machinery. Based on a screening we have found a specific decrease in active tRNA<sup>Gly</sup> under oxidative stress produced by either paraquat or H<sub>2</sub>O<sub>2</sub>. Under these conditions we have not observed a decrease in other tRNAs specific for other 9 amino acids. We think this decrease correspond to a chemical modification of the tRNA as it does not affect the tRNA levels as measured by Northern blot, but it induce an important *in vivo* decrease in the aminoacylation level of all tRNA<sup>Gly</sup> isoacceptors when cells are exposed to paraquat. Changes in tRNA<sup>Gly</sup> levels induce an increase in production of diadenosine tetraphosphate (Ap<sub>4</sub>A), an nucleotidic alarmon previously shown to affect cell cycle, motility, invasion of epithelial cells and consumption of carbohydrates. We have also found that it alters the efficiency of translation of specific Gly codons. Over-production of tRNA<sup>Gly</sup> alters diverse phenotypes, the strongest of all being the levels of paraquat required to inhibit fermentation of carbohydrates. Additionally, over-production of some tRNA<sup>Gly</sup> isotypes alter the bacterias' growth curve and its motility. Based on all this data, we propose that changes to tRNA<sup>Gly</sup> of *E. coli* participate in the regulation of the response to oxidative stress.

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## 262 When antisense makes sense: exploring the role of RNA polymerase-binding RNA aptamers in control of bacterial antisense transcription

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RNA polymerase (RNAP) is the enzyme responsible for transcription in *Escherichia coli*. Its activity is tightly controlled by multiple factors, such as proteins, RNAs and small molecules. We have identified a new class of RNA regulatory elements that modulate transcription in cis, either by leading to premature termination or antitermination. RNAP-binding RNA aptamers (RAPs) are relatively short RNA sequences (30-100 nucleotides) binding to the RNAP with high affinity. There are approximately 15,000 RAPs 'encoded' in the *E. coli* genome and the majority (~60%) is found antisense to annotated genes (asRAPs). Since the *E. coli* genome is pervasively transcribed from both strands, we hypothesised that antisense RAPs play a significant role in controlling antisense transcription, both positively and negatively. We studied the potential role of asRAPs in modulating antisense transcription. We particularly focused on how RAPs modulate transcriptional interference (TI) happening from two convergent promoters. Differential expression analyses of the *E. coli* transcriptome were performed and several datasets were obtained (i.e. RNA 3' end mapping and RNA deep-sequencing). Combining these data, we were able to identify several asRAP candidates with regulatory potential. The activity of these candidates was studied in depth on TI plasmid-based reporter systems by diverse methods (RT-qPCR, Northern blotting, and fluorescence reporter assays). Our preliminary data suggest that certain asRAPs are able to terminate transcription from the antisense strand, reducing TI and increasing the expression level of genes expressed from the sense promoter. We therefore suggest that asRAPs are widespread modulators of transcription interference.

## 263 The role of matchmaker protein Hfq in promoting the interactions between complementary small RNAs

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Bacterial small regulatory RNA (sRNA) affect gene expression by base pairing with complementary sequences in target mRNAs, which leads to translation activation or repression. The ring-shaped matchmaker protein Hfq facilitates their interactions by simultaneous binding to sRNAs and mRNAs using different RNA binding sites on its ring. However, recent studies showed that sRNAs can also basepair with other sRNAs, in this way inhibiting their function. Among such anti-sRNAs are, for example, tRNA derivative 3'ETS<sub>leuZ</sub> that inhibits RybB and RyhB sRNAs, anti-sRNA AgvB acting on GcvB sRNA, and a transcript of intergenic region chbBC targeting ChiX sRNA. These anti-sRNAs, also named sponges, were found to co-immunoprecipitate with Hfq. This suggests that Hfq could be involved in their interactions and raises a question of how Hfq interacts with sRNAs and anti-sRNAs to promote their pairing.

To explain how Hfq contributes to these interactions, the kinetics of annealing of selected anti-sRNA to their complementary sRNA targets in the presence of wild type Hfq and its variants with mutations in RNA binding sites was compared. The results showed that for ChiX annealing to complementary anti-sRNA either the proximal or the distal binding site of Hfq was sufficient, while for RybB annealing either the proximal or the rim site. However, the annealing of GcvB sRNA to AgvB anti-sRNA was impaired by both the distal and the rim mutation of Hfq. These results suggest flexible use of RNA binding sites on Hfq to promote the pairing of different partly complementary RNAs.

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## 264 Small regulatory RNA networks in *Staphylococcus aureus*: impact on adaptive processes, metabolism and virulence

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*Staphylococcus aureus* is a commensal bacterium of the nose and skin in humans and an opportunistic pathogen responsible for various infections with a predominant extracellular multiplication. It has evolved a large number of strategies to regulate the synthesis of virulence factors, which are produced in response to the host's immunity, stress and various environmental changes. We have demonstrated that RNAs are key intracellular effectors in these adaptive processes and co-regulate the expression of a large number of genes at the post-transcriptional level [1,2]. Using a variety of methods (bioinformatics coupled with expression studies, high throughput sequencing), more than 200 small regulatory RNAs (sRNAs) have been identified so far. The current challenge is to define their direct or indirect targets, the functions they control, the regulatory networks in which they belong, the RNA chaperones assisting them and to establish the interactions existing between sRNAs, two-component systems and regulatory proteins. We have recently applied and adapted the MS2-affinity purification approach [3] coupled to RNA sequencing (MAPS) to determine the targetome of different *S. aureus* sRNAs. Using a combination of *in vivo* and *in vitro* approaches, the co-purified mRNAs are validated as direct sRNAs targets. We use quantitative differential proteomics of wild type and mutant strains to corroborate the MAPS results and Co-IP experiments of different RNA binding proteins to investigate their involvement in the regulatory complexes.

Our analysis shows that *S. aureus* sRNAs act mainly as translational repressors linking metabolism and virulence.

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## 265 The contributions of matchmaker protein Hfq to the interactions of *Escherichia coli* MgrR sRNA with *eptB* and *ygdQ* mRNAs

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The Sm-like protein Hfq facilitates the pairing of *trans*-encoded small RNAs (sRNAs) to their target mRNAs. This ring-shaped protein possesses three independent RNA binding sites. Canonical sRNAs bind to the proximal face of Hfq using their 3'-terminal uridine tails, while their internal AU-rich sequences contact the rim site. mRNAs regulated by these sRNAs bind to the distal face of Hfq using repeated ARN sequences. However, recent studies of the effect of Hfq mutants on gene expression regulation and sRNA stability in bacterial cells showed an alternative mode of sRNA binding to Hfq. It involves simultaneous binding of sRNA's U-rich and A-rich sequences with proximal and distal faces of Hfq, respectively. mRNAs regulated by this subset of sRNAs bind to the rim of Hfq, instead of the distal site, using AU-rich sequences.

MgrR sRNA belongs to those sRNAs, which use the alternative mode of interactions with Hfq for translation regulation. Its transcription is dependent on the PhoP/PhoQ system and it is induced in response to low extracellular Mg<sup>2+</sup> concentration. Among its targets are negatively regulated *eptB* and *ygdQ* mRNAs. Here, the MgrR secondary structure, its binding to Hfq, and the role of Hfq in MgrR annealing to *eptB* and *ygdQ* mRNAs were analyzed to elucidate how Hfq affects this sRNA pairing with target mRNAs. The data showed that MgrR structure consists of three stemloops with an A-rich sequence located in a single-stranded region connecting SL1 and SL2. The proximal face of Hfq was especially important for the equilibrium binding of MgrR to Hfq. However, when the kinetics of MgrR annealing to *eptB* and *ygdQ* mRNA fragments was measured the data showed the essential role of the rim site, while the mutations in the proximal and distal faces of Hfq were less detrimental for the annealing. Moreover, the study of *eptB* and *ygdQ* mRNA mutants allowed confirming the essential role of particular AU-rich sequences for the Hfq contribution to MgrR annealing.

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## 266 A new pipeline to find noncoding RNA (ncRNA) in prokaryotes by using the RiboGap database

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Noncoding RNAs (ncRNAs) play important roles in regulation. To date, in prokaryotes they have been found mostly in 5' UnTranslated Regions (UTR). Therefore, UTRs harbor a lot of useful information regarding sequences and structures of ncRNAs. By associating a gene function to an ncRNA UTR, it is possible to find *de novo* ncRNAs for specific functions. Therefore extracting all 5' UTRs for a chosen gene function can lead us to find *de novo* ncRNAs like riboswitches for particular gene functions. Finding and extracting sequences from these regions (UTR) on a large genomic scale is challenging in itself because there are no direct annotations for these regions in genomic databases such as NCBI or Ensembl, but instead positions of coding sequences. Several ncRNAs were discovered by bioinformatics tools through prediction of structure based on conserved sequences. These approaches are challenging for intergenic sequences because most of them are over 100 nucleotides in length. In order to get around these challenges we propose to use the RiboGap database first to extract and find all intergenic sequences in 5' UTRs for one specific function and then to use prediction tools for secondary structure of RNA. By using this pipeline we have found three potential candidates for riboswitches that could bind some cations.

## 267 Competition for Hfq by CrcZ cross-regulates Hfq-dependent physiological processes unrelated to carbon metabolism

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*Pseudomonas aeruginosa* is a major cause of nosocomial infections and a persistent pathogen in the lungs of cystic fibrosis (CF) patients. Transcriptome studies revealed known and unknown non-coding RNAs that were up-regulated in anoxic biofilms grown in medium resembling the milieu of CF lungs. Among them was the regulatory RNA CrcZ, which was previously shown to bind to, and to sequester the RNA chaperone Hfq. Our previous studies uncovered CrcZ and Hfq as key players in the regulation of carbon catabolite repression and that both impact on the susceptibility of Pae to different antibiotics during anaerobic growth. We show that Hfq is required for anoxic biofilm formation and that CrcZ RNA interferes with this process by competition for Hfq. Similarly, CrcZ cross-regulates susceptibility of *P. aeruginosa* to different antibiotics.

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## 268 Structure to signaling: Understanding roles and mechanisms of non-coding RNAs in bacteria

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To sense and respond to their environment is a fundamental requirement for all organisms. A major mode of signal sensing in response to changing environments is via non-coding RNAs. This is especially evident in bacteria, where ligand-sensing riboswitches and RNA-protein complexes control important processes such as growth, metabolism, adaptations and stress response. Previously, we discovered a class of small RNAs that specifically recruit the RNA-binding ANTAR domain, in response to metabolic cues in many gut bacteria. Metabolite induced phospho- cascades activate the ANTAR protein for RNA recognition and regulation of genes that reside downstream of the RNA. Our broad bioinformatic analyses suggest that the ANTAR protein-RNA regulatory network is widely prevalent across bacteria, and that the central tenets for gene regulation by ANTAR may be conserved in nature. Small RNAs in actinomycetes have been especially hard to identify due to their cryptic promoters and unusual transcription terminators. We have found that actinomycetes, in particular mycobacteria, harbor a number of ANTAR domain proteins and their target ANTAR RNAs. The mycobacterial ANTAR protein undergoes phospho-activation to bind a host of small RNAs in vitro and in mycobacterial cells. These small RNAs share structural but not sequence-based similarity. Using a combination of RNA-protein biochemistry, biophysics and genetics we uncover the mechanism by which these small RNAs function. Pathogenic strains of mycobacteria appear to use ANTAR based regulation for controlling very specific pathogenesis related genes, suggesting a role for these RNA-protein complexes in disease.



## 269 Characterization of *Salmonella* small RNA PinT using MS2 Affinity Purification and RNA-Seq

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Small RNAs (sRNAs) are key mediators of bacterial gene expression, through post-transcriptional regulation. sRNAs regulate the expression of target genes through short imperfect base-pairing interactions, by either promoting or inhibiting transcription or by regulating the transcripts stability and degradation. Over the last decades, bacterial sRNAs have been recognized as a major class of regulatory molecules involved in infection. *Salmonella* Typhimurium has 280 sRNAs, some of which have been implicated in virulence, while many remain to be studied. Many of *Salmonella*'s sRNAs are highly upregulated during infection of mammalian cells.

Dual RNA-seq allows for the simultaneous capture of all classes of coding and noncoding transcripts in both pathogen and host, leading to a better understanding of the physiological changes in host and pathogen during the course of infection. This method has been applied to *Salmonella* infection models, leading to the identification of a previously uncharacterized sRNA, PinT. PinT is an 80 nucleotide long sRNA conserved among the *Salmonella* genus that is consistently highly induced in 14 distinct cell types. PinT is activated by the PhoP/Q two-component system and acts as a regulator of the expression of the SPI-1 and SPI-2 virulence programs. Moreover, PinT has a widespread effect on the host response, with approximately 10% of all human transcripts being differentially expressed after pulse expression of PinT and having an effect on subcellular localization of mitochondria.

Even though some of its targets are known, we still lack understanding on the role of PinT and its complete interacting network during infection. Computational prediction of targets can be difficult and unreliable. To overcome this, we have adapted MAPS (MS2 affinity purification followed by RNA-sequencing) to identify new targets of PinT in infection-relevant conditions. Using this approach we were able to select a group of PinT candidate targets, potentially involved in infection. One of the most promising being secreted effector kinase (*SteC*). SteC is a SPI2 effector protein that has been shown to promote bacterial survival in host tissues. With this work we aim to complement the knowledge on the biological function and regulatory network of PinT and contribute to a better understanding *Salmonella* infection.

## 270 sRNAs in transcription termination control in *E. coli*

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Remarkably, more than a quarter of *E. coli* genes contain an annotated long 5'UTR (>80 nt) – a ribosome-free segment of the nascent transcript located upstream of the translation start site that serves as a hub for various regulatory signals. Here we provide evidence that Rho termination factor acts within 5'UTRs of many bacterial genes and therefore functions as a global attenuator of gene expression.

Bacterial small RNAs (sRNAs) have been implicated in the modulation of translation initiation and/or mRNA stability by base-pairing with regulatory motifs of mRNAs. Here we demonstrate that sRNAs also act at the level of transcription termination. We use the *rpoS* gene, a general stress sigma factor  $\sigma^S$ , as a model system, and show that sRNAs (DsrA, ArcZ, and RprA) bind the *rpoS* 5'UTR to suppress premature Rho-dependent transcription termination, both *in vitro* and *in vivo*. sRNA-mediated antitermination markedly stimulates transcription of *rpoS* during the transition to the stationary phase of growth, thereby facilitating a rapid adjustment of bacteria to global metabolic changes. Next generation total RNA sequencing and bioinformatic analysis suggest that sRNAs-mediated suppression of Rho is a widespread mode of bacterial gene regulation. Comprehensive characterization of sRNAs-Rho interactome is underway.

**271 The mRNA sequence adjacent to sRNA binding sites affects the kinetics of sRNA annealing***Daria Sobanska<sup>1</sup>, Elzbieta Kierzek<sup>2</sup>, Ryszard Kierzek<sup>2</sup>, Mikolaj Olejniczak<sup>1</sup>*<sup>1</sup>**Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznan, Poland;** <sup>2</sup>**Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland**

The regulation of translation by bacterial small RNAs depends on their pairing to complementary sequences in regulated mRNA. Several studies showed the dependence of translation regulation on the thermodynamic stability of small RNA pairing to mRNA. However, it was also proposed that the kinetics of small RNA association to mRNA could be important for translation regulation. Interestingly, the binding sites of several sRNAs are flanked on the 3' side by a conserved adenosine residue. One example is *Salmonella enterica* RybB sRNA, which pairs to mRNA using its 5'-end sequence. Previous UV-melting studies of short model RNA duplexes showed that a single-stranded purine 3' adjacent to a GC-pair ending duplex had a stabilizing effect on the duplex.

Because the complex of RybB 5'-end seed sequence with its binding site in mRNA resembles such a model duplex, we decided to measure the equilibrium binding of RybB sRNA to mRNA fragments with different 3' adjacent nucleotide residues. Moreover, the kinetics of annealing of RybB to these mRNA fragments was also measured. In both types of experiments electrophoretic mobility shift assays were used. Besides, UV melting experiments were applied to measure the thermodynamic stability of short model duplexes. The results showed that when the 3'-adjacent nucleotide was adenosine or guanosine the association rates were faster than when it was cytosine or uridine. Moreover, the data showed that the changes in the thermodynamic stability dependent on the identity of 3' adjacent nucleotides correlated with the changes in the rates of sRNA-mRNA association, while the calculated dissociation rates were uniform. These data suggest that mRNA sequence adjacent to sRNA binding sites evolved to increase the rates of sRNA annealing to mRNA.

**272 Auxiliary Factors of the RNA helicase Prp43 in ribosome biogenesis***Priya Bhutada, Stefan Unterwieser, Lisa Kofler, Brigitte Pertschy***Institute of Molecular Biosciences, University of Graz, Graz, Styria, Austria**

Ribosome biogenesis is a complex, tightly regulated and highly energy demanding process. It needs participation of RNA-polymerases, 82 ribosomal proteins, 66 (small nucleolar RNA) snoRNAs and more than 200 non ribosomal proteins which carry out molecular rearrangements, modifications and rRNA processing steps within ribosomal precursors for maturation and functional assembly of ribosome. These include assembly factors, endo -and exonucleases, ATPases, transport factors, RNA helicases.

Among the RNA helicases, Prp43 DEAH/RHA helicase plays multiple roles at different stages in both 40S and 60S ribosomal subunit biogenesis and also participates in mRNA splicing. As Prp43 is responsible for essential processes, it necessitates the engagement of many coordinating auxiliary factors for the accomplishment of its functions. These co-factors of Prp43 include a group of proteins sharing a glycine-rich domain, termed G-patch domain. G-patch proteins Ntr1, Pxr1, Pfa1 and Cmg1 are known to stimulate ATPase and RNA helicase activity of Prp43. In mRNA splicing G-patch protein Ntr1 is responsible for Prp43 helicase activation, while the non G-patch auxiliary factor Ntr2 functions as adaptor for the recruitment of Prp43 to the spliceosome. Pxr1 and Pfa1 are involved in ribosome biogenesis.

Here we show that Prp43 is a component of ribosome precursor particles from 90S to both 60S and 40S subunit biogenesis. Pxr1 and Pfa1 activate Prp43 in ribosome biogenesis, with Pxr1 being mainly involved at the level of 90S particles, and Pfa1 presumably functioning at later stages of both 40S and 60S maturation. This finding indicates distinct roles of Pxr1 and Pfa1 during ribosome biogenesis. Along with Prp43 co-factors we also identified a G-patch protein Associated Factor named Gaf1. Gaf1 is a novel ribosome biogenesis factor involved at the level of 90S particles, as well as at early stages of 60S maturation. It is interesting to investigate the functional role of Gaf1 in ribosome biogenesis in association with Prp43.

## 273 Three Major Remodeling Events Within Assembling Ribosomes are Required for Irreversible Removal of a Pre-rRNA Spacer Sequence

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Three interdependent processes drive ribosome biosynthesis *in vivo*: pre-ribosomal RNA (pre-rRNA) folding and processing and binding of ribosomal proteins to the pre-rRNA. In yeast, ribosome assembly is facilitated by the actions of ~200 protein assembly factors. Assembly is hierarchical and progresses through establishment and restructuring of ribonucleoprotein (RNP) interaction networks. A major goal at this time is to establish what these networks are, how they are remodeled, and how their reconstruction drives assembly forward in an efficient and accurate manner. We have focused on the events required for one particular remodeling event, irreversible removal of the internal transcribed spacer 2 (ITS2) RNA from the pre-60S ribosomal subunit. Our approach has been to isolate mutants that cannot carry out this pre-rRNA processing event and assay effects of these mutations on pre-ribosome protein composition. Critical to our analysis was our recent determination of near atomic resolution cryo-electron microscopy (cryo-EM) structures of three consecutive late nuclear pre-60S subunit assembly intermediates. From these structures, we were able to identify RNP networks affected in mutants that cannot process ITS2. Based on their mutant pre-rRNA processing phenotype, ~30 ribosomal proteins and assembly factors are known to be required for endonucleolytic cleavage at the C<sub>2</sub> site, which initiates processing and removal of ITS2. Analysis of these mutants revealed that there are three major remodeling events that are necessary for C<sub>2</sub> cleavage to occur. (1) A group of assembly factors bound proximal to ITS2 must be released. (2) Several assembly factors that bind to the nascent peptidyl transferase center must associate with the pre-60S subunit. (3) Seven ribosomal proteins surrounding the exit of the polypeptide exit tunnel must become stably associated with the pre-60S subunit. We believe that these three events reveal the existence of a complicated checkpoint that prevents pre-60S subunits with improperly structured functional centers from undergoing the irreversible step of ITS2 removal.

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## 274 A novel pre-ribosomal subcomplex required for pre-rRNA processing and turnover

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The production of eukaryotic ribosomes is one of the most energy-consuming cellular processes during which, a myriad of enzymatic proteins catalyses important maturation events, including ribosomal (r)RNA processing, modification and folding. Such irreversible steps drive the directionality of the assembly pathway and careful spatial and temporal regulation of the enzymes that catalyse them is required. RNA helicases play major roles in structural remodelling of pre-ribosomal complexes and their activity is often regulated by dedicated cofactors, such as G-patch proteins. Here, we show that the human G-patch protein NF-κB repressing factor (NKRF) forms a nucleolar subcomplex with the DExH-box helicase DHX15 and the 5'-3' exonuclease XRN2. Using UV crosslinking and analysis of cDNA (CRAC), we demonstrate that NKRF crosslinks to the transcribed spacer regions of the pre-rRNA transcript and that depletion of NKRF, XRN2 or DHX15 impairs an early pre-rRNA cleavage step, the metazoan-specific processing at the A' site in the 5'-external transcribed spacer (5'ETS). Our data further show that the catalytic activity of DHX15 is required for this processing step and that NKRF functions as a cofactor of DHX15 and stimulates its ATPase and unwinding activity, suggesting that a structural remodelling event may facilitate processing at the A' site. Furthermore, depletion of NKRF or XRN2 leads to the accumulation of excised pre-rRNA spacer fragments and we show that NKRF is essential for recruitment of the exonuclease to nucleolar pre-ribosomal complexes. Our findings therefore reveal a novel pre-ribosomal subcomplex that plays various roles in the maturation of pre-rRNAs and the turnover of excised spacer fragments.

## 275 Towards the molecular characterization of the DExH-box helicase DHX37 involved in eukaryotic ribosome biogenesis

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Eukaryotic ribosome biogenesis is a highly complex process that relies on the stepwise processing of a large precursor rRNA by at least 200 trans-acting protein factors and snoRNPs. Among those accessory factors are ~20 DExD/H-box helicases that use their ATP-dependent RNA unwinding activities to dissociate snoRNAs from pre-rRNA or to mediate structural remodeling of pre-ribosomal particles. Most DExD/H-box helicases do not possess intrinsic substrate specificity for their RNA substrate and instead rely on specific cofactors that target them to their sites of action and modulate their ATPase and helicase activities. DHX37 is involved in the assembly of the 40S ribosomal subunit, but precise molecular functions and interaction networks remain elusive. The yeast homologue of DHX37, Dhr1, dislodges U3 snoRNA from pre-18S rRNA to promote formation of the central pseudoknot, a key architectural feature of the 40S subunit<sup>1</sup>. Whether DHX37 fulfills similar functions in mammalian 40S biogenesis, and how it is regulated during ribosome biogenesis, remains to be elucidated. To shed light on the molecular functions of DHX37 in 40S assembly, we aim to obtain crystal structures of DHX37 in different catalytic states and complexes. To this end, we expressed and purified DHX37 recombinantly in insect cells. *In vitro* binding assays showed that RNA binding is independent of ATP and ADP. We currently focus on determining the crystal structure of DHX37 in complex with RNA. Here we present our ongoing work towards the biochemical and structural characterization of DHX37.

1. R. Sardana *et al.*, The DEAH-box helicase Dhr1 dissociates U3 from the pre-rRNA to promote formation of the central pseudoknot. *PLoS Biol* **13**, e1002083 (2015).

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## 276 Study of Efg1 in the yeast *S. cerevisiae* revealed a novel endonucleolytic pathway participating in the decay of accumulated preribosomes

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Ribosome biogenesis requires the coordinated action of at least 200 maturation factors. It begins in the nucleolus, with the transcription of the rDNA by RNA Pol. I and the co-transcriptional assembly of the SSU Processome. The correct assembly of this pre-ribosomal particle is needed for A0, A1 and A2 cleavages which lead to the separation of the 43S and 66S pre-ribosomal particles, precursors of the mature subunits. Here we report that the *Saccharomyces cerevisiae* Efg1 is a non-essential protein localized in the nucleolus. Co-immunoprecipitation experiments showed that Efg1p is associated with the 35S pre-rRNA, the 23S RNA and U3 snoRNA, suggesting that it is a bona fide component of SSU Processome. Depletion of Efg1 resulted in a massive accumulation of 23S RNA due to a large delay in A0, A1 and A2 cleavages. This observation gave us the opportunity to study the fate of the 23S RNA in yeast. Our results indicated that 23S RNA is targeted by a novel degradation pathway involving the endonuclease Utp24 and the TRAMP/exosome complexes.



## 277 High-resolution analysis of native pre-ribosome translocation through the nuclear pore complex by electron tomography

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The nuclear pore complex (NPC) forms a large channel through the nuclear envelope and filters the macromolecules circulating between the nucleus and the cytoplasm. In addition to ensuring the proper nucleocytoplasmic transport of numerous proteins involved in nuclear functions, it mediates nuclear export of ribonucleoprotein particles (RNP)s, including messenger RNPs and pre-ribosomes. Understanding the functioning of the NPC in nuclear export of RNPs requires a knowledge of its 3D structure at high resolution and the interactions established by RNPs with the hundreds of nucleoporin proteins (Nups) that compose it. Nevertheless, translocation of RNPs is a rapid process difficult to record at single molecule level. Using fast high-pressure freezing and electron tomography, we could detect native RNPs translocating through the NPC in yeast and analyze their trajectory at high resolution. Particles were detected in 5-6% of more than 700 NPCs. Their resemblance with nucleolar RNPs and their absence in RNA polymerase I mutants indicated that these particles are primarily pre-ribosomes. They were detected with equal frequency in NPCs adjacent to the nucleolus and in those adjacent to the nucleoplasm. They followed the central axis of the NPC through the nuclear and inner rings. In contrast, they deviated from the central axis in the cytoplasmic ring, suggesting interactions with the cytoplasmic nucleoporins. By applying a Jackson queuing model to these electron microscopy data, we could estimate the translocation time of pre-ribosomes through NPCs to ~90 ms. These data deliver a nanometer scale image of pre-ribosome translocation through the NPC.

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## 278 Poly(A)-specific ribonuclease is a nuclear ribosome biogenesis factor involved in human 18S rRNA maturation

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Poly(A)-specific ribonuclease (PARN) owes its name to its deadenylase activity and has been extensively studied for its role in poly(A) tail shortening in the context of mRNA degradation. However, the functional repertoire of PARN has meanwhile been extended to the degradation and maturation of different types on non-coding RNAs, including scaRNAs and box H/ACA snoRNAs, the human telomerase RNA component, miRNAs, and piRNAs. Mutations in PARN were recently linked to dyskeratosis congenita and pulmonary fibrosis. Since we had previously identified PARN as a component of human pre-40S particles, we set out to test whether PARN assists 40S ribosomal subunit biogenesis. We now demonstrate that PARN is part of the enzymatic machinery that matures the human 18S ribosomal RNA (rRNA). Consistent with its nucleolar steady-state localization, PARN is required for 40S ribosomal subunit production and co-purifies with 40S subunit precursors. Depletion of PARN or expression of a catalytically-compromised PARN mutant result in accumulation of 3' extended 18S rRNA precursors. Analysis of these processing intermediates revealed a defect in 3' to 5' trimming of the internal transcribed spacer 1 (ITS1) region, subsequent to endonucleolytic cleavage at site E. Consistent with a function of PARN in exonucleolytic trimming of 18S-E pre-rRNA, recombinant PARN can process the corresponding ITS1 RNA fragment *in vitro*. Trimming of 18S-E pre-rRNA by PARN occurs in the nucleus, upstream of the final endonucleolytic cleavage by the endonuclease NOB1 in the cytoplasm. These results identify PARN as a new component of the ribosome biogenesis machinery in human cells. Defects in ribosome biogenesis could therefore underlie the pathologies linked to mutations in PARN.



## 279 Characterizing the ribosome assembly pathways in *E. coli* using time-resolved RNA footprinting methods

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Bacterial ribosome assembly has been studied *in vitro* for decades, however, we still have limited information on the rRNA folding pathway in the cell. Previous studies either assembled the ribosome in the test tube, or stalled assembly by depriving the cell of essential ribosomal proteins and assembly factors. However, neither of these methods can reveal how the rRNAs are folded in the cell in real time. To study the path of ribosome assembly under normal growth conditions, we used time-resolved dimethyl sulfate (DMS) and X-ray hydroxyl radical footprinting to monitor pre-rRNA structural changes in real time in *E. coli* cells. In addition, we characterized the kinetics of nascent rRNA synthesis and assembly using sucrose gradients and primer extension. Our preliminary data shows that the premature ribosomes can be captured when cells are growing at a lower temperature, and our methods reveal structural changes within the pre-rRNAs at early time points. These results provide nucleotide specific conformational dynamics on the rRNAs as they are transcribed, folded and processed *in vivo*. Also, the techniques in this study can be used to characterize ribosome assembly in other organisms and examine other large RNA-protein complex assembly in general.

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## 280 The T-ALL related *rpl10-R98S* mutant traps the 60S export adapter Nmd3 in the ribosomal P site

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Mutations in the ribosomal protein Rpl10 (uL16) can be drivers of T-cell acute lymphoblastic leukemia (T-ALL). We previously showed that these T-ALL mutations, including *rpl10-R98S*, disrupt late steps in cytoplasmic maturation of the 60S subunit in yeast, preventing the release of the nuclear export adapter Nmd3 and the subunit antiassociation factor Tif6. Consequently, these mutant ribosomes do not efficiently pass the quality control checkpoint and are blocked from engaging in translation. Here, we characterize suppressing mutations that bypass this block and show that the molecular defect of *rpl10-R98S* is a failure to release Nmd3 from the P-site. We recently presented the atomic structure of Nmd3 on the 60S subunit and showed that domains of Nmd3 span the joining face of the subunit, from the L1-stalk, through the E- and P-sites with the N-terminus of Nmd3 interacting directly with Tif6. Based on this structure, we proposed that Nmd3 interaction with Tif6 must be broken to permit the retraction of Nmd3 from the P site by the L1 stalk. This retraction would allow the P-site ligand Sdo1 to bind in the P site to trigger the release of Tif6 by the EF-G-like GTPase Efl1. Thus, a model emerged that the defect of *rpl10-R98S* may be a failure in retraction of Nmd3 from the P site. We identified *rpl10-R98S* suppressing mutations in multiple domains of Nmd3 as well as in Tif6. These mutations disrupt the interaction between Nmd3 and the ribosome or between Nmd3 and Tif6. Using an *in vitro* system with purified components, we found that while Nmd3 does not compete with Sdo1 for binding to the P-site of wild-type ribosomes, Nmd3 does inhibit Efl1 activity on mutant *rpl10-R98S* ribosomes. Importantly, this inhibition is overcome *in vitro* by mutations in Nmd3 that suppress *rpl10-R98S* *in vivo*. These results strongly support the model that Nmd3 must be dislodged from the P-site to allow Sdo1 activation of Efl1 and define a failure in the removal of Nmd3 as the molecular defect of the T-ALL-associated *rpl10-R98S* mutation. Identifying how this defect leads to T-ALL remains to be determined.

## 281 **S<sub>ynthesis</sub>T<sub>akes</sub>O<sub>ne</sub>P<sub>ause</sub>! Screening for novel inhibitors of eukaryotic ribosome biogenesis**

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Ribosomes are outstanding molecular nanomachines that synthesize all cellular proteins and hence form the basis for cellular growth. Eukaryotic ribosome biogenesis, an enormously intricate and highly dynamic process, requires not only 80 ribosomal proteins and four ribosomal RNAs (rRNAs) but more than 200 ribosome assembly factors. In the course of this process these assembly factors perform molecular rearrangements, rRNA processing and modifications or are involved in the nuclear export of maturing ribosomal particles. Due to the high complexity and immense speed of ribosome synthesis, the specific functions of most ribosome assembly factors remain still elusive.

A valuable tool to gain deeper insights into these mechanisms is to block the rapidly ongoing ribosome formation at a certain step. To this end, we developed a screen to identify novel inhibitors that target either the 40S or 60S ribosomal subunit maturation. We discovered several compounds blocking ribosome biogenesis leading to various rRNA processing defects. Treatment with these individual drugs might provide

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## 282 **Elucidating the assembly and quality control mechanisms of peptidyl transferase center and peptide exit tunnel construction within the 60S subunit of *S. cerevisiae* ribosomes**

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Efficient and accurate protein synthesis requires proper assembly of ribosomal functional centers. Defective ribosome assembly disrupts protein homeostasis and causes ribosomopathies and cancer. Two essential functional centers, the peptidyl transferase center (PTC) and the peptide exit tunnel (PET), regulate protein synthesis. Mechanisms involved in PET and PTC assembly have remained elusive due to lack of structural data. However, recent advances in cryo-EM technology enabled us to visualize the Nog2-TAP, Arx1-TAP and Nmd3-TAP pre-60S structures in *S. cerevisiae*, an unprecedented feat. These structures informed hypotheses regarding PET and PTC construction. For example, the Nog2-TAP structure shows that the essential assembly factor Nog1 may have multiple roles in ribosome assembly; it probes the rRNA of the PTC with its N-terminal helix bundle domain and reaches around 2/3 of the ribosome to probe the PET with its C-terminus. Adjacent to the helix bundle, Nog1 has an Obg-like GTPase domain whose function in ribosome assembly remains unknown. Furthermore, when the Nog2-TAP, Arx1-TAP and Nmd3-TAP structures are compared, a theme arises: the C-terminal extensions of three assembly factors (first Nog1, followed by Rei1, then Reh1) probe the exit tunnel and reach to the peptidyl transferase center (PTC). Interestingly, both Rei and Nog1 bind the export factor Arx1 at the PET exit. To give mechanistic insight into PET and PTC assembly, we are assessing ribosome biogenesis defects that result from mutations in the helix bundle, GTPase domain and C-terminal domains of Nog1, using northern blot analysis, affinity purification of assembly intermediates and western blot analysis. We propose that the helix bundle domain of Nog1 holds the rRNA of the PTC in an immature conformation, and that GTP hydrolysis catalyzed by the GTPase domain proximal to the helix bundle creates structural rearrangements in Nog1 that remove the helix bundle of Nog1 from the PTC. Additionally, we are testing whether the C-termini of Nog1, Rei1 and Reh1 inspect the PET for proper structure and stabilize the pre-ribosome. Finally, we report that the entry of Nog1 into the PET is a prerequisite for Arx1 binding. Thus, the C-terminus of Nog1 may license the pre-ribosome for nuclear export.

## 283 Structural Analysis Reveals the Features of Ribosome Assembly Factor Nsa1/WDR74 Important for Localization and Interaction with the AAA-ATPase Rix7/NVL2

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Ribosome assembly is a complex process that requires hundreds of essential assembly factors, including Rix7 (NVL2 in mammals) and Nsa1 (WDR74 in mammals). Previous studies in *Saccharomyces cerevisiae* suggest that Rix7 mediates the release of Nsa1 from nucleolar pre-60S particles however the underlying mechanisms of this release are unknown. Here, we solved the first crystal structure of *S. cerevisiae* Nsa1 at 1.3 Å resolution and used small angle X-ray scattering to determine the solution structure of full length Nsa1. Through a series of co-immunoprecipitation and immunofluorescence assays with the mammalian homologues we found that the N-terminal WD40 domain of WDR74 is required for interaction with NVL2 while the flexible C-terminal tail is required for nucleolar localization. Intriguingly we found that the interaction between WDR74 and NVL2 is not dependent upon RNA or pre-60S particles demonstrating that WDR74 and NVL2 can associate with one another both on and off pre-60S particles. We further show that WDR74 associates with the D1 AAA domain of NVL2, which represents a novel mode of binding of a substrate with a type-II AAA-ATPase.

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## 284 New Methods for Studying Ribosome Biogenesis in the Model Organism *X. tropicalis*

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The study of ribosome biogenesis has far-reaching consequences for revealing principals of basic biology and elucidating mechanisms of ribosomopathies, a family of disorders characterized by dysfunctions in making ribosomes. In order to fully understand this process in humans, assays in a whole organism are necessary. The development of the frog *Xenopus tropicalis* as a model organism provides a marked advantage over previously used tissue culture and yeast models because they are multicellular vertebrates with a close phylogenetic relationship to humans. Additionally, their ex-utero development provides ease in administering treatments and examining gross embryonic morphology, allowing the visual analysis essential to phenotypically understand ribosomopathies as developmental disorders. In order to probe the molecular basis of aberrant ribosome biogenesis, we developed a luminescence-based reporter assay to measure RNA polymerase I (RNAPI) transcription of the ribosomal DNA in *X. tropicalis*. This assay will give insight into understanding how depletion of proteins may affect RNAPI activity during embryonic development and the extent to which this grossly affects embryogenesis. This is significant because nucleolar maintenance and the development of multicellular organisms are dependent on pre-rRNA transcription. Additionally, we developed a puromycin incorporation assay previously used in cultured cells for use in *X. tropicalis*. By using an optimal concentration that does not kill the embryos or fully inhibit translation, puromycin incorporates into nascent proteins that can then be detected by western blot. This is used as a proxy to quantify global protein synthesis. We will use this new assay to quantify how depletion of proteins influences the activity of mature ribosomes. The development and optimization of these assays for use in *X. tropicalis* is an important advancement to gaining a better understanding of ribosome biogenesis at the level of the whole organism.

## 285 Role Of The Rpf2/Rrs1 Heterodimer In The Coupling Between RNA Polymerase I Transcription And Pre-ribosome Maturation In Yeast

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The biogenesis of ribosomes, is a vital multistep process consuming an important part of the cellular energy. Ribosome biogenesis begins with the transcription of the ribosomal DNA (rDNA) units by RNA polymerase I (Pol I). This process generates a primary transcript that associates with a large number of assembly and maturation factors in order to generate a large initial pre-ribosomal particle. This particle undergoes a complex maturation pathway which gives rise to the pre-40S and pre-60S particles, precursors to the small and large ribosomal subunits, respectively. Assembly and maturation of pre-ribosomal particles occur co-transcriptionally, suggesting a close coordination in space and time between Pol I transcription and pre-ribosome assembly. However the factors and molecular mechanisms involved in this coordination remain poorly understood. We show that the Rpf2-Rrs1 heterodimer, known to be essential for the maturation of the large ribosomal subunit through the recruitment of the 5S RNP, is involved in the functional coupling between rDNA transcription and pre-rRNA maturation. Contrarily to other pre-60S factors also associating co-transcriptionally with nascent pre-ribosomes, inactivation of Rpf2 and Rrs1 strongly affects Pol I occupancy on the rDNA and leads to strong perturbations of rDNA unit organization observed by Miller spreads. We show that Rpf2 and Rrs1 directly affect Pol I function through interactions with rDNA chromatin, with several rDNA-associated chromatin remodeling factors and with proteins affecting Pol I function.

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## 286 rRNA degradation in Escherichia coli under the starvation stress

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Bacterial cells of the liquid butch culture enter into the stationary phase with growth arrest when the available nutrient is consumed up, and can remain viable for several days under the starvation conditions. In the case of Escherichia coli, physiological state of cells in the exponential growth phase (log phase) is quite different from that in the growth arrested phase (stationary phase).

Our previous data showed that in some nutrient condition, the amount of total RNAs in the cells of stationary phase is degraded quickly. Considering that the rRNA is the most abundant RNA in the cells, it is likely that the degradation rate of rRNA is varying according to the nutritional condition, and which may be the important strategy of physiological adaptation to the starvation stress.

To understand the change of the state of RNA degradation and turnover in the bacterial cells, we have tried to investigate the degradation pattern of RNAs in the course of the life time of E. coli cells under the various nutrient conditions. The results showed that the ratio of rRNA species (5S, 16S and 23S) were almost stable through cell cycle, but the relative amount of 3' region of 16S rRNA was unstable, suggesting that the degradation of rRNA is not uniformly occurred through the entire region of rRNA. Based on these results and the analysis of gene-knockout mutants, we will discuss the bacterial strategy of adaptation to the starvation.

## 287 Identification of Karyopherins involved in the nuclear import of RNA exosome subunit Rrp6 in *Saccharomyces cerevisiae*

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The exosome is a conserved multiprotein complex essential for RNA processing and degradation. The nuclear exosome is a key factor for pre-rRNA processing through the activity of its catalytic subunits, Rrp6 and Rrp44. In *Saccharomyces cerevisiae*, Rrp6 is exclusively nuclear and has been shown to interact with exosome cofactors. With the aim of analyzing proteins associated with the nuclear exosome, in this work, we purified the complex with Rrp6-TAP, identified the co-purified proteins by mass spectrometry, and found karyopherins to be one of the major groups of proteins enriched in the samples. By investigating the biological importance of these protein interactions, we identified Kap95 and Sxm1 as the most important karyopherins for Rrp6 nuclear import and the nuclear localization signals recognized by them. Based on the results shown here, we propose a model of multiple pathways for the transport of Rrp6 to the nucleus.

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## 288 Determining the roles of the nuclease Nob1 in regulating translation initiation and ribosome quality control

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Normal cell function relies on the proper assembly and quality control of ribosomes. Ribosome assembly involves over 200 transiently binding proteins called assembly factors, which not only aid in ribosome formation, but also ensure that immature and defective ribosomes do not initiate translation. The last stage of ribosome maturation, known as the translation-like cycle, is thought to act as a series of quality control steps to inspect major functions of the maturing small subunit.

The assembly factor Nob1 is responsible for 3' –end cleavage of 20S pre-rRNA to produce the mature 18S rRNA. Interestingly, in Nob1 deficient cells, pre-40S subunits containing 20S pre-rRNA enter polysomes and initiate translation. However, when a dominant-negative, catalytically-inactive Nob1 is overexpressed, 20S-containing pre-40S ribosomes accumulate in 80S-like ribosomes but are not recruited to the polysomes, indicating that the presence of Nob1 blocks entry into the polysomes. The growth defect of cells overexpressing this Nob1 mutant protein is rescued by overexpressing Rio1, a serine kinase that associates with late pre-40S ribosomes and is thought to be involved in processing of 20S pre-rRNA into the mature 18S rRNA. Our findings support previous findings that Rio1 is involved in the Nob1-mediated rRNA cleavage step, and suggest that Nob1 regulates recruitment of pre-40S ribosomes to the polysomes. Ongoing experiments will further test the hypothesis that Nob1-mediated rRNA cleavage and dissociation serves as a quality control checkpoint in ribosome biogenesis.



## 289 The ribosome biogenesis factor yUtp23/hUTP23 coordinates key interactions in the yeast and human pre-40S particle and hUTP23 contains an essential PIN domain

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One of the biggest challenges and energy-demanding processes in the cell is the production of the four mature ribosomal (r)RNA components of the small (40S) and large (60S) ribosomal subunits. During pre-rRNA processing, a number of timely and spatially regulated endo- and exonucleolytic cleavages release the mature 18S, 5.8S and 25S/28S rRNAs from a single precursor molecule. Three early pre-rRNA cleavages in yeast (y) and human (h) 18S rRNA maturation (at sites A0, A1 and A2 in yeast and sites A0, 1 and 2a in humans) require the correct assembly of a large ribonucleoprotein complex known as the SSU processome, which contains two proteins with evolutionarily conserved PIN endonuclease domains, yUtp24(Fcf1)/hUTP24 and yUtp23/hUTP23.

The yUtp24/hUTP24 PIN endonuclease is proposed to cleave at sites A1/1 and A2/2a, but the enzyme cleaving at site A0 is not known. In budding yeast, yUtp23 contains a degenerate PIN domain (only 2 out of 3 required catalytic residues are present) and likely plays a non-enzymatic role. In contrast, hUTP23 harbours three PIN domain active site residues, which could be sufficient for enzymatic activity. The yUtp23 protein functions together with a H/ACA snoRNP, snR30, while hUTP23 is associated with U17, the human snR30 counterpart.

Here, using *in vivo* RNA-protein crosslinking and gel shift experiments, we show that yUtp23/hUTP23 makes direct contacts with expansion sequence 6 (ES6) in the 18S rRNA sequence and that yUtp23 interacts with the 3' half of the snR30 snoRNA. Protein-protein interaction studies further demonstrate that yUtp23/hUTP23 directly interacts with the H/ACA snoRNP protein yNhp2/hNHP2, the RNA helicase yRok1/hROK1(DDX52) and the ribosome biogenesis factors yRrp7/hRRP7 and yUtp24/hUTP24. yUtp23/hUTP23 could therefore be central to the coordinated integration and release of ES6 binding factors and likely plays a pivotal role in remodelling this pre-rRNA region in both yeast and humans. Studies using RNAi-rescue systems in human cells further reveal that intact PIN domain and Zinc finger motifs in hUTP23 are essential for 18S rRNA maturation. This raises the exciting possibility that hUTP23 may be an active nuclease.

## 290 A conserved metastable rRNA junction essential for bacterial 30S biogenesis

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The bacterial ribosome faithfully assembles co-transcriptionally from its rRNA and protein components. Conserved motifs in the bacterial rRNA form essential structural domains in the mature ribosome that guide the path of assembly. The junction between helices 5 and 6 (J5/6) of the 16S rRNA in the 30S ribosome has a consensus sequence of a Right Angle<sup>1</sup> motif. However, J5/6 adopts a splayed structure in the mature 30S ribosome, suggesting that J5/6 encodes a metastable structure that changes during the assembly process. J5/6 mutations that destabilize the right angle motif are recessive lethal in *E. coli*. Tagged 30S subunits containing J5/6 mutations are unable to support growth and never mature. *In vitro* assays show that mutations in J5/6 cause local structural perturbations and favor a non-native conformation of 16S helix 3. *In vivo* footprinting of tagged pre-30S ribosomes revealed higher exposure of helix 35-37, suggesting the absence of r-protein S2, which was confirmed by mass spectrometry. The central pseudoknot, an essential structure that forms active site in the 30S, is not formed in the mutant ribosome. Negative stain electron microscopy suggests that the structure of the mutant ribosome is severely distorted. Together, our data indicate that the mutations in J5/6 perturb a conformational switch in the 16S 5' domain that negatively influences packing of helix 15 against helix 6, favoring a non-native structure of helix 3. This effect is communicated to the central pseudoknot and from there to the rest of the 30S ribosome, blocking the addition of key ribosomal proteins and preventing maturation of the 16S rRNA. These results show how conformational switches in peripheral regions of the 16S rRNA are linked with organization of the decoding center and subunit maturation.

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## 291 Assembly of the Ribosomal Large Subunit Requires the Internal Loops of uL4 and uL22 in the Polypeptide Exit Tunnel

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Construction of the ribosomal large subunit (LSU) requires correct folding and positioning of ribosomal RNA (rRNA) and ribosomal proteins (r-proteins) into functionally competent centers. This process requires the activity of assembly factors that associate transiently with pre-ribosomes. Failure to properly construct these functional centers can activate checkpoints that halt ribosome assembly. Few of these checkpoints are understood on a mechanistic level. The polypeptide exit tunnel (PET) is a functional center of the LSU that acts as a conduit for nascent chains to exit translating ribosomes. The PET possesses rRNA and r-protein components that actively participate in translational stalling. Necessary for this stalling, the internal loops of two r-proteins, uL4 and uL22, create a constriction site in the PET. In order to assess the role these internal loops play in assembly events, we assayed pre-rRNA processing and pre-ribosomal particle composition using biochemical and mass-spectrometric methods to show that the PET-inclusive regions of the uL4 and uL22 internal loops are required for assembly of the LSU. uL22 internal loop deletions result in impaired growth and lethality while all analogous uL4 deletion mutants are not viable. Pre-ribosomal particles containing mutant uL4 accumulate nucleoplasmic 7S pre-rRNA intermediates and fail to recruit the essential assembly factor Sda1, indicating a block in remodeling events normally occurring just prior to nuclear export. Our results suggest that correct construction of the PET requires the placement of the uL4 and uL22 internal loops in the PET and failure to do so activates a previously uncharacterized checkpoint that affects necessary maturation events in other functional centers through an unknown mechanism. We speculate that the newly visualized GTPase Nog1 may sense these defects in PET construction. The eukaryote-specific C-terminal extension of Nog1 occupies the entirety of the PET, reaching all the way to the peptidyl transferase center (PTC). In addition, the N-terminal domain of Nog1 binds to the PTC. Thus, Nog1 appears to interact with uL4 and uL22 in the PET, but also with the PTC, possibly coordinating construction of both functional centers.

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## 292 Investigating the role of lncRNA HOTAIR in heterochromatin establishment via a match-making mechanism

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The human long noncoding RNA (lncRNA) HOTAIR is thought to act in trans to recruit the Polycomb repressive complex 2 (PRC2) to the HOXD gene cluster where it contributes to gene silencing in cell differentiation. It has remained unclear what factors determine HOTAIR-dependent PRC2 activity at specific genomic loci, particularly when high levels of HOTAIR result in aberrant gene silencing and cancer phenotypes. Using quantitative mass spectrometry to profile the HOTAIR interactome, we identified the heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1 as the most enriched nuclear protein specifically bound to HOTAIR. A2/B1 is a member of a family of proteins involved in nascent mRNA processing and RNA matchmaking. Our data suggest that A2/B1 are key contributors to HOTAIR-mediated chromatin regulation in breast cancer cells: A2/B1 knockdown reduces HOTAIR-dependent breast cancer cell invasion and decreases PRC2 activity at the majority of HOTAIR-dependent loci. We found that the B1 isoform (which differs from A2 by 12 additional amino acids) binds with the highest specificity to HOTAIR. B1 also binds chromatin and associates preferentially with RNA transcripts of HOTAIR gene targets. We also demonstrate a direct RNA–RNA interaction between HOTAIR and the target transcript JAM2, which is enhanced by B1. Together, these results suggest a model where B1 matches HOTAIR with nascent transcripts of target genes on chromatin, leading to H3K27me3 by PRC2 and gene silencing.

## 293 Cytosine methylation by DNMT2 facilitates stability and survival of HIV RNA in the host cell during infection

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DNA methyltransferase 2 is a predominantly nuclear protein that localizes to stress granules during cellular stress. Since DNMT2 lacks any canonical nuclear localization or export signal, cytoplasmic localization of DNMT2 and its role in stress granules remains enigmatic. Here we show that in response to stress, DNMT2 shows phosphorylation-dependent relocalization to stress granules and it binds and methylates various RNA species in sequence independent manner. Moreover, overexpression of DNMT2 causes aberrant expression of several genes involved in host response to HIV infections. We show that upon HIV-1 infection, DNMT2 relocalizes from the nucleus to the stress granules. Importantly, DNMT2 binds and methylates HIV-1 RNA at both CpG and non-CpG sites. Unexpectedly, overexpression of DNMT2 lead to increase in HIV-1 titre. Further analysis revealed that this increase in HIV-1 titre correlated with the increase in HIV-1 RNA stability due to DNMT2 overexpression. Our results show that through its methyltransferase activity DNMT2 provides post-transcriptional stability to cellular RNA. However, DNMT2 dependent HIV-1 mRNA stability facilitates the HIV-1 infection to detriment the host. Thus, our findings provide for a novel mechanism by which virus tries to modulate the host cell machinery to its own advantage.

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## 294 Epigenomic analysis of the complete human tRNA gene set reveals an unprecedented view of regulatory complexity

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Despite mounting evidence for the importance of tRNA regulation in diverse biological processes, relatively little is known about the regulation of specific tRNA loci in complex multicellular organisms. In humans, as in most eukaryotes, tRNA genes comprise one of the largest single gene families that produce more transcripts than any other type of RNA. However, estimates of transcriptional activity of the 500+ individual human tRNA genes across multiple tissue types are almost entirely absent due to difficulty measuring specific tRNA transcript abundance on a large scale, as well as uncertain assignment of most transcripts among multiple identical loci.

We present analyses of epigenomic states associated with 622 predicted tRNA genes and pseudogenes across 127 reference cell types derived from the Roadmap Epigenomics Project, providing an unprecedented genome-scale view of tRNA gene regulation. These analyses segregate tRNA loci into constitutively active “housekeeping” genes (Group A: 147 loci), highly variable / differentially regulated genes (Group B: 100 loci), differently regulated genes that are mostly restricted to embryonic and induced pluripotent stem cells (Group C: 95 loci), intronic and other tRNA pseudogenes (Group D: 59 loci), and apparently silent genes showing little or no activity (Group E: 222 loci). Strikingly, tRNA covariance models scores (based on conserved primary and secondary structure features) are not reliable indicators of epigenetic activity group: identical copies of genes are found among both the constitutive and tightly regulated gene groups. Thus, nearby transcription factor binding patterns are important for understanding tRNA tissue-specificity and regulation. We note that a disproportionate share of highly active tRNA genes occupy the promoter regions of RNA polymerase II-transcribed protein coding genes -- an unexpected result given that many tRNA genes also act as insulator elements. Together, this data and newly developed tRNA-seq methods are providing one of the most comprehensive maps of tRNA gene regulation, and should be an important resource for ongoing studies of tRNA biology and human disease.

## 295 Integrative genomics approach to identify novel regulators of RNA methylation

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N6-methyladenosine (m6A) is a pervasive modification of RNA that is associated with multiple forms of post-transcriptional gene regulation. A few regulators of m6A have been identified individually, however no genome-wide assay has been reported. This is in part to the difficulty of designing call-based reporter systems for RNA methylation. In this study we take an integrative genomics approach to address this problem. We combine ENCODE gene expression data from HepG2 and K562 cells depleted in one of hundreds of RNA binding proteins (RBPs) with RNA methylation data to identify significant associations between RBPs and methylation. Gene ontology analysis revealed over-representation of several RBPs involved in cellular stress response and RNA splicing. We demonstrate via multiple biochemical assays that several of these genes are indeed regulators of RNA methylation and their dysregulation may be associated with human disease.

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## 296 m6A modification is important for the U3 snoRNA localization

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Cellular RNA has different chemical modifications, which are important for its biological function. m6A is one of the most abundant modifications in mRNA and non-coding RNAs [1]. Protein complex of two methyltransferases METTL3-METTL14 and supportive proteins performs m6A modification in human [2]. The U3 snoRNA is an abundant box C/D snoRNA that is required for the pre-rRNA processing and small ribosomal subunit assembly. We showed that METTL3 knockdown influence on the localization of U3 snoRNA in human cell. METTL3 knockdown resulted in accumulation of U3 in the cytoplasm at the expense of the reduction in nuclear localization. The dependence of U3 nuclear localization on METTL3 was confirmed by IP analysis using m6A antibody.

It's known that U3 snoRNA can shuttle between nucleus and cytoplasm. Early U3 monoparticles assemble in nucleoplasm or Cajal bodies and then imported to nucleolus for complete maturation [3]. So, we suggest that U3 maturation should involve METTL3 dependent methylation, which is necessary for the transport to nucleus/nucleolus.



## 297 E3 ubiquitin ligase Hakai is required for m<sup>6</sup>A mRNA methylation and normal growth and development in *Arabidopsis*

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RNA modifications, which collectively constitute the epitranscriptome, have been found to play a crucial role in regulating gene expression. One kind of RNA modification, N<sup>6</sup>-methyladenosine (m<sup>6</sup>A), is a ubiquitous base modification found internally in the mRNA of most eukaryotes and is implicated in multiple biological processes. The formation of m<sup>6</sup>A is catalysed by the methyltransferase (MTase) complex, with MTA (METTL3) as the major mRNA adenosine methylase in *Arabidopsis* closely associated with FIP37 (WTAP). Additional components have recently been identified which expand the complex to include MTB, FIP37, Virilizer and Hakai (an E3 ubiquitin ligase). As a novel member of the MTase complex, the role of Hakai in mRNA methylation both in plants and mammals is not yet understood. The aim of this study is to elucidate the function of Hakai and how it interacts with other components in the MTase complex. Homozygous *hakai* knockout mutants have been generated via CRISPR-Cas9, and a homozygous *hakai* intron-located T-DNA insertion mutant was also characterised. m<sup>6</sup>A levels in these lines was 33% to 40% reduced compared with the wild type and normal m<sup>6</sup>A levels could be restored by complementation with a wild-type *Hakai* transgene. *hakai* mutants demonstrated variable root phenotypes under different conditions but generally showed more lateral roots on higher concentrations of sucrose (>3%). In addition, a *hakai mta* double mutant demonstrated more severe developmental defects than an *mta* single mutant whilst a *hakai fip37* double mutant appears to be lethal. Taken together, *Hakai* is implicated to be essential for full m<sup>6</sup>A methylation and affects the normal growth and development of *Arabidopsis*, acting synergistically with other members of the MTase complex.

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## 298 Group II introns revisited: family relationships as revealed by sequence and/or structure clustering

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Group II introns are large RNAs that consist of a catalytically active self-splicing ribozyme and an intron-encoded protein (IEP). This IEP has several activities, including a maturase, which helps in the intron's RNA folding, a reverse transcriptase and a DNA endonuclease. With their IEP, GII introns can retrohome to specific DNA target sites, as well as retrotranspose to other sites of the genome. This high mobility capacity explains their existence in all three domains of life, and is the reason they have been used as novel gene targeting vectors, "targetrons". GII introns are also believed to be ancestors of spliceosomal introns in eukaryotes.

GII introns are characterized by a conserved secondary structure, organized into six domains, DI-DVI. These domains interact with each other to form a conserved tertiary structure that brings together distant elements to form a catalytic core, where the conserved active site of the intron is.

GII introns can be divided into three families A, B and C. 3D structures were determined for members of all three families, revealing a common structural core and different peripheral elements.

All GII intron sequences were extracted from the Mobile GII Intron Database. We performed all against all comparisons of GII intron sequences using BLAST and BlastR. We have also considered the secondary structures for all sequences, and performed all against all secondary-structure-based clustering. We processed the results using our in-house tool CLANSTIX, and we analyzed and visualized the results using CLANS. As a result, we obtained three visual representations of GII intron family relationships, where the representation considering sequence and secondary structure seems to point to an improved separation between the above-mentioned families. The clustering analysis that takes sequence and secondary structure into account is more robust than sequence comparisons alone, because it can take into account more remote evolutionary similarities. It can be used to validate existing classifications and to understand where unclassified sequences fit.



**299 General rules for functional microRNA targeting**

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The functional rules for microRNA (miRNA) targeting remain controversial despite their biological importance, because only a small fraction of distinct interactions, called site types (STs), have been examined among an astronomical number of STs which can occur between miRNAs and their target mRNAs. To systematically discover functional STs and to evaluate the contradicting rules reported previously, we utilized large-scale transcriptome data and statistically examined whether each of ~2 billion STs is enriched in differentially downregulated mRNAs responding to overexpressed miRNAs. Accordingly, we identified 7 functional noncanonical STs, most of which are novel, in addition to 4 canonical STs, while also removing numerous false positives reported by previous studies. Extensive experimental validation and significantly elevated 3'UTR sequence conservation indicate that these noncanonical STs may play biologically relevant roles. Our expanded catalogue of functional STs illuminates that the gene regulatory network controlled by miRNAs may be far more complex than currently perceived.

**300 Topological structure determination of RNA using small angle X-ray scattering**

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Although RNAs play a critical role in all cellular processes, the elucidation of their 3D structures is a daunting task. Naked RNAs are difficult to crystallize, and NMR spectroscopy is generally limited to small RNA fragments. As there is little apparent correlation between RNA primary sequences and three-dimensional folding, the usefulness of a pure computational structure prediction approach is also limited. Currently, there is an acute need for a robust, high throughput method that can determine topological structures of RNAs guided by some experimental data. We present here a novel method (RS3D) that can assimilate the RNA secondary structure information, SAXS data, and any readily available tertiary contact information to determine the topological fold of RNA. Starting from an open conformation that satisfies the secondary structure information in a glob model, where each glob represents a specific nucleotide, the algorithm carries out natural hierarchical moves evident from the structural composition of RNAs. Every new move is guided towards satisfying the SAXS data fit, secondary structure constraints, and any additional long-range interaction information. The best-ranked glob models are then converted to explicit all-atom coordinates and refined against the SAXS data and solvent accessibility data (if available) under the constraints of robust force fields using the Xplor-NIH program. Our method is widely applicable to a variety of RNA folding architectures currently present in the structure database. Furthermore, we demonstrate the applicability and feasibility of the program to derive low resolution topological structures of relatively large multi-domain RNAs.

### 301 Noise in RNA structural profiling data and its impact on reactivities and structure prediction

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The diverse functionalities of RNA can be attributed to its capacity to form complex and varied structures. The recent proliferation of new structure profiling techniques coupled with high-throughput sequencing has helped RNA studies expand in both scope and depth. However, the complexities inherent to such experiments and their subsequent outputs require careful read mapping and reactivity estimation strategies. We extend previous modeling studies for automated reactivity estimation from structure profiling data to include recent developments in the field. Using insights gained from our results, we characterize how various sources of bias in current experimental protocols contribute to noise in reactivity estimates. In addition, we examine the impact of noise in reactivities on performance of data-directed secondary structure prediction, a predominant application of structure profiling data.

We develop a model retaining steps common to most protocols for transcriptome-wide structure profiling and provide analytical solution for reactivity estimation using a maximum-likelihood approach. Analytical solutions allow us to identify systematic sources of noise in reactivity estimates due to experimental steps that differ between protocols. In addition, we highlight issues in mapping reads that contribute to noise in reactivity estimates. Since several of the examined experimental and read mapping steps are inevitable components of current profiling methods, we propose computational approaches to mitigate their negative impact on reactivity estimation.

Noise in reactivity estimates due to any of the experimental or analysis steps gets carried over to data-directed structure-prediction. We develop several noise models to examine how noise in reactivity estimates affects the performance of structure prediction algorithms. We find that while such algorithms may be able to tolerate decent amounts of fluctuations, conditions that depress reactivities or mask information altogether may have more pronounced effects. Our study demonstrates the utility of data-derived modeling in structure analysis.

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### 302 Computational investigation of the circular RNA scenario in hypoxia stress

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Hypoxia is associated with several diseases, including tumorigenesis, and occurs when tissues are deprived of adequate oxygen supply. Tissues react by activating endothelial cells and expressing specific growth factors, such as VEGF-A, to stimulate angiogenesis, improving oxygen delivery to cells.

Circular RNAs (circRNAs) are a novel class of long non-coding RNAs that are produced through back-splicing. So far, little is known about the biogenesis and function of circRNAs in hypoxia.

We have previously identified circRNAs that are highly expressed in endothelial cells and have a pro-angiogenesis phenotype *in vitro* (1). To further study the role of circRNAs in response to hypoxia and to understand the mechanism of their generation and regulation, we performed RNA-Seq of human HeLa cells. Differential expression analysis revealed their transcriptional and splicing response to decreased oxygen levels.

Using a combination of two established computational pipelines, find\_circ and CIRCexplorer, we identified thousands of circRNAs in hypoxic and normoxic conditions, finding several highly expressed circRNAs that change in hypoxia. Candidate circRNAs will now be investigated for their biosynthesis and their potential roles in the hypoxia response.

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### 303 PyRy3D: software for modeling macromolecular complex structures and its application to the *Trypanosoma brucei* 20S editosome

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One of the major challenges in structural biology is to determine the structures of macromolecular complexes and to understand their function and mechanism of action. However, compared to structure determination of the individual components, structural characterization of macromolecular assemblies is very difficult. To maximize completeness, accuracy and efficiency of structure determination for large macromolecular complexes, a hybrid computational approach is required that will be able to incorporate spatial information from a variety of experimental methods (like X-ray, NMR, cryo-EM, cross-linking and mass spectrometry, etc.) into modeling procedure. For many biological complexes such an approach might become the only possibility to retrieve structural details essential for planning further experiments. We developed PyRy3D, a method for building and visualizing low-resolution models of large macromolecular complexes. The components can be represented as rigid bodies (e.g. macromolecular structures determined by X-ray crystallography or NMR, theoretical models, or abstract shapes) or as flexible shapes (e.g. disordered regions or parts of protein or nucleic acid sequence with unknown structure). Spatial restraints are used to identify components interacting with each other, and to pack them tightly into contours of the entire complex (e.g. cryo-EM density maps or ab initio reconstructions from SAXS or SANS methods). Such an approach enables creation of low-resolution models even for very large macromolecular complexes with components of unknown 3D structure. Our model building procedure applies Monte Carlo approach to sample the space of solutions fulfilling experimental restraints.

The editosome is a 0.8MDa protein complex that catalyzes the RNA editing process in African trypanosomes. We applied the hybrid modeling approach implemented in PyRy3D software in order to build ensembles of structural models of the *Trypanosoma brucei* 20S editosome that agree with currently available experimental and theoretical data.

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### 304 A Pipeline for Computational Riboswitch Detection with Experimental Verification Using Inverse RNA Folding

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One of the biggest challenges in riboswitch research is to find additional eukaryotic riboswitches since more than 20 riboswitch classes have been found in prokaryotes but only one class has been found in eukaryotes. Moreover, this single known class of eukaryotic riboswitch, namely the TPP riboswitch class, has been found in bacteria, archaea, fungi and plants but not in animals. We present a computational approach based on inverse RNA folding that transforms structure-based search into a sequence-based search, thereby enabling the utilization of well-established sequence-based search utilities such as BLAST. The transformation to sequence space is obtained by using a fragment-based inverse RNA folding solver with sequence and structure constraints, available within RNAfbinv. More generally, the method that consists of a pipeline with various adjustable components (e.g., BLAST can be replaced by Infernal) is described along with its findings in prokaryotes and an additional interesting candidate in eukaryotes that is not detected by other methods commonly used for riboswitch detection. The prokaryotic candidate was verified to be a purine riboswitch by in-line probing. The 5' radiolabeled RNA ran on a denaturing gel to infer secondary structure. With the addition of the guanine ligand we observed reduce scission in the primary multi-loop which fits results seen in naturally occurring well-known riboswitch aptamers. Potential improvements in 3D RNA structure prediction will allow us to run RNA-ligand binding simulation for both the synthetic riboswitch design process and post-processing filtering stage in the search pipeline.

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### **306 Elucidating the targets of tRNA fragments**

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Transfer RNA fragments (tRFs) are a class of small RNA molecules derived from mature or precursor tRNAs. Although tRFs have been characterized very recently, gradually they have been attracting more attention. Similar to microRNAs, there is evidence that tRFs are found across a wide range of organisms and tissues in cytoplasmic compartments or loaded to RISC complexes, often in numbers comparable to microRNAs. Despite clear differences between tRFs and microRNAs, there is accumulating evidence that tRFs may play a vital role in post-transcriptional gene regulation and RNA silencing.

Here we describe potential interactions of human tRF with their putative target RNAs associated with human Argonaute complexes as elucidated from CLASH (Crosslinking, Ligation, And Sequencing of Hybrids) sequencing results. We found that Argonaute-loaded tRFs target a wide range of transcripts corresponding to various gene types, from rRNA to lincRNA to protein-coding transcripts. In the latter, 3' UTR regions are the likely primary target of tRFs, although there is a significant number of interactions of tRFs with coding and 5' UTR regions. Furthermore, we observed a novel phenomenon - a large number of putative interactions between tRFs and intronic sequences. We provide ample evidence supporting the notion that tRF "seed" sequences can be located on either end of a tRF molecule in agreement with experimentally validated results from earlier studies.

We performed extensive computational analysis, including k-mer search in the UTR regions conserved across multiple species and testing for motif enrichment in tRF-targeted sequences derived from the CLASH experiments. Contrary to the traditional view of microRNAs "seed" sequences, located on the 5' end of the microRNAs molecule, we found that tRF "seeds" can be located both on the 5' and 3' end of the tRF molecule as well as in the middle of the fragment. Last, we observed that in many cases such interactions occur in specific locations where the sequence of the targeted RNA molecule is enriched for palindromic motifs likely driving the secondary structure formation.

### 307 Computational identification of novel terminal exons with TECtool

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Mammalian genes typically have multiple transcription initiation and termination sites as well as multiple exon forms that are used in a cell type specific manner to generate distinct transcript isoforms. Improving the accuracy of inferring transcript isoform abundance leads to an improved understanding of cellular processes, for example through a more accurate prediction of transcript interactions. However, despite great efforts to annotate eukaryotic transcripts, many transcript isoforms are still to be identified. mRNA-seq-based methods for transcript reconstruction have only started to emerge and are insufficiently accurate [1]. In particular, mRNA 3' end sequencing data indicates that thousands of poly(A) sites are located in regions that are currently annotated as intergenic and intronic [2]. In this study we have developed TECtool (Terminal Exon Characterization tool), a method that reconstructs terminal exons and associated transcript isoforms based on sequenced mRNA 3' ends and RNA-seq data. Applying TECtool to various datasets, we have identified many novel terminal exons and transcripts, which are particularly frequent in immune cells. Ribosome profiling data indicates that these novel transcript isoforms lead to the production of novel proteins. TECtool is a method that enriches the existing transcript annotation, thereby leading to improved transcript isoform abundance estimation [3], identification of regulatory (miRNAs, RBPs) binding sites, and annotation of protein domains.

1. Hayer KE, et al. Bioinformatics. 2015
2. Gruber AJ, et al. Genome Research. 2016
3. Kanitz A, et al. Genome Biology. 2015

### 308 Multifaceted Effects of Splice Altering Mutations in Cancer

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Although it is known that splicing alterations affect the landscape of mRNA isoforms present in both tumor and normal tissue, *determining the functional consequences of all variants in a splicing context is still understudied in cancer genomics*. Here we use SpliceInator a semi-automated tool to systematically detect splicing phenotypes using mutation and gene expression data. SpliceInator combines two lines of evidence to assess mutant specific aberrant splicing events and their implications, one based on interpretation of RNA-Seq fragment mapping using TopHat and another based on standard statistical hypothesis testing of RSEM expression values. We interrogated 1,146 conserved splice site mutations across 19 cancer types, revealing a wide range of complex splicing phenotypes. 521 variants in our dataset conferred a measurable splicing alteration, with 70% associated with only one splicing defect, while the remaining were a combination of two to four different splicing events. Another 624 splice site mutations did not confer any measurable splicing defects, although 75% were classified as having a low variant allele fraction, low exon expressivity, or both and 25% are still undetermined. Our case control based analysis highlighted 19 highly expressed splice variants that induce alternative transcripts which are not degraded by RNA surveillance mechanisms and potentially contribute to the tumor phenotype. Furthermore, we identified several mis-annotated variants in cancer genes (including *PTEN* and *PARP1*) that create and strengthen nearby alternative splice sites, further justifying the demand for a tool that can systematically evaluate all mutations in a splicing context. Our study highlights the importance of having individualized RNA-Sequencing to complement mutational analysis to evaluate patient specific splicing alterations and improves on current annotation methods broadening our understanding of variants that affect splicing and their biological contribution to oncogenesis.



**309 microRNAs at the spliceosome – why so many?***Shelly Mahlab<sup>1</sup>, Ayelet Peretz<sup>2</sup>, Liran Carmel<sup>2</sup>, Ruth Sperling<sup>2</sup>, Michal Linial<sup>3</sup>***<sup>1</sup>The Rachel and Selim Benin School of Computer Science and Engineering, The Hebrew University of Jerusalem, Jerusalem, Israel; <sup>2</sup>Department of Genetics, The Hebrew University of Jerusalem, Jerusalem, Israel; <sup>3</sup>Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel**

MicroRNAs (miRNAs) are short ncRNAs that via base-pairing with target mRNAs negatively regulate expression and translation in healthy and diseased tissues. In human, over 2500 mature miRNAs are reported which originate from ~1900 genes. Most studies focus on the function of miRNAs in the cytoplasm, however, evidence suggests that miRNAs are also present and have novel function in the nucleus. When isolated from cell nuclei under native conditions, intact Pol II transcripts are found assembled in a huge (21 MDa) and dynamic macromolecular machine, the supraspliceosome. It is composed of four active native spliceosomes, each resembling the *in vitro* spliceosome, interconnected by pre-mRNA. The supraspliceosome functions in coordinating pre-mRNA processing steps in the nucleus including removal of introns, processing of the 5' and 3' ends, RNA editing and processing intronic short ncRNAs.

In this study, we investigate precursor miRNAs (pre-miRNAs) and characterize the sequences derived from them within the context of the spliceosome isolated from HeLa cells. Specifically, following isolation of the endogenous spliceosome from cell nuclei (in triplicates), the associated small RNAs (<200 nt) were extracted and subjected to next generation sequencing. We found ~400 different expressed pre-miRNAs with the endogenous spliceosome, ~60% of them are located within introns of their host genes. Comparing the miRNAs' composition to the profile from different cell compartments shows the highest correlation with nuclei miRNAs ( $r=0.48$ ). Remarkably, 51% of reads that are mapped to pre-miRNA sequences are actually matured miRNAs. These are most likely shuttled back to the nucleus from the cytoplasm following processing by Dicer. For a small but significant fraction of the detected mature miRNAs, the relative abundance of sequences associated with the 5'p relative to 3'p miRNAs markedly differ from the relative ratio observed in the cytoplasm. These genes are mostly located within introns. For example, for hsa-mir-151a which is highly expressed, we identified only the 3'p miRNA, while in the cytoplasm the 5'p miRNA dominates. The deviation of miRNA strand abundance from the appearance in the cytoplasm (for 5'p and 3'p) proposes an overlooked function for these miRNAs at the supraspliceosome.

**310 Protein side-chain conformational changes upon nucleic acid binding***Zhichao MIAO<sup>1,2</sup>***<sup>1</sup>EMBL-EBI, Hinxton, UK; <sup>2</sup>Wellcome Trust Sanger Institute, Hinxton, UK**

Protein-RNA/protein-DNA interactions, which mediate a great number of biological processes, are largely conducted by protein side-chains. Our previous study of side-chain conformational changes in non-nucleic acid binding state has quantitatively analysed the flexible nature of side-chain conformations. It pointed out that side-chain flexibilities are related to their degree of freedom, shape of the side-chain group, hydrophobicity and accessibility. Some other researches have highlighted the side-chain conformational changes upon ligand. However, the side-chain conformational changes upon DNA binding have not been quantitatively characterized. It is an urgent need to understand such conformational changes upon nucleic acid binding and the conformational preferences to help improve protein design, protein docking and function prediction.

Here, we provide a first quantitative analysis of side-chain conformational preferences and changes upon nucleic acid binding on large scale data sets and systematically compared these changes with the ones in non-nucleic acid binding states. We discovered the side-chain conformational flexibility exist even on RNA/DNA binding interfaces, but similar types of interactions need to be sustained. Some side-chains need to rearrange their conformations either to make space for the binding of the nucleic acid or to optimize the interactions with the RNA/DNA molecule. These side-chains need to be more flexible to adapt to the shape of the RNA/DNA molecule to render better binding free energies, being less rotameric in conformational space. Some of the conformational changes also affect the conformations of the neighbouring residues to make rearrangements. Such residues constitute an interaction network that need to be solved in the side-chain packing problem. Still, more than half of the residues need to keep the same conformation in both bound and unbound states, which means the majority of the residues need to get prepared for nucleic acid binding even in unbound state. So the 'induced-and-fit' model only needs minimum conformational changes for the RNA/DNA binding.

This study points out the importance of side-chain conformational changes upon nucleic acid binding; quantitatively demonstrates the conformational changes and conformational preferences and provides useful data resource for benchmarking protein-RNA/DNA docking and side-chain packing.

### 311 RNA-Puzzles: advance in RNA 3D structure prediction and perspectives

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RNA-Puzzles is a world-wide collective experiment in blind 3D RNA structure prediction, which is similar to CASP for protein. Predictors are entitled to predict RNA 3D structures with only sequence information, and assessments of prediction are performed based on crystal structures after their release. In the past 6 years, 19 Puzzles have been predicted, including some breakthrough structures such as tRNA-T box complex, glutamine riboswitch and VS ribozyme. We have concluded three rounds of RNA-Puzzles papers. And we organized the 1st international RNA-Puzzles meeting in October 2016.

According to the previous puzzles, some key insights of RNA 3D structure have been demonstrated. The L-glutamine Riboswitch Puzzle clarifies that secondary structure alone without non-Watson-Crick interaction information is not enough in describing the real RNA structure. The Puzzle 4 implies that RNA structure prediction may help in generating better structural models for experimental structure. And prediction algorithms may help in structure optimization. Further, according to the puzzles (4, 8 and 13), ligand binding sites of the RNAs can be inferred by predicted 3D structure model. RNA conformational change is also possible to be predicted, as shown in Puzzle 14.

With these advances, we feel that RNA-Puzzles may take up more challenging tasks in helping the biologist to solve real-world problems. RNA structure crystallization is a difficult job that not easy to be achieved for each RNA, while structure models may offer insights to molecular mechanisms. RNA-Puzzles would like to endeavour community efforts to predict structurally uncharacterized RNA 3D structures and provide them as advisory/reference structures to biologists. With the help of Rfam, we have selected ~20 RNA families who are believed to have stable 3D structures but have not been solved yet. These RNAs will be predicted by RNA-Puzzles community, screened by quality prediction, remodelled according to experts' advice and top models will be deposited and demonstrated to RNA biologist as advisory structures. In this action, we hope to automate RNA structure prediction and assessment workflow and enable more collaborations in solving real-world problems.

### 312 Detection and Annotation of functional RNA structures in the Viral Domain

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Viruses are a tremendously diverse group of obligate intracellular parasites comprising many highly relevant pathogens such as the recently emerging Ebola and Zika Virus. Even though there are vast differences among most viral life cycles and their host interactions, every virus species has to undergo a stage in its life where RNA is indispensable for reproduction. In addition to RNA's function as carrier of genetic information, as seen in mRNAs or viral genomes entirely made out of RNA, one of the most striking properties of RNA is its ability to form well-defined structures with distinct chemical or biological functionality. A large number of viral RNA structures can be linked to pathogenicity, the latest example being the Zika Virus, where pathogenicity and replication is highly dependent on the strength of several RNA structures in the genomic 3'-UTR.

Due to the inherent utility of RNA structures for viral survival, computational *de novo* prediction and evaluation of potentially functional RNA structures has been a central topic in RNA bioinformatics, resulting in the development of programs as RNAz and AliDot by our group. Both programs allow for rapid screening of entire viral genomes and provide the user with a means to assess the relevance of predicted RNA structures.

In our study, we investigate the role of conserved RNA structures in viral evolution not only in a few isolated species, but in the context of the entire viral domain. Employing mentioned structure prediction tools in combination with powerful structure and sequence homology search tools as provided by cmSearch from the infernal package, we systematically screen every adequately annotated virus species for potentially functional RNAs. Our results show a surprising amount of to date unrecognized highly relevant RNA structures in most viruses. Furthermore, contrary to common expectations, we observe a large number of conserved RNA structures in annotated protein-coding regions. In addition to our findings, we show examples of structures that are found to be not only conserved within one virus species or even family, but across phylogenetically unrelated domains, emphasizing the versatility and importance of RNA structure for viral survival.

### 313 SPQR: a coarse-grained representation for RNA structure prediction

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The knowledge of the three-dimensional structure of a given RNA molecule is of fundamental importance for the understanding of its function. Considering the complexity of the experimental determination, computational tools have emerged as a convenient choice for this aim. Among them, the use of coarse-grained representations of RNA is of particular interest, due to the current limitations of all-atom approaches both in terms of their performance and of the accuracy of the available force fields. In addition, coarse-grained approaches offer the opportunity to learn which are the essential structural elements that must be included in their representation in order to fold a given set of relevant motifs.

We introduce the SPlit and conQueR (SPQR) model, a nucleobase centered coarse-grained representation for structure prediction. The choice of its interactions and degrees of freedom makes possible the inclusion of stacking, planar canonical and non-canonical base pairing and base phosphate interactions, while it allows the specification of sugar puckers and glycosidic bond angle conformations for each nucleotide. In the same spirit, we introduce a backmapping procedure which makes it possible to fully reintroduce atomistic details into the predicted structures.

We present the results of the model in a set of motifs and structures which contain both canonical and non-canonical base pairs, including all the possible sequences for the three most common tetraloop families. In addition, we show how the backmapping procedure can help to correct some inaccuracies of the model, which help to refine our predictions even more. We will also discuss the particular choice of the parametrization and how it can be improved, specifically in the case of base-phosphate interactions.

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### 314 Computational Identification of RNA Modifications

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The epitranscriptome is an emerging layer of RNA regulation and is characterized by over 100 different RNA modifications that are known to exist on different RNA species. As compared to the relatively more well-known RNA modification events like A to I and C to U RNA editing, there is emerging evidence to indicate that mRNAs also carry other RNA modifications like m<sup>6</sup>A, pseudouridine, m<sup>5</sup>C and m<sup>1</sup>A which play important roles in the regulation of embryonic stem cells pluripotency and stress response. Nonetheless, due to the novelty of these RNA modification events, there is currently only very limited number of methods to identify the large variety of RNA modification events in the transcriptome. To add on, these methodologies often also requires extremely specialized experimental protocols which can be difficult to establish. Here, we propose a computational methodology for the computational identification of RNA modifications events, which allows RNA modifications to be identified from RNA sequencing datasets with relatively ease. Through the use of a specialized statistical model and stringent filtering criteria, sites of RNA modifications were identified. RNA modifications were also found to produce a distinct and unique signature thereby providing an unique feature for the classification of putative RNA modification events. Results using simulated datasets also confirmed the relatively high sensitivity and specificity of our methodology. Overall, we believe the computational tool we establish would ease the study of RNA modifications and enable its study in a wide variety of contexts.

### 315 Efficient RNA 3D conformational space exploration and its applications

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The accurate prediction of RNA tertiary structure remains a largely unsolved problem for larger RNA molecules. We present Ernwin[1], a program for helix-centered, coarse-grained RNA 3D structure prediction. It assembles loop and helix based fragments and uses a coarse grained representation of these fragments for exploration of the RNA's conformational space via Metropolis Hastings Monte Carlo sampling. The use of fragments without internal degrees of freedom based on the RNA's secondary structure dramatically reduces the search space and increases sampling efficiency and computational speed. We use knowledge based potentials to evaluate the RNA's energy based on the interaction of coarse-grained fragments.

Thanks to coarse graining, we offer equivalent prediction accuracy to other tools in less time. We obtain predictions with accuracy of 10 Å or less for the majority of solved structures shorter than 100 nucleotides (20 Å for structures shorter than 200 nucleotides).

To achieve efficient exploration of the conformation space for molecules with complex secondary structure, we implemented different sampling strategies (including Simulated Annealing and Replica Exchange Monte Carlo), different move sets (exchange of one fragment or multiple fragments) and different fragment selection algorithms (based only on number of nucleotides, based on sequence similarity or based on motif search). The quality of the predictions of Ernwin is evaluated by comparison to experimental structures found in the PDB and optimal prediction protocols are proposed.

Furthermore, we evaluate how our predictions can be combined with other experimental methods, such as SAXS, FRET[2] and AFM. 3D maps and 3D models generated by single particle cryo electron microscopy have great benefits for structure prediction, but even 2 dimensional micrographs can be used to rule out certain structures and thus improve prediction accuracy.

Ernwin is open source and available at github.

A webserver of Ernwin will be available at [rna.tbi.univie.ac.at/ernwin/](http://rna.tbi.univie.ac.at/ernwin/) in spring 2017.

Reference:

[1] P. Kerpedjiev, C. Höner zu Siederdissen and I.L. Hofacker, RNA 2015; 21(6): 1110-1121 doi:10.1261/rna.047522.114

[2] N. Hecker et al., 2016, arXiv:1610.00340

### 316 Rlsearch2: Fast genome-wide computational screen for RNA-RNA interactions and its application in siRNA off-target discovery

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Many non-coding RNAs function through intermolecular interactions with other RNAs. These interactions play key roles in regulatory mechanisms, but are also a concern in therapeutics, e.g., undesired interactions of designed oligos. Being able to predict such interactions can be useful in several areas, including interpretation of RNAi application outcomes, integrative miRNA and gene expression analysis, and functional studies of non-coding RNAs. However, this requires large-scale genome- and transcriptome-wide RNA-RNA interaction predictions, a resource-demanding task. We have implemented an efficient method, Rlsearch2, which makes use of an efficiently integrated seed-and-extend framework. In Rlsearch2, we use suffix arrays to locate perfect complementary seed regions (including G-U wobble pairs) between given query and target sequences. These seeds are then extended in both ends for further base pairs, evaluated with a simplified energy model. The large-scale capability of Rlsearch2 is exemplified by a screen of ~2600 human miRNAs on the whole repeat-masked human genome, which takes about ~6h using 16 threaded cores. This is orders of magnitude faster than other currently available methods. Utilizing Rlsearch2 predictions, we designed an siRNA off-target discovery pipeline that also takes accessibility and expression abundance information into account. Combining all this information with a partition function approach, we can accurately predict individual off-targets of specific siRNAs as well as their overall off-targeting potential, which might further influence their repression efficiency. Rlsearch2 is available at <http://rth.dk/resources/rlsearch/>.



### 317 Cataloging RNA bioinformatics resources and their relationships

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The past decades have seen a growing interest in the study of non-coding RNAs and transcriptomics, which was both accompanied and advanced by the development of RNA bioinformatics tools and databases. However, with the large number of available resources that solve a wide variety of tasks, it can be challenging to find the one best-suited for a specific research problem. To address this issue, we, the RNA tools and software consortium, provide a comprehensive catalog of RNA bioinformatics tools and databases. In there, around 400 resources are extensively characterized by a mixture of controlled vocabulary and free text description. We also annotate relationships between resources, e.g., tools contained in a software suite are unambiguously linked to a specific version of such package. The terms describing application domains, as well as functions, and input/output data of the tools, are organized in an ontology. This ontology enables a precise classification of tools and enhances the searchability of the registry. For example, around 80 tools fall into the "RNA-seq" domain and are further categorized by their functions, spanning from "Read pre-processing" and "mapping" to "Differential expression analysis" and "Isoform quantification". A user searching for "RNA folding" will find the synonymous function "RNA secondary structure prediction", but also its subclasses such as "RNA consensus secondary structure prediction", describing tools that - rather than processing a single sequence - work on a set of sequences, either prealigned or simultaneously align and fold them. The user can navigate the ontology to find exactly the desired function. A list of relevant resources is presented to the user, who can then make a choice based on their descriptions and various attributes. This illustrates how the ontology helps non-specialists to find relevant tools for a given task at hand. Our tool collection will be integrated into the "ELIXIR Tools and Data Services Registry" to allow easy access. Check <http://rth.dk/resources/rnatools/> for further information and future updates.

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### 318 Integration of miRNA signatures into signaling pathway impact analysis supports the bioinformatic identification of de-regulated cellular networks in recessive dystrophic epidermolysis bullosa

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The monogenic disorder recessive dystrophic epidermolysis bullosa (RDEB) belongs to the family of rare genodermatoses characterized by mutations in the *COL7A1* gene, which codes for the structural anchoring protein type VII collagen. Missing or dysfunctional type VII collagen disrupts the ultrastructural linkage between epidermis and the underlying dermis. RDEB manifests in severe blister formation sublamina densa and erosions of skin and mucous membranes with impaired wound healing. Patients are highly exposed to developing particularly invasive squamous cell carcinomas (SCC).

MicroRNAs are small non-coding RNA molecules associated with post-transcriptional regulation of more than 30% of protein-coding genes in humans. An implication in complex diseases, including cancer, has been associated with changed expression levels of microRNAs. We recently identified a de-regulation of microRNA 10-b and let-7c in RDEB-SCC cell lines versus RDEB-keratinocytes and non-RDEB controls by means of a microRNA sequencing approach. In order to explore the impact of altered microRNA expression on cellular pathways, miRNA-mRNA relationships were derived from various relevant databases like miRTarBase. Microarray derived mRNA expression levels were integrated to perform a topology aware pathway analysis screen on expression data to highlight de-regulated molecular networks in RDEB patient cell lines.

As the majority of the traditional pathway analysis tools are so far not directly considering the influence of microRNAs, an augmented version of the signaling pathway impact analysis method developed by Draghici *et al* (2016) was applied to infer regulatory relationships between de-regulated microRNAs and cellular networks to unveil de-regulated KEGG pathways in RDEB-SCC vs controls.

The observed perturbation of several pathways indicates a putative functional contribution of de-regulated microRNAs in RDEB disease progression. Our data analysis strategy provides a solid basis to pursue disease relevant microRNA regulated pathways.



### 319 New Approaches to Determine RNA Pseudoknot Order

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RNAs are flexible and dynamic molecules. This often manifests itself in a complex fold of the 3D structure - with multiple motifs consisting of all kinds of canonical and non-canonical interactions. The longer the sequence of nucleotides is, the more likely it is to observe fascinating motifs called pseudoknots. Unlike real knots which would persist a forced melting of the molecule, pseudoknots are regular Watson-Crick base pairs which are formed between sequentially distant nucleotides and they would unwind during melting just as any other base pair. The name comes from the fact that any planar representation of the pseudoknotted RNA structure results in a crossing arc. It represents an interaction linking two distant locations which are separated by a well-structured fragment such as a helix.

Pseudoknots are evolutionary conserved and they play a major role in the global stability of an RNA structure. Furthermore, pseudoknots are diverse and in larger structures they can form complex networks of inter-dependencies. There have been a few attempts at pseudoknot classification: by type, by family or by topological genus. Our addition to this is a notion of pseudoknot order - a measure of structural complexity - defined as a minimum number of base pairs set decompositions which result in a nested (i.e. non-pseudoknotted) structure [1]. Despite the terseness of the definition, it is a difficult problem to determine this minimum decompositions set. Here we present our results which show that the computational side of finding the pseudoknot order comes down to a - well-known and proven to be challenging - graph theory problem of vertex coloring. The order corresponds to the chromatic number while color assignments represent levels of pseudoknots. We also show a complementary way of assigning the levels which could be potentially more accurate in terms of RNA folding pathways. Finally, we present the statistics for both approaches gathered in a set of RNA 3D structures deposited in the PDB database.

[1] M. Antczak, T. Zok et al., **2014**. *RNApdbee--a webserver to derive secondary structures from pdb files of knotted and unknotted RNAs*. **Nucleic Acids Research**, 42(W1), pp.W368-W372

### 320 ModeRNA 2.0: the use of multiple templates improves accuracy of RNA 3D structure models in automated homology modeling

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The functions of many RNA molecules are dependent on their ability to form complex three-dimensional (3D) structures. However, experimental determination of RNA 3D structures is laborious and challenging, and therefore, the majority of known RNAs remain structurally uncharacterized. To address this problem, computational structure prediction methods were developed. One of the approaches utilizes information derived from known 3D structures of other RNA molecules used as structural templates. This approach, referred to as comparative modeling or homology-modeling, enables 3D structure modeling of RNA of any size, as long as a suitable template exist and the sequence of the target RNA to be modeled can be aligned correctly to the template sequence and structure.

We have previously developed ModeRNA, a fully automated method for comparative modeling of RNA 3D structures, which allows for modeling RNA sequences that contain posttranscriptional modifications.

Here, we present ModeRNA version 2.0, which introduces modeling based on multiple templates. This new feature can be used to model complex RNA 3D structures with elements that are evolutionarily variable (present in some members of the family and not in the others), the use of partial templates (for instance individual subdomains or motifs), and mixing templates with different level of resolution and completeness.

We demonstrate on structures of group I and group II introns, and c-di-AMP riboswitches. That the use of at least two templates that are partially complementary leads to significant improvement in the accuracy of the resulting models, as compared to models built on individual templates. The improved accuracy of initial models persists through the stages of model optimization, which in our case includes the use of SimRNA (for refolding of poorly modeled structure fragments) and QRNAS (for full-atom optimization of geometry). Our software is available from <http://genesilico.pl>.

## 321 Ligand dependent accessibility of the Shine-Dalgarno sequence under the control of riboswitches in mesophilic and thermophilic bacteria

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In bacteria, translation is initiated by binding of the 30S ribosomal subunit to the Shine-Dalgarno (SD) sequence upstream of the start codon in a messenger RNA (mRNA). A translational riboswitch, commonly embedded in the 5' untranslated region of a bacterial mRNA, regulates translation initiation by controlling access to the SD sequence in response to a metabolite that reshapes the riboswitches' secondary structure. Despite numerous studies done to understand the ligand mediated dynamics of isolated riboswitches, including using single molecule FRET, there is still a dearth of understanding of how a riboswitch functions in the context of a long native mRNA due to a lack of available probing tools. To address this limitation, our lab recently developed an assay called Single Molecule Kinetic Analysis of RNA Transient Structure (SiM-KARTS) that monitors the secondary structure fluctuations of single RNA molecules of arbitrary length through their accessibility to transiently binding fluorescent probes. SiM-KARTS was used to investigate the ligand-dependent accessibility of the SD sequence of an mRNA hosting the 7-aminomethyl-7-deazaguanine (preQ<sub>1</sub>)-sensing riboswitch from the thermophilic bacterium *Thermoanaerobacter tengcongensis* (*Tte*), using an anti-SD probe analogous to the 3' end 16S rRNA of *Tte* ribosome. Spike train analysis revealed that individual mRNA molecules alternate between two conformational states, distinguished by 'bursts' of probe binding associated with increased SD sequence accessibility. Addition of preQ<sub>1</sub> decreases the lifetime of the SD's high-accessibility (bursting) state and prolongs the time between bursts. Such complex ligand sensing by individual mRNA molecules rationalizes the nuanced ligand response observed during bulk mRNA translation. We are further focusing on the role of a homologous translational preQ<sub>1</sub> riboswitch regulating an mRNA from the mesophilic pathogenic bacterium *Bacillus anthracis* (*Bas*). Based on the shorter SD:anti-SD complementarity in *Bas* compared to *Tte*, faster kinetics are expected. Such a difference in kinetic profile would be consistent with the expected evolutionary adaptation of gene regulation in mesophilic (*Bas*) and thermophilic bacteria (*Tte*). We propose that SiM-KARTS is a sensitive tool to detect localized conformational changes in virtually any large RNA at the single molecule level.

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## 322 Twister ribozymes in eukaryotes are encoded by non-LTR retrotransposons of the RTE superfamily

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Small self-cleaving ribozymes such as the Hammerhead, the Hepatitis- $\delta$  and the Twister motifs are a group of catalytic RNAs with a widespread and numerous occurrence in genomes from all domains of life. Previous data showed that Hammerhead and Hepatitis- $\delta$  ribozymes are frequently found in mobile genetic elements such as retrotransposons, which have efficiently colonized most eukaryotic genomes. In this work, bioinformatic mining of Twister ribozymes reveals their conserved occurrence in LINE retrotransposons of the RTE superfamily. Twister motifs are encoded in the 5' UTR of most RTEs of plants, as well as in several metazoan families of this clade of retroelements. The illustrative case of the RTEs present in the apple genome (*Malus domestica*) reveals the presence of ~1500 Twister ribozymes of the type P1 that can be classified into three similar variants. Most genomic ribozyme sequences correspond to 5'-truncated versions at the precise cleavage site, which suggests that the self-cleaved RNA of the retroelement would be the template for retrotranscription and genome integration. Moreover, kinetic analysis of several of these genomic twisters reveals efficient co-transcriptional self-processing in the absence of stable P1 stems or canonical self-cleavage sites. Consequently, and in a similar way as observed for the Hepatitis- $\delta$  ribozymes found in other LINEs, these retroelements would seem to process their 5' RNA end through self-cleaving motifs regardless of the upstream sequences where they are embedded. Altogether, our data confirm the conserved presence of self-cleaving ribozymes in eukaryotic retrotransposons, which explains the widespread occurrence of these catalytic RNAs and suggests that more catalytic RNAs may be found encoded in other mobile elements.

**323 Probing the hatchet ribozyme by atomic mutagenesis***Christoph Falschlunger, Ronald Micura***Institute of Organic Chemistry and Center for Molecular Biosciences, Innsbruck, Tyrol, Austria**

Recently, the ribozyme field was reinvigorated by 'comparative genomic analysis'-based identification of novel nucleolytic ribozyme motifs, termed twister, twister sister, pistol, and hatchet [1,2]. These discoveries have opened an opportunity to undertake structure-function studies to expand on our understanding of self-cleaving ribozyme-mediated catalysis. To this end, we set out to explore catalytic features of the hatchet ribozyme by targeted atomic mutagenesis. We focus on the replacement of highly conserved nucleosides in hatchet RNAs by the corresponding deazanucleosides to evaluate their impact on cleavage activity and putative roles as general acid-base catalysts. Our study aims at new insights into the catalytic strategies employed by the hatchet ribozyme.

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**324 Cooperative Interactions in the Hammerhead Ribozyme Drive  $pK_a$  Shifting of G12 and its Stacked Base C17***Erica Frankel<sup>1</sup>, Christine Keating<sup>1</sup>, Philip Bevilacqua<sup>1,2</sup>*

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General acid-base catalysis is a key mechanistic strategy in protein and RNA enzymes. Ribozymes use metal ions, nucleobases, and organic cofactors to carry this out. In most small ribozymes, a guanosine is positioned to participate in proton transfer with the nucleophilic 2'OH. The unshifted  $pK_a$  values for these nucleobases and solvated metal ions are far from neutrality, however, and thus non-ideal for general acid-base catalysis. Remarkably, ribozymes often exhibit cooperative interactions between nucleobases and metal ions to shift these  $pK_a$ s closer to neutrality. Herein, evidence is provided for cooperative interaction in the hammerhead ribozyme amongst G12-the guanine that interacts with the nucleophilic 2'OH-the -1 nucleobase C17, and  $Mg^{2+}$  ions. We introduce global fitting as a method to analyze rate-pH data parametric in  $Mg^{2+}$  concentration and benchmark it on previously published data from the HDV ribozyme. We then apply global fitting to new rate-pH data for the hammerhead ribozyme using a minimal three-dimensional, four-channel cooperative model, which describes the interaction amongst C17, G12, and  $Mg^{2+}$  ions. The value for the  $pK_a$  of G12 that we obtain is channel-dependent and varies from 8.1 to 9.9, shifting closest towards neutrality in the presence of two cationic species:  $C17H^+$  and a  $Mg^{2+}$  ion. The value for the  $pK_a$  of the -1 nucleotide, C17, is raised an astonishing 3.5-5 units towards neutrality under low  $Mg^{2+}$  conditions. Shifting of the  $pK_a$  of C17 appears to be driven by an electrostatic sandwich of C17 between carbonyl groups of the 5' neighboring U and of G12 and involves cation- $\pi$  interactions. The relationship among all three species leads to a "waviness" in rate-pH profiles that has been observed previously in literature on both minimal and full-length constructs but never explained. Rate-pH profiles reveal that the major reactive channel under biological  $Mg^{2+}$  and pH involves a cationic C17 rather than a bound  $Mg^{2+}$  ion. Lastly, we have expanded the global fitting technique to systematically conduct kinetic simulations to predict rate-pH profiles that can be applied to other ribozymes to lend critical insight into their catalytic mechanisms.

**325 Exploring the structure and function of the *moaA* riboswitch**Fabio Amadei<sup>1</sup>, Sofia Gallo<sup>1</sup>, Katrin Schrader<sup>2</sup>, Jose Santamaria<sup>2</sup>, Günter Schwarz<sup>2</sup>, Roland K. O. Sigel<sup>1</sup><sup>1</sup>University of Zürich, Zürich, Switzerland; <sup>2</sup>University of Köln, Köln, Germany

The Moco RNA motif was discovered in 2007 by a large comparative screening of bacterial genomes.<sup>1</sup> Its characteristics strongly suggested that this widespread and highly conserved RNA motif is a riboswitch. Further studies on the *moaA* riboswitch, a Moco RNA motif regulating the *moaA* operon in *E. coli*, proved indeed its involvement in gene regulation.<sup>2</sup> This finding fortified the assumption of this RNA to be indeed a riboswitch and therefore to directly sense Molybdenum cofactor (Moco) or one of its metabolites.<sup>2</sup>

The fact that to date there is no proof for a direct interaction between the *moaA* riboswitch and a metabolite is due to the high instability and oxygen sensitivity as well as the scarce availability of Moco. In fact, Moco and many of its precursors are only available by biosynthesis, which involves several enzymes encoded in the *moaA* operon.<sup>3</sup>

We are aiming to understand if the *moaA* riboswitch is indeed able to directly bind a small cellular metabolite, and which intermediate along the Moco biosynthetic pathway it would be. We have therefore applied a wide range of biochemical assays including in-line probing, Terbium(III) cleavage and hydroxyl radical footprinting. Our results show that some metabolites along the Moco biosynthetic pathway can be excluded as direct binding partner because they do not promote any structural change in the RNA conformation. Structural investigations complete our study, as they are needed to find the appropriate assay for each metabolite. We could confirm the proposed secondary structure<sup>2</sup> of the *moaA* riboswitch as well as assign its Mg(II) binding sites.

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**326 Coupling of Mn(2+) sensing and global structural rearrangements of *yybP-ykoY* riboswitch explored by molecular dynamics simulations**Krishna C. Suddala<sup>1</sup>, Ian R. Price<sup>2</sup>, Michal Janeczek<sup>3,4</sup>, Petra Kuhrova<sup>3</sup>, Qian Hou<sup>1</sup>, Shiba Dandpat<sup>1</sup>, Jiri Sponer<sup>3,4</sup>, Pavel Banas<sup>3,4</sup>, Ailong Ke<sup>2</sup>, Nils Walter<sup>1</sup>

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Riboswitches are RNA structural elements which regulate gene expression at level of translation, transcription or splicing. As they mainly occur in bacteria, they are considered as promising targets of new class of antibacterial drugs. Thus, detailed understanding of their mechanism of action is important. One of the crucial points of the mechanism is how the local structural changes induced by ligand binding are propagated toward the expression platform. Important insight can be provided by classical molecular dynamics which simulates structural dynamics at atomic-level resolution.

The *yybP-ykoY* riboswitch is an RNA element that selectively senses presence of Mn(2+) cation, and in response to its binding impedes transcription termination. Under high concentration of Mn(2+) a part of the terminator loop sequence is firmly sequestered within aptamer domain and thus transcription can carry on. But as the Mn(2+) concentration diminishes, the aptamer domain become more flexible which allows the terminator to form and to inhibit the transcription.

Here, we employed the classical molecular dynamics to investigate the role of the A-minor interaction between L1 and P3.1 stem in transferring the information about presence or absence of Mn(2+) in the ion-binding pocket toward the expression platform. We studied the stability of the L1 loop and P1.1 stem containing the sequestered part of the terminator loop sequence from *Lactococcus lactis* and *Xanthomonas oryzae* under different ionic conditions and within different structural contexts.



**327 Conditional control of splicing by RNA device***Adam Mol, Florian Groher, Beatrix Suess***Technical University of Darmstadt, Department of Biology, Darmstadt, Germany**

Alternative splicing of pre-mRNA in mammals is one of the important cellular processes and it is responsible for protein diversity. The accuracy of the splicing process involves the recognition of short sequences within the pre-mRNA that delimit the exon-intron boundaries. Nearly 90% of the human genes are subjected to alternative splicing and disruption of the splicing machinery lead to genetic diseases and cancer. Reprogramming of aberrant splicing could provide novel approaches to the development of molecular therapy. For this purpose, we want to use aptamers as a promising tool to control splicing events. We established a TetR-aptamer regulated intron, which is able to control the genetic output of a gene of interest by placing the defined switching module upstream the coding sequence. The TetR-aptamer is inserted into an intron close to the 5' splice site (5' ss). In absence of TetR, U1 snRNP recognize the 5' ss leading to constitutive splicing. Recognition of the 5' ss is inhibited in the presents of TetR resulting in an intron retention. The binding of TetR to the aptamer is reversible due to conformational changes of TetR through addition of doxycycline (DOX). Different reporter and natural human genes will be used to proof the switching system.

**328 Discovery and characterization of new S-adenosylmethionine (SAM) riboswitch structures***Balasubramanian Sellamuthu, V Kornikova, X Yang, F Khalfaoui, M. R. Naghdi, J Perreault***INRS-Institut Armand-Frappier, Laval, Quebec, Canada**

Riboswitches are structured RNA elements located in the 5' untranslated regions of bacterial mRNA. Their aptamer domain is responsible for selectively binding their cognate metabolite ligand, which exerts gene control following a conformational rearrangement of the expression platform. We have discovered through bioinformatics (CMfinder, Infernal, RNARobo) new SAM II-clan riboswitch structures upstream of several genes involved in SAM and sulfur metabolism widespread in bacteria.

S-adenosylmethionine (SAM) is currently the metabolite for which there is the largest number of different classes of riboswitches reported, at least four different types, and up to seven, depending on the classification used. We discovered variant riboswitches of the SAM-II clan conserved in close to a hundred species of Betaproteobacteria, including many pathogenic bacteria in the *Burkholderia* and *Bordetella* genera. Based on the new SAM-II clan consensus that we could establish, additional examples in numerous genera were found.

Many of these newly found aptamers also contain an additional stem of a varying length (10-104 nt). Our discovered riboswitches contain an aptamer domain which is believed to adopt a distinct unbound conformation with a conserved alternative stem in the absence of its ligand (SAM). To decipher the putative unbound structure of one of these unique SAM riboswitches and to elucidate the role of the new additional stem, we have conducted mutational studies to understand the ligand-bound and unbound structures in the 100 nt SAM riboswitch in *Burkholderia thailandensis* E264. A series of 20 mutations aimed at weakening or strengthening these two state were tested by in-line probing. Our results indicate that all mutant structures, even those weakening the unbound structure, decrease binding affinity for SAM by 100 to 1000 fold compared to the wild-type  $K_d$  of 0.3  $\mu$ M. This suggests the alternative stem does not compete with the ligand-bound structure, but would rather participate in the folding pathway to help attain the active aptamer conformation. Furthermore, the alteration or deletion of the new additional stem modestly decreases SAM binding affinity.



### 329 Dynamic features of the twister ribozyme revealed by single-molecule FRET imaging

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The twister RNA is a recently discovered nucleolytic ribozyme that is present in both bacteria and eukarya. While its biological role remains unclear, crystal structure analyses and biochemical approaches have revealed critical features of its catalytic mechanism. We set out to explore dynamic aspects of twister RNA folding that precede catalysis. To do so, we have employed both bulk and single-molecule fluorescence resonance energy transfer (FRET) methods to investigate a set of twister RNAs with labels that were strategically positioned at communicating segments. The data obtained reveal that folding of the central pseudoknot (T1), the most crucial structural determinant to promote cleavage, exhibits reversible opening and closing dynamics at physiological  $Mg^{2+}$  concentration. Uncoupled folding of the twister ribozyme, in which T1 formation precedes proper and complete structuring of the closing segment that includes stem P1, was confirmed using pre-steady state three-color smFRET experiments initiated by  $Mg^{2+}$  injection. These findings shed important new insights into the dynamic processes associated with the  $Mg^{2+}$ -dependent twister ribozyme folding path and their contribution to the cleavage mechanism.

N. Vušurović, R. B. Altman, D. S. Terry, R. Micura, S. C. Blanchard, Dynamic features of the twister ribozyme revealed by single-molecule FRET imaging, 2017, submitted.

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### 330 Ligand-dependent ribozymes for controlling gene expression in the nematode *C. elegans*

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Aptazymes are artificial riboswitches composed of aptamers fused to ribozymes. They have been established for the conditional control of gene expression in many single cell systems. We show that aptazymes are versatile tools for the ligand-dependent control of protein expression in different tissues of the nematode *C. elegans*.

Aptazymes have been shown to function as flexible expression platforms allowing for the construction of various ligand-dependent gene regulators especially in bacterial, yeast and mammalian cell contexts when inserted into mRNAs of interest. Conditional gene expression is achieved by binding of the ligand to the aptamer connected to the ribozyme expression platform and therefore dose-dependent cleavage of the respective transcript. Implemented in a eukaryotic expression system, aptazyme-mediated cleavage of mRNA within the untranslated region leads to degradation of the transcript and therefore downregulation of gene expression. Several aptazymes have been developed by different groups in the past allowing both up- and down-regulation upon addition of a small molecular effector.

For the model organism *C. elegans* convenient methods for conditional gene expression are still lacking. In order to expand the aptazyme technology towards this important research animal model, we tested several different riboswitches from our and other groups. Tetracycline-dependent HHR switches originally designed by Suess and co-workers allowed us to turn on gene expression in response to the addition of the effector in the nematode. The utilized tetracycline concentrations are non-toxic and conditional gene expression is demonstrated over the course of development from embryo to young adulthood. Finally, we can apply aptazyme-mediated control in different tissues of the worm. Our study in *C. elegans* shows that ribozyme-based switches of gene expression can be utilized as valuable tools in many different organisms.

### 331 Cell penetrable peptides with nanomolar affinity against intracellular RNA targets

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Structured RNA molecules in various viruses have been known as important therapeutic targets. To inhibit RNA-protein interactions, such as TAR RNA-Tat protein in HIV-1, therefore, are crucial for reducing pathogenicity of viruses. Specific small molecules against these RNA targets, however, cannot provide enough surface area, providing only micromolar affinity modulators. As efficient modulators, we suggested amphipathic  $\alpha$ -helical peptides, because they can provide large surface area to generate strong nanomolar binders against the structured target RNA molecules. However, these novel-modulating peptides should penetrate into eukaryotic cells to reach intracellular RNA targets, while most of peptides have poor cell penetrating ability in their nanomolar concentrations. We have developed a strategy for these peptides to get into cells in their nanomolar concentrations, without help of other cell penetrating tools. The strategy relies on constructions of bulky peptides that could aggregate themselves to form nanoparticles, which can penetrate into host cells by various endocytosis mechanisms. We showed several examples of the cell penetrable peptide modulators that showed low nanomolar IC<sub>50</sub> values, which are same as their binding affinity against intracellular RNA targets. These peptides could be used as novel family of future drug candidates against pathogenic viruses.

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### 332 Designing small molecules targeting CUG repeats causing Myotonic Dystrophy type 1

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Development of small molecules that can recognize specific RNA secondary and tertiary structure is currently important research topic for developing tools to modulate gene expression and therapeutic drugs. Expanded CUG trinucleotide repeats, known as toxic RNA repeats cause the human disease, myotonic dystrophy. The expanded r(CUG)<sub>n</sub> repeats formed a hairpin structure and sequestered the muscleblind-like 1 (MBNL1) protein leading to mis-splicing regulation. Because DM1 is an incurable inherited neuromuscular disease. Several groups have reported the small molecules binding to r(CUG)<sub>n</sub> repeats and carried out the competitive binding of developed molecules with MBNL1 protein. To develop new ligand to recognize the CUG trinucleotide repeats, here, we reported a series of ligands binding to the CUG repeats.

We designed and synthesized four new molecules for CUG repeat binding. The designed 2,9-dialkylamino substituted phenanthroline (DAP) was anticipated to bind to uracil with two hydrogen bonds in AA-DD motif to reducing the repulsive interaction.

The melting temperature (T<sub>m</sub>) of trinucleotide repeats RNA was measured with ligands. r(CUG)<sub>9</sub> repeats showed  $\Delta$ T<sub>m</sub> increasing in presence of DAP, whereas other control ligands did not show increase in T<sub>m</sub>.

We also investigated the binding of ligands to the repeat sequence by SPR assay. SPR assay for r(CUG)<sub>9</sub> showed increase of the SPR signal. In contrast, all control ligands didn't show any increase of SPR response. These data indicated that three ligands should have a much lower affinity to r(CUG)<sub>9</sub> than that of DAP.

To assess the effect of DAP to r(CUG) repeat on the biological processes, an in vitro dual luciferase reporter assay was designed and carried out. For the translation reaction, the Fluc/Rluc ratio showed a gradually decrease as the DAP concentration increased.

We have developed DAP binding to r(CUG)<sub>n</sub> repeat. The structure activity study showed that the tricyclic ring system and suitable hydrogen bonds was important for the r(CUG)<sub>9</sub> repeat binding. The in vitro translational assay indicated that suggesting DAP is a promising candidate for further development. These results would be useful for the design in the next generation of ligands eventually applicable in the study and possible treatment of DM1 and other repeat diseases.

### 333 sTREAT: a fluorescent protein based reporter system for monitoring RNA nuclease processing events in cells

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Many classes of RNA, such as miRNA, tRNA, and mRNA, mature through nuclease mediated events. These nuclease driven events can have a profound impact on metabolism and are essential in determining cell fate through alternative splicing, alternative polyadenylation, and miRNA maturation. Nucleases often act in combination with other proteins as part of larger complexes that are able to recognize elements of RNA primary and secondary structure. Purifying significant amounts of active nuclease complexes *in vitro* is a significant challenge for performing biochemical assays and compound screens at scale. Current methods to assay nuclease activity in cells, such as 3'UTR reporter systems, lack critical well to well assay controls and bidirectional promoters result in the production of multiple mRNAs that may have different message stabilities. Here we present a stoichiometrically controlled fluorescent reporter for monitoring RNA nuclease processing events *in cell* that we term sTREAT. Applications of this reporter system to genetic screens, small molecule screens, and RNA structure function studies will be presented and discussed. Overall we expect that our sTREAT reporter system will enable new avenues for uncovering RNA biology and provide another entry point for RNA-centric drug discovery.

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### 334 A small molecule that target UGGAA pentanucleotide repeats in spinocerebellar ataxia 31

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Spinocerebellar ataxia 31 (SCA31) is autosomal dominant spinocerebellar degeneration caused by insertion of complicated pentanucleotide repeats, (TGGAA)<sub>n</sub>, (TAGAA)<sub>n</sub>, (TAAAA)<sub>n</sub> and (TAGAATAAAA)<sub>n</sub>. It has been shown that (TGGAA)<sub>n</sub> is involved in the pathogenesis of SCA31 among these pentanucleotide repeats. Pentanucleotide repeats are inserted to an intron between *BEAN1* and *TK2* in human chromosome 16q22.1. Transcription of pentanucleotide repeats in *BEAN1*-direction produces toxic (UGGAA)<sub>n</sub> repeats that form abnormal RNA structures called RNA foci. Previous studies have shown that nuclear RNA foci containing (UGGAA)<sub>n</sub> form in Purkinje cell nuclei of SCA31 patients, leading to Purkinje cell neurodegeneration in SCA31.

SCA31 is an incurable inherited neurodegenerative disease mediated by toxic (UGGAA)<sub>n</sub> transcripts. Currently, there is no cure for SCA31. Applications of a small molecule that binds to toxic (UGGAA)<sub>n</sub> repeats are a possible strategy for alleviation of SCA31 symptoms. However, a small molecule that target (UGGAA)<sub>n</sub> repeats has not yet been demonstrated.

Previously we have designed and synthesized small molecules that selectively bind to mismatch base pairs of nucleic acids by complementary hydrogen bonding with nucleobases. For example, naphthyridine carbamate dimer (NCD), consisting of two N-alkyloxycarbonyl-2-amino-7-methyl-1,8-naphthyridine heterocycles binds to CGG/CGG motif containing G–G mismatch in DNA duplexes. The putative hairpin structure of (UGGAA)<sub>n</sub> repeats consists of two A–U base pairs and GGA/GGA triad containing G–G and two G–A mismatches. Therefore, we have focused on G–G mismatch-binding naphthyridine carbamate derivatives as (UGGAA)<sub>n</sub> repeat binders. In this study, we investigated the binding of naphthyridine carbamate derivatives to UGGAA/UGGAA pentad-containing RNA duplexes and (UGGAA)<sub>n</sub> repeats by melting temperature measurements, circular dichroism spectroscopy, cold-spray-ionization time-of-flight mass spectrometry and so on. These results indicated that a naphthyridine carbamate derivative showed strong binding to (UGGAA)<sub>n</sub> repeats. In addition, we examined the bioactivity of the (UGGAA)<sub>n</sub> repeat-binding molecule for SCA31 disease model animals. Administration of the (UGGAA)<sub>n</sub> repeat-binding molecule alleviated neurodegeneration caused by the expression of (UGGAA)<sub>n</sub> repeats. The (UGGAA)<sub>n</sub> repeat-binding small molecule reported here will be an useful chemical tool for SCA31 studies.

### 335 Synthesis and characterization of novel cyclic dinucleotides

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Nature uses a well-established system of nucleotide-derived second messengers. The latest to enter the list were cyclic dinucleotides. In addition to the long known c-di-GMP, it was found that c-di-AMP is phylogenetically widespread and, like c-di-GMP, involved in the regulation of a plethora of cellular processes in bacteria. [1]

The hybrid c-AMP-GMP was detected in two different forms. The 3'-5' / 3'-5'-linked c-AMP-GMP is produced by certain bacteria such as *Vibrio* and *Geobacter*, whereas the 2'-5' / 2'-5'- version signals bacterial infections in mammalian cells. [2] The bacterial versions of the cyclic dinucleotides trigger pronounced but specific immune responses, making them promising candidates as vaccine adjuvants. [3] For all known bacterial cyclic dinucleotides riboswitches selectively recognizing the second messengers have been identified. Furthermore, single nucleotides in the binding pocket of a riboswitch class that binds c-di-GMP are varied, enabling recognition of c-di-AMP and c-AMP-GMP. [2b, 4]

We speculated that nature could make use of further combinations of nucleotides in additional cyclic dinucleotides. We therefore synthesized the so-far undescribed compounds c-AMP-CMP, c-AMP-UMP, c-di-CMP, c-CMP-GMP, c-CMP-UMP, and c-di-UMP. We analyzed the immunogenic potential, their binding affinity towards some variations of class I c-di-GMP riboswitches, and utilized them as standards in order to search for the presence of these novel compounds in nature.

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### 336 How Oxidation of the Ribosome's Active Site Affects Translation

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The ribosome is the central ribozyme responsible for protein biosynthesis. The exact working principle of the peptidyl transferase center (PTC), its active site, is still a matter of ongoing debate and research. However, significant contribution to catalysis stems from the ribose-phosphate backbone of rRNA, specifically the 2'OH of A2451 [1]. Recent findings in Alzheimer's disease indicate oxidized ribosomes could be a contributing factor to neurodegenerative diseases [2]. However, the consequences of direct oxidative stress to the ribosome and its effects on translation are unknown. Investigating oxidation in the PTC could provide insight into the mechanism of peptide bond formation, and also elucidate disease-relevant effects of the oxidized translation machinery.

Using the technique of atomic mutagenesis, non-natural nucleosides can be introduced at any position of the 23S rRNA during *in vitro* reconstitution of the ribosome. This method surpasses conventional mutagenesis and effectively enables us to alter single atoms in the context of the ribosome [3]. We replaced the universally conserved PTC nucleobases A2451, A2601, U2585, U2506 and C2063 with synthetic counterparts carrying the most common oxidations 8-oxoA, 5-HOrU and 5-HOrC, respectively. The chemically engineered ribosomes were then studied in various functional assays to investigate the consequences of nucleobase oxidations on translation

Incorporation of different oxidized bases into the PTC affected ribosome's functions in diverse ways. Oxidizing an individual nucleobase either resulted in a radical deceleration of peptide bond formation, alteration of tRNA binding to A- and P-site or even an increase of the translation rate due to facilitated translocation. These results expand our current understanding of the roles of PTC residues in ribosomal function. This study is the first investigation of site-specific oxidation of central PTC residues, and provides diverse, interesting findings for both the ribosome field and research on neurodegenerative disease.

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**337 Novel RNA end modifications detected by innate immune RNA sensors***Rachel Ancar, Jay R Hesselberth***University of Colorado School of Medicine, Aurora, CO, USA**

Innate immune sensors must respond rapidly to a variety of pathogens while avoiding aberrant detection of normal cellular molecules. RIG-I-like receptors (RLRs) are cytosolic dsRNA sensors that bind viral RNA transcripts. RLRs such as retinoic acid inducible gene I (RIG-I) and Melanoma Differentiation-Associated protein 5 (MDA5) couple detection of viral RNAs to the expression of type I interferon and pro-inflammatory cytokine genes. Structural and biochemical studies previously identified short viral dsRNAs with 5'-triphosphate moieties as RIG-I ligands. The 5'-triphosphate is a unique feature of viral replication, suggesting a mechanism by which RIG-I would specifically identify viral RNAs while avoiding recognition of self-RNAs. Recent studies show that RNA cleavage products of the endoribonucleases Ire1 and RNase L can activate a RIG-I-dependent type I interferon response. However, these endoribonucleases produce RNA products with 5'-OH and 2',3'-cyclic phosphate termini, so it is unclear how these RNA cleavage products activate RIG-I.

We hypothesize that RNA end modification enzymes remodel the termini of these RNA cleavage products to promote their recognition by RIG-I. We use genetic and biochemical approaches to determine the impact of RNA modification enzymes on RNA detection by RIG-I and define the chemical requirements for RIG-I mediated detection of RNA cleavage products. Our preliminary studies suggest that the 5' and 3'-ends of RNA are differentially detected by RIG-I, possibly by 3'-end RNA modification enzymatic activity. We show that synthetic dsRNAs with 3'-monophosphates are capable of activating interferon expression, while 5'-monophosphates do not activate the antiviral response. Furthermore, interferon expression is dependent on the position of the phosphate at the 3'-end of the RNA. These studies suggest that host RNA end modification enzymes modulate detection of intracellular RNA by RIG-I.

**338 The RNA helicase YTHDC2 regulates the transition from proliferation to differentiation in the germline stem cell lineage***Alexis Bailey<sup>1</sup>, Pedro Batista<sup>2,3</sup>, Rebecca Gold<sup>1</sup>, Y Grace Chen<sup>2</sup>, Dirk G de Rooij<sup>4</sup>, Howard Chang<sup>2</sup>, Margaret Fuller<sup>1</sup>*<sup>1</sup>Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA, USA;<sup>2</sup>Center for Personal Dynamic Regulomes, Stanford University School of Medicine, Stanford, CA, USA;<sup>3</sup>Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA;<sup>4</sup>Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

The switch from mitotic proliferation to meiosis is the key event marking onset of germ cell differentiation to produce haploid gametes. Here we show that in mammals the RNA helicase YTHDC2 is required for a clean transition from mitosis to meiosis in male and female germ cells. *Ythdc2*<sup>-/-</sup> male germ cells attempt to enter meiotic prophase but appear to have a mixed identity, maintaining expression of mitotic Cyclin A2 and failing to properly express many meiotic markers. Instead of executing meiotic prophase, the cells attempt an abnormal mitotic-like division then quickly die. YTHDC2 protein localizes to germ granules in spermatocytes entering the initial stages of meiotic prophase. Comparison of the mRNAs expressed in *Ythdc2*<sup>-/-</sup> and age-matched wild-type testes revealed several transcripts expressed at lower levels in *Ythdc2*<sup>-/-</sup> than in wild-type testes. The transcripts most under-expressed in *Ythdc2*<sup>-/-</sup> compared to age-matched wild-type testes were frequently up-regulated in spermatocytes compared to spermatogonia based on data from sorted germ cells (Soumillon et al., 2013), suggesting widespread failure to express the spermatocyte transcription program in *Ythdc2*<sup>-/-</sup> male germ cells. In addition, expression of 27 transcripts remained relatively high in *Ythdc2*<sup>-/-</sup> testes compared to age-matched wild-type controls. Based on published datasets, these were genes that tend to be expressed in spermatogonia but normally down-regulated in wild-type spermatocytes, suggesting that the mitotic program may fail to properly shut down in the absence of YTHDC2 function. YTHDC2 binds several RNAs involved in cell cycle and cell division, and YTHDC2 interacting RNAs are enriched for m<sup>6</sup>A targets. We propose that action of YTHDC2 through post-transcriptional control of RNA is required during the early stages of meiotic entry in mice to both down-regulate the previous mitotic program and facilitate proper expression of meiotic and differentiation genes.



**339 Alternative polyadenylation patterns for novel gene discovery and classification in cancer***Oguzhan Begik, Merve Oyken, Tuna Cinkilli Alican, Tolga Can, A. Elif Erson Bensen***Middle East Technical University, Ankara, Turkey**

Certain aspects of diagnosis, prognosis and treatment of cancer patients are still important challenges to be addressed. Therefore, we propose a pipeline to uncover patterns of alternative polyadenylation (APA), a hidden complexity in cancer transcriptomes, to further accelerate efforts to discover novel cancer genes and pathways. Here, we analyzed expression data for 1,045 cancer patients and found a significant shift in usage of poly(A) signals in cancers. Using machine-learning techniques, we further defined subsets of APA events to classify cancer types. Furthermore, detected APA patterns were associated with altered protein levels in patients, revealed by antibody-based profiling data. Overall, our study offers a computational approach for the use of APA in novel gene discovery and classification in cancers, with important implications in basic research, biomarker discovery and precision medicine approaches.

**340 RCas9 mediated site-directed RNA editing allows programmable base conversion of human mRNA***Kristopher Brannan<sup>1</sup>, Ryan Marina<sup>1</sup>, David Nelles<sup>1</sup>, Gene Yeo<sup>1,2</sup>***<sup>1</sup>University of California, San Diego, San Diego, CA, USA; <sup>2</sup>National University of Singapore, Singapore, Singapore**

CRISPR/Cas mediated genome editing has revolutionized biological research by enabling the site-specific manipulation of genetic information at the DNA level. Repurposing of nuclease-dead Cas9 for the direct targeting of RNA (RCas9) has been successfully used to localize cellular mRNAs of interest, but no system exists for RCas9 mediated site-directed RNA base editing. For the purpose of programmable RNA-editing, we fuse an effector protein to RCas9 and show that this fusion can direct conversion of specific targeted bases on both reporter and cellular mRNAs. Our RNA-editing platform improves upon the efficiency and specificity of previous RNA-editing strategies, and provides a powerful tool for the targeted and reversible manipulation of gene expression.

**341 Impact of Modified Nucleobases on Base Pairing in RNA Experimental Structures***Mohit Chawla<sup>1</sup>, Romina Oliva<sup>2</sup>, Janusz Bujnicki<sup>3</sup>, Luigi Cavallo<sup>1</sup>*<sup>1</sup>King Abdullah University of Science and Technology (KAUST), Jeddah, Saudi Arabia; <sup>2</sup>Department of Sciences and Technologies, University Parthenope of Naples, Naples, Italy; <sup>3</sup>Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cellular Biology, Warsaw, Poland

Posttranscriptional modifications greatly enhance the chemical information of RNA molecules, contributing to explain the diversity of their structure and functions. A significant fraction of RNA experimental structures available to date present modified nucleobases, with half of them being involved in H-bonding interactions with other bases, i.e. 'modified base pairs'. To this end, we did a systematic investigation of modified base pairs, in the context of experimental RNA structures. In addition, non-natural (synthetic) modifications have been introduced in nucleic acid structures for targeted applications. This prompted us to also analyze the impact of synthetically prepared non-natural modifications on the H-bonding propensity in the context of nucleic acid structures. For this, set of PDB structures solved by X-ray crystallography at a resolution of 3.5 Å or better and containing RNA molecules with posttranscriptional modifications were analysed, in order to identify the modified base pairs and classify their geometry. As a result of this analysis, we obtained 573 base pairs containing at least one modified base. Base pairs containing non-natural modifications were also modelled starting from available experimental structures in the PDB. The geometries of the base pairs were optimized with a Density Functional Theory (DFT) approach. Interaction energies were evaluated at the MP2 level of theory. Our structural analyses show that most of the modified base pairs are non Watson-Crick like and are involved in RNA tertiary structure motifs. Similar analyses were also performed on base pairs involving some non-natural modifications of particular biotechnological interest, in the context of nucleic acids. The combined bioinformatics and quantum mechanics studies we performed help provide a rationale for the impact of the different modifications on the geometry and stability of the base pairs they participate in, and possibly predict the effect of newly designed modifications. (1-4)

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**342 Spatio-temporal profiling of Filamin B RNA-editing in mouse***Philipp Czermak<sup>1,2</sup>, Laura Cimatti<sup>2</sup>, Michael F. Jantsch<sup>1,2</sup>*<sup>1</sup>Division of Cell Biology, Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria; <sup>2</sup>Department of Chromosome Biology, Max F. Perutz Laboratories, University of Vienna, Vienna, Austria

RNA editing is a post-transcriptional RNA modification which leads to nucleotide alterations in RNA molecules. Adenosine to Inosine (A-to-I) editing is the main form of RNA editing in mammals. A-to-I editing occurs in regions of double-stranded and structured RNA where the deamination of adenosine to inosine is performed by Adenosine Deaminases acting on RNA (ADARs). Inosines are recognized as guanosines by many cellular processes, such as folding, splicing and translation.

Among the known coding targets of ADARs the A-to-I editing event on the filamin mRNAs is highly conserved in vertebrates and leads to a nonsynonymous glutamine (Q) to arginine (R) amino acid substitution in a highly interactive domain of the encoded proteins. Filamins are able to bind F-actin forming a network and therefore serve as cytoskeletal proteins that also function as scaffolds for a variety of different interactors.

In particular, we are interested in the A-to-I editing of Filamin B mRNA. To determine where Flnb editing might display its maximum effect we aimed to determine the editing level of Flnb, tissues from neonate (P0), juvenile (P21) and adult (P120) wild type (wt) C57BL/6 mice. The extracted RNA was reverse transcribed and the edited Flnb region enriched by PCR and subjected to bar-coded NGS analysis. Data analysis is currently under way. With this approach we aim at laying the foundation for further research on the impact of RNA-editing on Filamin B functions.

**343 Editing Inducer Elements: a requisite for A-to-I editing efficiency at specific sites***Chammiran Daniel, Ditte Rigardt, Marie Öhman***Molecular Biosciences Wennergren Institute, Stockholm, Sweden**

Adenosine deaminases acting on RNAs (ADARs) convert adenosine to inosine (A-to-I) in double stranded RNA. A-to-I RNA editing occurs post-transcriptionally and editing of coding sequences can lead to expression of different protein isoforms, since I is read as G during translation. Most recoding events induced by ADARs are found within the pre-mRNA transcript of neuronally expressed genes where editing expands the variety of protein isoforms required for neuronal function. The transcript of the excitatory AMPA glutamate receptor subunit A2 (GluA2) is subjected to editing at several sites, creating receptor isoforms with altered kinetics. Editing at the Q/R site, where a glutamine is recoded to an arginine, is the most efficient site of editing known with almost 100% edited transcripts. ADARs require double stranded RNA for binding and deamination, but it is not clear why this substrate is so extraordinarily efficiently edited. We have previously shown that Gabra-3 transcript, coding for the  $\alpha 3$  subunit of the GABA<sub>A</sub> receptor, requires a distant intronic editing inducer element (EIE) to be efficiently edited. Here we show that an EIE also is required for editing at the Q/R site in GluA2. Common between these EIEs is a long double stranded stem structure without any sequence homology. The EIEs can replace each other in the induction of editing efficiency. Furthermore we show that the efficiently edited kainite glutamate receptor subunit GluK2 also holds an EIE within its pre-mRNA, proposing that this is a general requisite for efficiently edited substrates. We propose a model where two regions of dsRNA are required for efficient editing: first, a long dsRNA to recruit the ADAR enzymes and increase the local concentration of the enzyme and secondly a more structured duplex ideal for specificity and catalysis.

**344 Absence of the Fragile X Mental Retardation Protein results in defects of RNA editing of neuronal mRNAs in mouse***Alice Filippini<sup>1</sup>, Daniela Bonini<sup>1</sup>, Caroline Lacoux<sup>2</sup>, Laura Pacini<sup>2</sup>, Maria Zingariello<sup>3</sup>, Laura Sancillo<sup>4</sup>, Daniela Bosisio<sup>5</sup>, Valentina Salvi<sup>5</sup>, Jessica Mingardi<sup>1</sup>, Luca La Via<sup>1</sup>, Francesca Zalfa<sup>3</sup>, Claudia Bagni<sup>2,6</sup>, Alessandro Barbon<sup>1</sup>*

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The fragile X syndrome (FXS), the most common form of inherited intellectual disability, is due to the absence of FMRP, an mRNA-binding protein involved in RNA metabolism. Recently, an unexpected function of FMRP in modulating ADAR enzymes activity has been reported both in *Drosophila* and Zebrafish. ADARs are RNA-binding proteins that catalyze the RNA editing process, a post-transcriptional mechanism that increases protein diversity, present mainly in the central nervous system (CNS). Changes in editing patterns are frequently associated with neuropsychiatric and neurodevelopment disorders.

We analysed several RNA editing sites in *fmr1* knockout (KO) mice, in order to evaluate the possible functional ADAR2-FMRP interaction in mammals and to understand the impact of FMRP on the editing process. We found an increase in editing levels of *fmr1* KO mice for several edited transcripts mainly expressed in the brain, indicating that FMRP might act as an inhibitor of editing activity. This evidence was then supported by an editing assay on HEK293T cells stably expressing ADAR2 and transiently transfected with FMRP.

To test the possible physical interaction of ADAR2 and FMRP, we performed Proximity Ligation Assay (PLA) in mouse primary cortical neurons and in HEK293T cells over-expressing ADAR2 and FMRP. The results showed a co-localization of ADAR2 and FMRP mainly in the nucleus. A similar co-localization of ADAR2-FMRP was also observed by double-immunogold Electron Microscopy (EM) in hippocampal sections of adult mice.

The editing levels alterations in *fmr1* KO mice and the physical interaction of ADAR2 and FMRP strongly support, for the first time in mammals, the presence of a link between the editing process and FMRP functions.

### 345 Is (Cytosine-5) RNA Methylation linked to Genome Instability?

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In the last decade, a (cytosine-5) methyltransferase called DNA methyltransferase 2, (Dnmt2) was identified in a position effect variegation screen for genes involved in chromatin establishment and maintenance and found to affect only reporters at loci corresponding to transposable elements (TE). Importantly, rather than methylating DNA, Dnmt2 has robust RNA methylation activities modifying a subset of tRNAs in all tested organisms reaching from bacteria, to flies, to plants to man. This observation suggested a link of RNA methylation and genome instability.

To test if (cytosine-5) RNA methylation affects transposon control, another RNA (cytosine-5) methyltransferase, NOP2/Sun domain family member 2 (NSun2), was analyzed using biochemical approaches including binding assays, gene expression studies and a reporter assay screen. The results indicate that NSun2 is also influencing transposon expression and silencing.

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### 346 The tRNA Identity Elements of Certain Aminoacyl-tRNA Synthetases are Necessary for Human Pus10-mediated Ψ54 Formation in tRNAs

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The TΨC loop of tRNAs has T (ribothymidine or 5-methyluridine) at position 54 in most Bacteria and Eukarya, and in some Archaea. Most Archaea have a pseudouridine (Ψ) or its modified version at this position. A nearly universal Ψ is present at tRNA position 55. Members of the TruB family produce Ψ55 in bacteria and eukaryal tRNAs, but Pus10 produces both tRNA Ψ54 and Ψ55 in Archaea. Pus10 is the only known member of one of the six families of Ψ synthases. *Pus10* homologs are present in Archaea and most Eukarya, but not in Bacteria and yeast. This coincides with the presence of Ψ54 in archaeal tRNAs and some specific tRNAs of mammals and its absence in tRNAs of Bacteria and yeast. We show that tRNA Ψ54 activity is present in mammalian cell extracts and Pus10 is involved in this process. This activity is specific to only certain tRNAs, e.g., those for Gln, Trp and Pro. U54 of Ala, Phe and Asp tRNAs are not modified to Ψ54. Changing the Trp-tRNA synthetase identity elements of Trp tRNA to those of Ala-tRNA synthetase drastically reduces Ψ54 formation in this tRNA. In the reverse case, i.e., changing identity elements of Ala tRNA to those of Trp tRNA significantly brings back this activity. This suggests that certain aminoacyl-tRNA synthetases cooperate with Pus10 in producing Ψ54 in specific tRNAs.

### 347 The ribosome code to differentiation

*Sophia Häfner<sup>1</sup>, Martin Jansson<sup>1</sup>, Ulf Birkedal<sup>2</sup>, Henrik Nielsen<sup>2</sup>, Anders Lund<sup>1</sup>*

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We are currently witnessing the dawn of epitranscriptomics: the incredible number of RNA modifications and their crucial importance for the fine-tuning of all cellular processes is merely starting to be recognized. In this context, ribosomal RNA (rRNA) is quickly shifting into the limelight. Indeed, for a long time, ribosomes were thought to be constitutive machineries without active control on translation; however, this belief has been challenged over the last decade by the concept of ribosome heterogeneity. One potential source of ribosome variation are the numerous posttranscriptional modifications of rRNA, the most abundant being 2'-O-methylation and pseudouridylation, which are crucial for correct ribosomal biogenesis and translational fidelity.

The epitranscriptomics toolbox was expanded recently by the RiboMeth-seq method, which allows for fast, global and quantitative mapping of the 2'-O-Me status of rRNA (Birkedal et al. 2015). We applied the method to many cell lines and cellular processes, allowing us to unveil three cardinal facts: some positions are only fractionally methylated, the methylation profile differs between cell types, and it displays dynamics upon cellular processes. Thus we hypothesize that some of the fractionally methylated positions may represent fine-tuning buttons to adjust the ribosome to different situations.

Hence, the differentiation of human embryonic stem (ES) cells constitutes a particularly relevant model system, as cell fate commitment represents a fundamental change for the entire transcriptome. We investigated the 2'-O-methylation profile of human ES and induced pluripotent stem (iPS) cells during differentiation into the three embryonic stem cell layers. Stunningly, all cell lines displayed clear and coherent changes of several methylated positions upon differentiation. Moreover, while some changes are identical to all types of differentiation, others are very specific, allowing us to hypothesize the existence of a "methylation code of differentiation".

Modulation of the methylation levels by tuning the expression levels of the linked snoRNAs *via* gain and loss mutant stem cell lines and the assessment of their impact on the stemness and differentiation potential are currently ongoing and will ultimately allow us to decipher the underlying molecular mechanisms linking the 2'-O-methylation profile to the differentiation process.

### 348 News from an ancient relative: The Elongator complex and Elongator-dependent anticodon modifications in *Dictyostelium discoideum*

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The Elongator complex plays an important role in the chemical modification of tRNA anticodon uridines at position 34 (U34) and it is conserved amongst eukaryotic organisms. Here we report the identification and characterization of the Elongator complex in *Dictyostelium discoideum*, as the first organism of the evolutionary supergroup of the Amoebozoa. The two Elongator subcomplexes Elp123 and Elp456 appear to exist mostly independently, and they can readily be co-purified upon overexpression. Native PAGE and size exclusion chromatography suggest a size of around 450 kDa for the Elp456 subcomplex (HAP), which may form a heterotrimer in *D. discoideum* instead of the heterodimer shown for yeast.

Investigations on the modification pathway via loss of function studies revealed a fully conserved modification pathway. The thiouridylation at U34 of tK<sup>UUU</sup>, tE<sup>UUC</sup> and tQ<sup>UUG</sup> is conditionally dependent on the 5-carbamoyl-methyluridine (mcm<sup>5</sup>) and 5-methoxy-carbonyl-methyl-2-thio-uridine (mcm<sup>5</sup>) modification. We investigated the contribution of mcm<sup>5</sup>s<sup>2</sup> tQ<sup>UUG</sup> towards the decoding efficiency of the CAA and CAG codons. For this purpose, we fused poly glutamine leaders with different CAA/CAG ratios to GFP and expressed these constructs in the background of different mutant strains. We measured expression differences via flow cytometry and western blot. Our data suggest that wobbling of tQ<sup>UUG</sup> over the CAG codon can occur in *D. discoideum*.

Strains lacking Elongator proteins displayed only weak phenotypes under normal lab conditions.

The AT rich (77%) genome of *D. discoideum* features a high frequency of A-ending codons and therefore a high dependency on Elongator modified tRNA. Unlike in yeast, statistic analysis shows no global codon usage differences between cellular pathways. We suggest that the high copy number of certain tRNA genes in the genome of *D. discoideum* counteracts any phenotypes that result from the loss of U34 modification.



### 349 Single molecule FRET unravels stepwise conformational changes during H/ACA RNP assembly and catalysis

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Eukaryotic H/ACA RNP complexes play an essential role in the maturation of ribosomal and spliceosomal RNA. During this process, they catalyze the pseudouridylation of target RNAs in a unique sequence-specific fashion. The central element is the pseudouridine synthase Cbf5; together with three auxiliary proteins (Nop10, L7Ae, Gar1) and a guide RNA, they constitute the core H/ACA complex. A number of available structures of H/ACA RNP complexes from various organisms have provided a good understanding about the architecture of this complex; however, information on structural dynamics throughout the cycle of assembly, substrate recruitment and catalytic turnover are sparse.

We have developed and established pipelines to site-specifically label proteins and RNA to be reconstituted into catalytically active H/ACA complexes for single molecule FRET spectroscopy. By analyzing different distance vectors, we find that upon incorporation into various subcomplexes, RNA conformation differs significantly from the conformation found in the active, fully assembled complex. We thereby show how stepwise assembly of the complex distorts the guide RNA to facilitate substrate binding.

By expanding our analysis to various structural elements (Gar1, thumb-loop domain) as well as different substrate RNA analogues, we identified various novel states required for catalytic turnover. We can correlate these states with predicted intermediates of the respective reaction pathways, and show that distinct conformational rearrangements are correlated with catalysis. Our results explain in detail the functional contribution of individual proteins and their domains for H/ACA-mediated pseudouridylation, an insight going beyond the current understanding from existing structural and biochemical data.

In summary, we identify and characterize several steps of conformational dynamics during assembly and catalytic turnover in H/ACA complexes, significantly enhancing our understanding of the structural dynamics of sequence-specific pseudouridylation.

### 350 Enzymatic detection of activities of m6A RNA methyltransferases and demethylases

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RNA methylation at the N6 position on adenine (m6A) is most prevalent internal RNA modification, which is mainly found at the RRACH sequence (R: G or A, H: U, A or C). The modification, which has been reported to regulate various physiological processes, is dynamic and reversible. It has been shown that FTO and ALKBH5 demethylate m6A and that METTL3 and METTL4 catalyze the methylation. To characterize these demethylases or methyltransferases and to find their inhibitors, it is required to develop new methods to easily detect their enzymatic activities. Here, we propose a convenient method to detect enzymatic activities of these demethylases and methyltransferases without using any specific apparatus or radioisotopes.

MazF is a bacterial toxin that plays an important role in growth regulation. MazF works as an endoribonuclease that specifically cleaves RNA at the 5'-end of ACA sequence. We found that MazF cleaved a single-stranded RNA fragment containing GGACA but not GG(m6A)CA by a conventional gel electrophoresis. After being reacted with FTO or ALKBH5, the oligo RNA including an m6A was cleaved by MazF. The cleavage was inhibited by addition of known inhibitors of FTO or ALKBH5. In addition, the oligo RNA including a GGACA sequence was not cleaved by MazF after being reacted with the METTL3/METTL4 complex. These results indicate that the activities of both RNA demethylases and methyltransferases can be detected by this new method. Though the sequence is limited to "ACA", this method is easy to assay the enzymatic activities of RNA methylation/demethylation at the N6 position on adenine.

**351 High-throughput identification of C/D box snoRNA targets with CLIP and RiboMeth-seq**

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High-throughput sequencing has greatly facilitated the discovery of long and short non-coding RNAs (ncRNAs), which frequently guide ribonucleoprotein complexes to RNA targets, to modulate their metabolism and expression. However, for many ncRNAs, the targets remain to be discovered. In this study, we developed computational methods to map C/D box snoRNA target sites using data from core small nucleolar ribonucleoprotein crosslinking and immunoprecipitation and from transcriptome-wide mapping of 2'-O-ribose methylation sites. We thereby assigned the snoRNA guide to a known methylation site in the 18S rRNA, we uncovered a novel partially methylated site in the 28S ribosomal RNA, and we captured a site in the 28S rRNA in interaction with multiple snoRNAs. Although we also captured mRNAs in interaction with snoRNAs, we did not detect 2'-O-methylation of these targets. Our study provides an integrated approach to the comprehensive characterization of 2'-O-methylation targets of snoRNAs in species beyond those in which these interactions have been traditionally studied and contributes to the rapidly developing field of 'epitranscriptomics'.

**352 Global Interplay of A-to-I RNA Editing and pre-mRNA Splicing**

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Post-transcriptional RNA-processing events allow for transcriptome diversity in complex organisms. A-to-I RNA editing and pre-mRNA splicing are two major post-transcriptional nuclear RNA-maturation events that drive transcriptome variation. A-to-I RNA editing is mediated by adenosine deaminases acting on RNA (ADARs) by hydrolytic deamination of adenosines. Editing sites are typically defined by double stranded structures that are frequently formed by exon-intron base pairing. It is therefore expected that splicing-efficiency will affect editing rates. Obviously, sequence changes introduced by ADAR-mediated RNA editing may potentially also perturb splice patterns. In this study, we probe how these two processes affect each other at a transcriptome-wide scale. We determine changes in editing patterns following treatment of primary cell culture systems with a splicing inhibitor. So far, we show that editing levels of sites exclusively dependent on exonic elements are less affected by splicing inhibition while editing levels of sites defined by exonic and intronic sequences are strongly affected by reduced splicing rates. Intriguingly, significant differences in editing levels are observed between pre-mRNA and mRNA of target substrates, suggesting additional mechanisms regulating the stability, processing or nuclear export of edited transcripts. Currently, transcriptome-wide analysis of splice patterns in editing deficient cells is being analyzed. Together, this will provide insights into the cross-talk between these two major post-transcriptional RNA-processing events. Moreover, biochemical experiments will be performed to determine the mechanisms leading to different editing levels in pre-mRNA and mature mRNA.

**353 RNA folding by RNA modifying enzymes**

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Non-coding RNAs typically have to adopt a distinct three-dimensional structure in order to be functional. In addition, most non-coding RNAs are subjected to post-transcriptional chemical modifications. However, many of the RNA modifying enzymes are not essential, and the modifications within the RNA are frequently dispensable for RNA function. This raises the question why many RNA modifying enzymes are conserved across all domains of life.

Here, we report a critical link between tRNA folding and tRNA modification. First, we demonstrate that the tRNA pseudouridine synthase TruB targeting U55 in the TΨC arm of all tRNAs acts as a tRNA chaperone by helping tRNA to fold into an active form. Second, this tRNA chaperone function of TruB is crucial for bacterial fitness in contrast to the actual RNA modification. Third, we use kinetic studies to unravel the molecular mechanism of tRNA folding by TruB. By unfolding critical tertiary interactions in the elbow region of tRNA, that might be prone to misfolding, TruB enables tRNA to re-fold into the correct structure.

Many RNA modifying enzymes locally unfold their substrate RNA to gain access to the target nucleotide for modification. Therefore, we hypothesize that other enzymes modifying the tRNA elbow region are also tRNA chaperones. Indeed, we show here that the tRNA methyltransferase TrmA which generates T54 is also promoting tRNA folding. We are currently investigating the potential tRNA chaperone activity of other tRNA modifying enzymes representing different enzyme families.

Together, our results discover a functional link between RNA modification and folding that impacts cellular fitness. This finding has wide-spread implications not only for tRNA maturation, but also ribosome biogenesis, formation of the spliceosome and possibly other RNAs and ribonucleoproteins as all these processes may benefit from RNA folding by RNA modifying enzymes. Moreover, it can be envisioned that many RNA binding proteins, that utilize an induced fit mechanism when interacting with RNA, could also act as RNA chaperones influencing the 3D structure of the cellular transcriptome.

**354 The N<sup>6</sup>-methyladenosine (m<sup>6</sup>A) RNA modification modulates neuronal functions and sex determination in *Drosophila* via its nuclear reader protein YT521-B**

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N<sup>6</sup>-methyladenosine RNA (m<sup>6</sup>A) is among the most abundant mRNA modifications in vertebrates that can regulate multiple aspects of the mRNA fate and is involved in several biological processes, including circadian clock, metabolism and embryonic stem cell differentiation. However, its precise roles during development of complex organisms remain unclear.

We carried out a comprehensive molecular and physiological characterization of the individual component of the m<sup>6</sup>A methyltransferase complex as well as of the YT521-B nuclear reader protein in *Drosophila melanogaster*. Components of the complex are ubiquitously expressed with clear enrichment in the nervous system, consistent with the high level of m<sup>6</sup>A in this tissue. Transcriptome wide m<sup>6</sup>A profiling in *Drosophila* S2R+ cells revealed a conserved distribution with vertebrates. Furthermore we found a number of genes required for neuroblast division, proliferation and differentiation that were mis-regulated upon m<sup>6</sup>A loss. Consistently, mutant flies for the catalytic subunits suffer from severe locomotion defects due to impaired neuronal functions. Components of the m<sup>6</sup>A methyltransferase complex also fine-tune the female-specific splicing of *Sex lethal* (*Sxl*) transcript and of its downstream targets, revealing a role for this modification in sex determination and dosage compensation. Remarkably, the knock out of YT521-B resembles the loss of catalytic subunits, implicating this protein as a main effector of m<sup>6</sup>A functions *in vivo*.

Lastly, we performed a screen to identify new components of the methyltransferase complex. We will present our current data regarding the interplay of these factors in the regulation of m<sup>6</sup>A-dependent RNA processing in *Drosophila*. Altogether, our study substantially extends the knowledge on m<sup>6</sup>A biology, demonstrating the crucial role of this modification in neuronal processes.

### 355 A-to-I editing regulation by human ADARs

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Adenosine-to-inosine (A-to-I) RNA editing, catalyzed by adenosine deaminases acting on RNA (ADARs), increases the complexity of transcribed RNAs and expands the diversity of genome functions. Although millions of human A-to-I editing sites have been profiled, we still have limited understanding of editing regulation by ADARs genome-wide. By taking advantage of different human stable HEK293 cell lines with hADAR1 knockdown, hADAR2 knockdown or hADAR1/hADAR2 double knockdown, we classify human A-to-I editing sites into different groups that are catalyzed by hADAR1 only (type I), hADAR2 only (type II), hADAR1 or hADAR2 (type III), or both hADAR1/hADAR2 (type IV), respectively. Surprisingly, with our de novo computational pipeline, we have identified a subset of A-to-I editing sites that are up-regulated in hADAR2, but not hADAR1, knockdown, suggesting a non-catalytic impact of hADAR2. Additionally, some previously-reported SNP sites are also rescued by our de novo bioinformatic pipeline and are proven to be A-to-I editing sites. Finally, we apply genome-wide iCLIP and fCLIP analyses to systematically survey different A-to-I editing sites and their association with hADAR1 and/or hADAR2. Collectively, this study provides new insights into A-to-I editing regulation by different hADARs.

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### 356 Evolution of RNA-modifying enzymes in Metazoa

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In bilaterian animals the 3' ends of microRNAs (miRNAs) are frequently modified by methylation, tailing and trimming. These modifications affect miRNA-mediated gene regulation by modulating miRNA stability. Here we analyzed data from three non-bilaterian animals: two cnidarians (*Nematostella vectensis* and *Hydra magnipapillata*) and one poriferan (*Amphimedon queenslandica*) and revealed that their miRNAs frequently undergo modifications like in bilaterians: the majority of miRNAs are expressed as different length isoforms and frequent modifications of the 3' end by mono U or mono A tailing are observed. However, unlike in bilaterians, we also find that the majority of miRNAs in *Nematostella* are methylated. This modification occurs in a constant rate throughout development. Moreover as the factors regulating miRNA modifications are largely uncharacterized in non-bilaterian phyla we investigated the evolution of 3' terminal uridylyl transferases (TUTases) and the MUT7/Nibbler exonuclease, factors that are involved in miRNA 3' modifications in Bilateria. Phylogenetic analysis on TUTases showed that TUTase1 and TUTase 6 are a result of duplication of in bilaterians and that TUTase7 and TUTase4 are the result of a vertebrate-specific duplication. We also find an unexpected number of *Drosophila*-specific gene duplications and domain losses in most of the investigated gene families. Overall, our findings shed new light on the evolutionary history of RNA modifier enzymes in Metazoa, as they reveal that this core set of enzymes already existed in the last common ancestor of all animals and was probably involved in modifying small RNAs in a similar fashion to its present activity in bilaterians.



**357 A strategy for selectively altering genetic information at the level of RNA***Maria Montiel-Gonzalez<sup>1</sup>, Isabel Vallecillo-Viejo<sup>1,2</sup>, Joshua J. C. Rosenthal<sup>1</sup>*<sup>1</sup>Marine Biological Laboratory, Woods Hole, USA; <sup>2</sup>Universidad de Puerto Rico, San Juan, Puerto Rico

RNA Editing is an enzymatic process that initiates site-directed mutagenesis within RNAs. This process is catalyzed by Adenosine Deaminases that Act on RNA (ADARs), a family of enzymes that convert adenosines (A) to inosines (I) in messenger RNAs. I is structurally similar to guanosine (G), and an A-to-I change is interpreted as an A-to-G change by ribosomes and other biological processes. As a result of this mechanism, protein function may be altered, particularly if editing occurs within mRNA coding regions. In our lab, we have engineered a recombinant ADAR that directs the editing reaction to a specific adenosine of our choosing within an RNA. This interaction between RNA and protein is guided by a complementary RNA oligonucleotide. By using this site-directed RNA editing (SDRE) strategy, we have attempted to correct the most common premature termination codons (PTCs) in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) transcripts (G542X, W1282X, R553X, R1162X, and Y122X). Our goal is to use SDRE for restoring functional CFTR chloride currents in all five CFTR PTCs. As a first step, we tried to maximize the editing efficiency of our strategy in human cells. We transiently co-transfected all the elements of our strategy in HEK293T cells and four days post-transfection, we measured correction at the level of RNA by RT-PCR and direct sequencing. Using SDRE, we have detected efficient editing ( $\geq 37\%$ ) in cellula at the target adenosine in all CFTR PTCs tested; CFTR G542X (UGAG; 37%) W1282X (UGAA; 63%), R553X (UGAG; 46%), R1162X (UGAG; 86%), and Y122X (UAA; 83 and 75%). However, there were various degrees of off-target editing events dependent on the sequence of the targeting area. Future experiments will focus on testing and delivering our strategy on Fischer Rat Thyroid epithelial cells that stably express these CFTR PTC constructs. We will also assess functional correction by testing our strategy in epithelial cell monolayers using the Ussing chamber. This work was funded by CFF Award ROSENT14XX0, R01 NS087726 from NINDS and RCMI Grant G12 RR 03051.

**358 Dynamic N<sup>1</sup>-methyladenine modification in the eukaryotic transcriptome***Sigrid Nachtergaele<sup>1</sup>, Dan Dominissini<sup>2</sup>, Gideon Rechavi<sup>2</sup>, Chuan He<sup>1</sup>*<sup>1</sup>University of Chicago, Chicago, IL, USA; <sup>2</sup>Sheba Medical Center, Tel-Hashomer, Israel

Gene expression can be regulated post-transcriptionally through dynamic and reversible RNA modifications. A noteworthy example is N<sup>6</sup>-methyladenine (m<sup>6</sup>A), which affects messenger RNA (mRNA) localization, stability, splicing, and translation. However, the repertoire of modifications found on mRNA continues to expand. We reported that N<sup>1</sup>-methyladenine (m<sup>1</sup>A), previously characterized in tRNA and rRNA, occurs on thousands of different gene transcripts in eukaryotic cells. m<sup>1</sup>A is chemically distinct from m<sup>6</sup>A, carrying a positive charge at physiological pH that can have dramatic consequences for RNA structure and folding. Employing newly developed sequencing approaches, we find that m<sup>1</sup>A is enriched around the start codon upstream of the first splice site, in contrast to the localization of m<sup>6</sup>A in the transcriptome. We find that its distribution and characteristics are also quite distinct from m<sup>6</sup>A, suggesting different cell biological functions for this modification. m<sup>1</sup>A is found primarily in more structured regions around canonical and alternative translation initiation sites, is dynamic in response to physiological conditions, and correlates positively with protein production. These unique features are conserved in mouse and human cells, but how m<sup>1</sup>A contributes to the regulation of gene transcription, processing, and translation remains an active area of investigation.



**359 Identification of a novel RNA 2',3'-cyclic phosphatase activity in human cells***Paola Pinto<sup>1</sup>, Stefan Weitzer<sup>1</sup>, Karl Mechtler<sup>2</sup>, Javier Martinez<sup>1</sup>*<sup>1</sup>Medical University of Vienna, Department for Medical Biochemistry, 1030 Vienna, Austria; <sup>2</sup>IMP, Research Institute of Molecular Pathology, 1030 Vienna, Austria

Processing of RNA molecules at the 3' end is crucial for their stability, function and quality control. More globally, defects in RNA processing have increasingly been linked to numerous pathologies. Therefore, it is essential to identify and characterize the responsible enzymes. Yet to be studied in deep, RNA terminal 2',3'-cyclic phosphates play important roles in RNA metabolism as intermediates in tRNA splicing, non-canonical mRNA splicing during the unfolded protein response and as products of endonucleolytic cleavage by ribonucleases. Interestingly, RNA terminal 2',3'-cyclic phosphates can also be generated *de novo* by the RNA 3'-terminal phosphate cyclase, RTCD1 - RtcA in bacteria. By characterizing RTCD1 we detected a seemingly associated, new enzymatic activity that further converts terminal 2', 3'-cyclic phosphates into nucleotides displaying 2', 3' OH groups. We purified the enzymatic activity from HeLa cytoplasmic extract through classical chromatography. A highly-enriched fraction was analyzed by Mass Spectrometry and genes encoding candidate proteins were silenced by RNAi. At this point we are confident in having identified the responsible gene. We are currently expressing the recombinant enzyme in *E.coli* and in HEK293 cells as a myc-tagged protein. The activity and functions of the novel enzymatic activity will be thoroughly investigated both *in vitro* and *in vivo*.

**360 Discovering the functions of 5-methylcytosine in mRNA biology***Ulrike Schumann<sup>1</sup>, Tennille Sibbritt<sup>1</sup>, Andrew Shafik<sup>1</sup>, Maurits Evers<sup>1</sup>, Chikako Ragan<sup>1</sup>, Thomas Preiss<sup>1,2</sup>*<sup>1</sup>Australian National University, Canberra, ACT, Australia; <sup>2</sup>EMBL Australia, Melbourne, VIC, Australia

5-methylcytosine is a post-transcriptional modification common in tRNA and rRNA, playing an important role in tRNA function and stability as well as ribosome biogenesis and assembly. The 5-methylcytosine modification is mediated by the NOP2/Sun (NSUN) family of RNA methyltransferases, which consists of seven members, presumably each with distinct targeting specificities. NSUN2, the best-understood family member, methylates tRNAs and tRNA methylation plays an important role in decoding, tRNA stability and stress responses. NSUN2 has also been shown to target other classes of RNA such as snoRNAs, lncRNAs and mRNAs, but the role of 5-methylcytosine in these RNAs remains poorly characterised.

Several aspects of mRNA biology could be affected by 5-methylcytosine such as splicing or alternative exon usage, stability, RNA degradation and miRNA targeting. Recently several studies suggested a role of 5-methylcytosine is to increase the stability and/or enhance translation of a small number of mRNAs involved in senescence.

We chose two approaches to investigate the role of 5-methylcytosine in mRNA biology. First, we analysed the effect of loss of 5-methylcytosine, by means of NSUN2 knock-down in HeLa cells, on transcriptome composition. Second, we used polysome gradient analysis of HeLa cell lysate, followed by transcriptome-wide bisulfite RNA sequencing to detect any correlation between translation state and methylation level of mRNAs, either globally or for individual examples. Findings from these two approaches will be presented.

**361 Elucidating the molecular basis for intellectual disability linked to a tRNA editing enzyme***Jillian Ramos, Dragony Fu***University of Rochester, Rochester, New York, USA**

Transfer RNAs (tRNAs) are subject to a diversity of post-transcriptional modifications that range from simple methyl groups to complex molecular additions. For example, adenosine nucleotides at the wobble position of tRNA undergo deamination to form the inosine modification, which can base pair with adenine, uracil and cytosine. Since there are no tRNA isoacceptors to decode certain codons, the inosine modification is essential for viability due to one tRNA isoacceptor decoding multiple synonymous codons. The human enzyme that catalyzes inosine formation consists of a heterodimeric complex composed of the ADAT2 and ADAT3 proteins. Recent studies have uncovered mutations in the ADAT3 subunit that cause autosomal-recessive intellectual disability (ID). However, how this ADAT3 mutant contributes to ID is unknown. Here we show that ADAT3 V144M forms foci inside HeLa cells indicative of aggregation and this phenotype can be alleviated by coexpression of ADAT2. Using coimmunoprecipitation, we found that ADAT3 V144M displays increased interaction with the HSP60 chaperone, providing further evidence that the V144M mutant displays defects in folding and aggregation. Profoundly, we see a reduction in inosine formation in cells harvested from individuals harboring the aforementioned mutation. In agreement with this, our *in vitro* assays using the recombinant protein complex shows reduced enzymatic activity on tRNA Val<sub>(AAC)</sub>. Our studies provide a mechanism for understanding the molecular basis for neurodevelopmental disorders associated with a mutant tRNA editing enzyme.

**362 m<sup>6</sup>A mRNA writers regulate development and hormonal response in *Arabidopsis* and include HAKAI***Kamil Ruzicka<sup>1,2</sup>, Mi Zhang<sup>3</sup>, Ana Campilho<sup>2</sup>, Zsuzsanna Bodi<sup>3</sup>, Muhammad Kashif<sup>2</sup>, Maria Saleh<sup>1</sup>, Sedeer El-Showk<sup>2</sup>, Geert De Jaeger<sup>4</sup>, Nigel Mongan<sup>3</sup>, Jan Hejatkó<sup>1</sup>, Yka Helariutta<sup>2</sup>, Rupert Fray<sup>4</sup>***<sup>1</sup>CEITEC, Brno, Czech Republic; <sup>2</sup>University of Helsinki, Helsinki, Finland; <sup>3</sup>University of Nottingham, Nottingham, UK; <sup>4</sup>Department of Plant Systems Biology, VIB, Gent, Belgium**

Despite recent stunning progress, our knowledge about the physiological role of N<sup>6</sup>-adenosine methylation (m<sup>6</sup>A) of mRNA and the identity of associated molecular machinery has still many gaps. It is also very little known about its role in pattern formation, in plants, as well as in other eukaryotes. Protein AHP6, a member of signaling cascade of a plant hormone cytokinin, plays a critical role during protoxylem development and its expression relies also on other phytohormone, auxin. We isolated a mutant *virilizer-1* (*vir-1*), which shows reduced *AHP6prom::GFP* activity, accompanied with altered vascular formation and hormonal resistance. Consistently, RNA sequencing revealed that *vir-1* shows altered expression of numerous genes associated with regulation of auxin dependent processes.

*vir-1* is a weak allele of an embryonically lethal and evolutionarily conserved gene, which was proposed to function in mRNA processing. We used tandem affinity purification to find proteins interacting with VIR. We co-purified proteins previously known to be involved methylation in mRNA, as well as a conserved homolog of human HAKAI. We demonstrate that genetic depleting of m<sup>6</sup>A levels leads to phenotypic defects similar to those seen on *vir-1*. We also found that depletion of expression of *VIR* and *HAKAI* in *Arabidopsis* results in strongly reduced m<sup>6</sup>A levels in mRNA.

### 363 An RNA editing/binding-independent gene regulatory mechanism of ADARs and its clinical implication in cancer

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Adenosine DeAminases acting on dsRNA (ADAR) are highly conserved family of enzymes catalysing adenosine to inosine deamination (A-to-I editing). Being the best-studied function associated with ADAR1 and ADAR2 (ADARs), A-to-I RNA editing contributes to multi-level gene regulation depending on where it occurs. The differential editing frequencies of these recoding sites are found to impact on human diseases such as neurological diseases and cancer. Adenosine-to-inosine (A-to-I) RNA editing, catalysed by ADAR, occurs predominantly in the 3' untranslated regions (3'UTRs). The contributions of 3'UTR editing by ADARs to cancer development have not yet been illustrated. Here we uncover an unanticipated link between ADARs and the expression of target genes undergoing extensive 3'UTR editing. Using METTL7A (Methyltransferase Like 7A), a novel tumor suppressor as an exemplary target gene, we demonstrate that its expression could be repressed by ADARs beyond their RNA editing and dsRNA binding functions. ADARs interact with Dicer to augment the processing of pre-miR-27a to mature miR-27a. Consequently, mature miR-27a targets the METTL7A 3'UTR to repress its expression level. In sum, our study unveils that the extensive 3'UTR editing is merely a footprint of ADAR binding, and is dispensable for the regulation of at least a subset of target genes. Instead, ADARs contribute to cancer progression by regulating cancer-related gene expression through their non-canonical functions independent of RNA editing and dsRNA binding. The functional significance of ADARs is much more diverse than previously appreciated and this gene regulatory function of ADARs is most likely to be of higher importance than the best-studied editing function. This novel non-editing side of ADARs opens another door to target cancer. This study is timely and represents a major break-through in the field of ADAR gene regulation and cancer biology.

### 364 N(6)-Methyladenosine RNA methylase-independent oncogenic role for METTL3 in acute myeloid leukemia

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N6-Methyladenosine (m6A) is the most abundant internal chemical modification in eukaryotic mRNA. This modification is dynamic and reversible and can control any aspect of mRNA post-transcriptional regulation, including splicing, export, stability and translation (Zhao et al., Nat Rev Mol Cell Biol., 2017). In mammals, the m6A writer is a multicomponent complex composed of the two methyltransferases METTL3 and METTL14 and the regulatory proteins WTAP, KIAA1429 and RBM15. Post-transcriptional m6A RNA modification is indispensable for cell viability and development, yet its role in cancer biology is still poorly understood. Acute myeloid leukemia (AML) is a heterogeneous hematopoietic malignancy that is characterized by genetic alterations in hematopoietic stem and progenitor cells that produce complete or partial blockage at different stages of myeloid differentiation and uncontrolled proliferation. Notably, WTAP has been recently found upregulated in AML, acting as an oncogenic factor (Bansal et al., Leukemia, 2014). Moreover, we have observed a general increase of METTL3 and METTL14 expression in primary AML samples. This leads us to speculate that m6A RNA modification might play crucial role in leukemogenesis. Thus, we analyzed the functional role of m6A in AML cell lines and primary CD34+ hematopoietic progenitor cells. Impairing the expression of the methylation complex components by RNAi affected consistently myeloid differentiation and induced massive apoptosis. Moreover, in AML cell lines METTL3 mislocalizes in the cytoplasm and it associates with translating ribosomes. Furthermore, we found that the cytoplasmic METTL3 may directly affects WTAP protein levels. Thus, suggesting additional catalytic-independent role for this protein in mRNA expression regulation, as recently described in lung cancer (S. Lin et al., Mol Cell, 2016). Notably, both the wild type and catalytic mutant derivative of METTL3 positively affect AML cells proliferation and they are able to rescue the apoptotic phenotype observed in RNAi assays. Altogether, these data indicate that the dysregulation of the m6A methylation may contribute to leukemogenesis and the catalytic function of METTL3 is dispensable for its oncogenic role in AML.

**365 Landscape of A-to-I RNA editing in human colorectal cancer**Yuanming Wang<sup>1,2</sup>, Raghuvaran Shanmugam<sup>1,2</sup>, Fan Zhang<sup>2</sup>, Xiujun Zhang<sup>1,2</sup>, Iain Tan<sup>2</sup>, Meng How Tan<sup>1,2</sup><sup>1</sup>Nanyang Technological University, Singapore, Singapore; <sup>2</sup>Genome Institute of Singapore, Singapore, Singapore

Adenosine-to-inosine (A-to-I) RNA editing is a fundamental post-transcriptional gene regulatory mechanism. It is pervasive in the human transcriptome and has been implicated in a variety of cancers. Despite increasing efforts to unravel the functions of RNA editing in tumorigenesis, our understanding of its role in colorectal cancer remains poor. Here, we comprehensively map the A-to-I editing landscape in colorectal cancer by analyzing paired normal-tumor RNA-seq data of 41 patients from The Cancer Genome Atlas (TCGA) project as well as sequencing matched normal-tumor samples from an additional 35 patients. Consistent with previous studies of other types of solid tumors, we observed a significant increase in ADAR1 expression and a significant decrease in ADAR2 expression. The editing of hundreds of sites are also significantly altered, the majority of which lie in non-coding regions. By integrating expression and editing profiles, we were able to identify putative regulators of editing and genes whose expression levels may be impacted by changes in their editing. Interestingly, a cluster of sites in the 3'UTR of the MDM2 oncogene exhibited a significant increase in their editing levels with a concomitant activation of MDM2. We demonstrated that this coupling between editing and expression of MDM2 was probably mediated through Staufen1 via a translational control mechanism. Collectively, our results highlight that A-to-I RNA editing serves as an important epitranscriptomic mechanism that contributes to human colorectal cancer.

**366 Small angle X-ray scattering studies of the human and plant adaptor protein from the m6A methyltransferase complex**Michal Taube<sup>1,2</sup>, Artur Jarmolowski<sup>2</sup>, Zofia Szweykowska-Kulinska<sup>2</sup>, Maciej Kozak<sup>1</sup><sup>1</sup>Joint Laboratory for SAXS studies, Faculty of Physics, Adam Mickiewicz University, Poznan, Poland;<sup>2</sup>Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

Recently, widespread N6-methyladenosine (m6A) modification of messenger RNA (mRNA) and non-coding RNA was discovered in yeast, flies, mammals and plants. It was shown that this modification could affect mRNA fate within the cell: changing its splicing pattern, promoting transport to the cytoplasm, increasing degradation rate and enhancing translation. Moreover, m6A modification of primary miRNA transcripts promotes miRNA biogenesis in human cell lines. At the functional level m6A modification plays an important role in many biological processes, including: stem cell differentiation, circadian clock in mammals, functioning of the nervous system and sex determination in flies, meiosis in yeast and development in plants.

In mammals two methyltransferases: METTL3 and METTL14, together with the adaptor protein WTAP are required for m6A methylation. In plants MTA and FIP37, in yeast IME4 and MUM2 were identified as functional homologs of METTL3 and WTAP, respectively. From structural studies it is known how methyltransferase subunits cooperate with each other for substrate recognition and methyl group transfer from the S-adenosylmethionine cofactor but little is known about the role of WTAP and FIP37 proteins in m6A methylation of mRNA at the molecular level.

Here we present the results of SAXS studies of WTAP and FIP37 proteins in solution. Our data showed that WTAP and FIP37 proteins form in solution high-order oligomers of various molecular weight. Ab-initio modeling revealed an elongated shape of oligomers of both proteins. This experiments have also shown that the degree of oligomerisation is concentration dependent. We observed similar behavior for a shorter fragment of FIP37 encompassing the coiled-coil domain, showing that this region is required for the FIP37 oligomerisation. At the functional level the oligomerisation of WTAP and FIP37 proteins may facilitate recognition of mRNA and engagement of the methyltransferase complex for catalytic activity and formation of functional speckles within the nucleus.

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### 367 Increased A-to-I editing of the angiomiR MicroRNA-487b alters target gene selection during ischemia

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**Introduction.** MiR-487b-3p has been shown to regulate angiogenesis despite having strikingly few conserved putative target genes. Adenosine-to-inosine (A-to-I) editing of microRNA precursors by ADARs can expand a microRNA's target pool if editing alters target gene selection of the mature microRNA. 2'-O-ribose-methylation (2'OMe) of adenosines in microRNA precursors, on the other hand, may shield them from A-to-I editing by ADAR2.

**Hypothesis.** We hypothesized that miR-487b-3p undergoes A-to-I editing and 2'OMe under ischemic conditions.

**Methods and Results.** cDNA was made from total RNA isolated from the calf muscles of mice at different time points after ischemia. Using Sanger sequencing and restriction fragment length polymorphism (RFLP) analysis, we identified and validated an A-to-I editing site in the miR-487b-3p precursor (pri-miR-487b), which alters the seed sequence from 5'-ATCGTAC-3' to 5'-ITCGTAC-3'. Strikingly, shortly after induction of ischemia, the amount of edited pri-miR-487b increased significantly from 6.5% to 12% of the total amount of pri-miR-487b. Using specific rt/qPCR kits, we showed that the amount of edited mature miR-487b-3p also increased significantly, demonstrating that processing of pri-miR-487b is not abolished by editing. We confirmed that this A-to-I editing site is completely conserved between mice and humans, using primary human arterial fibroblasts. In vitro luciferase reporter gene assays demonstrated that target site selection of miR-487b-3p is indeed altered by editing in its seed-sequence.

To determine potential 2'-O-ribose-methylation of the adenosine that can undergo A-to-I editing, we used Reverse Transcription at Low dNTP concentrations followed by qPCR (RTL-qP). We found the estimated fraction of specific adenosine 2'OMe increased from 9±32% before ischemia to 52±14% one day after ischemia. Next we confirmed that, like A-to-I editing, 2'OMe of miR-487b-3p is conserved between mice and humans.

Finally, using RBP-immunoprecipitation on cell lysates, we showed that pri-miR-487b indeed binds to both ADAR1 and ADAR2, but also to the 2'-O-ribose-methyltransferase Fibrillarin.

**Conclusions.** We discovered that A-to-I editing and 2'OMe of the first nucleotide of pri-miR-487b's seed sequence are strictly regulated during ischemia. Editing of miR-487b-3p alters and expands its set of target genes. Both miR-487b-3p A-to-I editing and 2'OMe are conserved between humans and mice.

### 368 Role of the TRM10 enzyme family in the methylation of position 9 of human tRNAs

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To date, more than 100 chemical RNA modifications have been described, most of them found in tRNAs. However, the pathways leading to the synthesis of many modifications, and their impact on the RNA molecule itself remain poorly understood. The TRM10 family is a widely conserved family of tRNA methyltransferases found in Archaea and Eukaryotes, responsible for the methylation of nitrogen 1 of purines at position 9 of tRNAs. The human genome encodes three forms of the TRM10 methyltransferase family; however, the specific role of the different forms remains poorly understood to date. Remarkably, the methyl group introduced by these enzymes at the Watson-Crick edge of the purine interferes with canonical base pairing, and causes misincorporation or abortion during reverse transcription. The presence of the modification can thus be identified as a hyper-error signature in Next-Generation Sequencing (NGS) data. Here we used NGS to identify the full repertoire of tRNAs methylated at position-9 in a human cell line. Furthermore, using CRISPR/Cas-mediated genome editing we have generated human cell lines deleted for members of the TRM10 family to investigate the role of the different isoforms in tRNA modification. With our study, we aim to elucidate the functional significance of the expanded TRM10 family in vertebrates, the role of the different members in tRNA modification, and ultimately the importance of position-9 methylation for protein translation.



### **369 tRNA 2'-O-methylation: a novel function of human box C/D RNPs**

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Mammalian cells express thousands of non-coding RNAs (ncRNAs) which function in almost all steps of gene expression. To get further insights into the functional and structural complexity of human ncRNAs, we study a large class of ncRNAs: the box C/D 2'-O-methylation guide RNAs. In human cells, box C/D guide RNAs are located either in the nucleolus (small nucleolar RNAs, snoRNAs) or in the nucleoplasmic Cajal-bodies (small Cajal body RNAs, scaRNAs) where they function mostly in 2'-O-methylation of ribosomal RNAs and spliceosomal snRNAs, respectively. We have recently identified two structurally closely related human box C/D RNAs which are conserved in all vertebrates. The two C/D RNAs share a common, evolutionarily conserved antisense element that is predicted to direct 2'-O-methylation of the elongator tRNAMet. Although the two box C/D RNAs seem to represent two isoforms of the same 2'-O-methylation guide RNA, one of them accumulates in the nucleolus (snoRNA), while the other concentrates in the Cajal bodies (scaRNA). CRISPR-Cas9-mediated knock-out of the "snoRNA isoform" or the "scaRNA isoform" of the new box C/D RNA each reduced the methylation state of tRNAMet at the predicted position by about 50%. Elimination of both isoforms fully abolished tRNA methylation, demonstrating that the two methylation guide RNAs, irrespective of their subnuclear localizations, function in 2'-O-methylation of the same tRNA. Thus, we have described a novel function for human box C/D RNAs, discovered the first guide RNA with two differently localized isoforms and finally, our work has first implicated Cajal bodies in tRNA maturation.

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### **370 Withdrawn**

### 371 Genome-wide study on the impact of defective splicing machinery on the establishment of SMN-dependent neurodegenerative phenotype

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Spinal Muscular Atrophy (SMA), a lethal neurodegenerative disorder, is characterized by low levels of the Survival of Motor Neuron (SMN) protein. This protein is essential for the assembly of small nuclear ribonucleoproteins (snRNPs), key components of the spliceosome - a large RNA-protein macromolecular complex in which splicing of pre-mRNA occurs. Strikingly, even though efficient splicing is a basal requirement for every cell population, low levels of the ubiquitous SMN protein mainly affect motor neurons (MNs).

*Drosophila melanogaster* models of SMA replicate the patient-observed neuromuscular junction phenotype and have allowed for a systematic screening of genetic modifiers of the disease. Still, despite robust knowledge of SMA's genetics, the exact molecular mechanisms connecting SMN to MN degeneration remain elusive.

We present a detailed analysis of the central nervous system transcriptome of a *Drosophila melanogaster* SMA model using high-depth RNA-Seq, providing novel insights into SMN-dependent changes in gene expression and their connection to MN degeneration. We further address the conservation of these mechanisms in human by correlating this data with the transcriptome profile of MN cell cultures derived from SMA patient fibroblasts. Our results identified conserved changes in the expression of genes revealing strong links to disease-relevant signalling pathways previously described as modulators of the SMN-dependent loss-of-function phenotype.

We expect to pinpoint novel candidates for future complementary therapeutics, but more importantly we are dissecting the molecular mechanisms linking SMN down-regulation to specific gene expression changes underlying the SMA-associated neurodegeneration profile.

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### 372 Decrypting Gephyrin splice variants function in developing brain

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Brain function relies on a balance between excitation and inhibition. While excitatory synapses are well described, relatively little is known about the molecular control of inhibitory synapse in terms of development and remodeling. The Gephyrin (GPHN) gene is the main molecular organizer of inhibitory synapses. It is involved in multiple protein-protein interactions, with GABA<sub>A</sub> and glycine neuroreceptors, the cytoskeleton, and various cell adhesion and signal transduction proteins. Our analysis of GPHN expression using long read sequencing by zero-mode waveguide (PacBio) techniques revealed an impressive diversity of GPHN transcripts (more than 300), in strong contrast to the only two alternative transcripts annotated in the *Ensembl* database. In this context, we identified several new exons, new cryptic splicing events, and new alternative splicing events, suggesting the existence of unprecedented variety of GPHN protein isoforms. In addition, we found that GPHN protein isoforms are regulated in a tissues specific manner and during mouse development. In particular, many of the splicing events were highly regulated in Cerebellum, Hippocampus, and brain, as compared to other mouse tissues. Importantly, alternative splicing regulation of some GPHN exons could be further correlated with inhibitory synapse formation during mouse post-birth development. Altogether, we will propose a working model suggesting how alternative splicing regulation of GPHN impacts the plasticity of inhibitory synapses, to be examined in the light of the micro-deletions within GPHN exons, associated with neuronal disorders as ASD, schizophrenia or seizures.

### 373 miR-182 Regulates Slit2-Mediated Axon Guidance by Modulating the Local Translation of a Specific mRNA

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During nervous system development, axons navigate a complex environment to establish connections with their targets. Extrinsic chemotropic cues guide the leading tip of the axon, the growth cone, by inducing its directional steering. Cue-induced growth cone steering is aided by localized mRNA translation. Different guidance cues are known to elicit translation of subsets of mRNAs that differentially regulate the cytoskeleton. Yet, how specific mRNAs are selected for translation is still poorly understood. miRNAs are critical translational regulators that act through sequence-specific mechanisms. We thus investigated the local role of miRNAs in mRNA-specific translation during pathfinding of *Xenopus laevis* retinal ganglion cell (RGC) axons to their midbrain target, the optic tectum.

We first determined which miRNAs are present within RGC axons using small RNA-seq. We identified miR-182 as the most abundant among a rich repertoire of axonal miRNAs. An *ex vivo* miRNA sensor assay further revealed that miR-182 is active locally in RGC axons but not in the cell soma, where this miRNA is also expressed albeit at low levels. We next investigated the functional roles of miR-182 in axon guidance using a loss-of-function approach. miR-182 depletion impaired growth cone response to Slit2 but not Sema3A, two tectal repellent cues. Additionally, loss of miR-182 caused RGC axon targeting defects *in vivo* specifically within the tectum where Slit2 is expressed. Together, this suggests that miR-182 regulates axon targeting by impinging on Slit2 signaling at the growth cone. Slit2 induces the local translation of cofilin-1 mRNA at the RGC growth cone to modulate steering. Luciferase assay showed that cofilin-1 is a direct target of miR-182. Loss of miR-182 altered cofilin-1 levels in unstimulated and Slit2-stimulated growth cones, revealing that miR-182 silences cofilin-1 prior to Slit2 exposure. We, finally, detected that Slit2 inactivates miR-182 specifically within RGC growth cones without degrading it, suggesting that Slit2-induced miR-182 loss-of-activity triggers burst of cofilin-1 translation.

Our data support a model whereby miR-182 reversibly gates the selection of transcripts for fast translation depending on the extrinsic cue.

### 374 Genome-wide analysis reveals that eIF2D coordinates moto-neuronal synaptic function *in vivo* by modulating translation of specific mRNAs

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Eukaryotic mRNA translation involves numerous factors that bind to ribosomes and/or mRNAs to promote the canonical cap-dependent scanning pathway, which functions on most mRNAs in essentially all cells. Work in cell-free translation systems has also revealed a class of "non-canonical" translation factors whose *in vivo* functions remain largely unclear. One such protein is eIF2D (a.k.a. Ligatin), which has been implicated in regulation of initiation via GTP-independent initiator tRNA delivery to the P-site, as well as recycling of post-termination complexes under certain conditions. Studies in yeast showed that this protein is not essential and does not seem to affect general translation. Moreover, biological functions of eIF2D *in vivo* in a multicellular organism have never been studied before, and the specific mRNA targets of its regulation in living cells remain unknown.

To address these questions, we generated and characterized eIF2D knockout flies. We found that they are viable, fertile, and do not show morphological defects but the larvae have a decreased locomotion speed. Rescue experiments reveal that eIF2D is sufficient on either side of the larval neuromuscular junction (NMJ) to promote normal locomotion. NMJ morphology appeared largely normal in eIF2D-KO larvae, but electrophysiology data revealed reduced baseline transmission and pre-synaptic homeostasis defects.

To identify mRNAs regulated by eIF2D *in vivo* we performed polyribosome profiling from temporally staged eIF2D-KO larvae and control animals, followed by genome-wide RNA-Seq ("Poly-Seq"). This revealed strong effects on translation of specific mRNAs. Prominent among these were mRNAs coding for proteins implicated in synaptic processes and locomotion, including both pre- and post-synaptic components, consistent with the observed phenotypes. Importantly, NMJ synapses in eIF2D-KO larvae have specific changes in protein composition that can be largely explained by the altered translation observed by Poly-Seq. Moreover, we observe common characteristics (e.g. cis-elements) in the transcript sequences of these mRNAs that could serve as regulatory elements for translation control by eIF2D.

Collectively, our results define a crucial *in vivo* role for eIF2D within the motor system to promote synaptic function via coordinating translation of specific mRNAs.

**375 RBM4 modulates cell and neuronal migration via alternative splicing of Dab1***D Dhananjaya<sup>1,2</sup>, Kuan-Yung Hung<sup>2</sup>, Woan-Yuh Tarn<sup>1,2</sup>*<sup>1</sup>Taiwan International Graduate Program in Molecular Medicine, National Yang-Ming University and Academia Sinica, Taipei, Taiwan; <sup>2</sup>Institute of BioMedical Sciences, Academia Sinica, Taipei, Taiwan

The RNA binding protein RBM4 modulates cell differentiation via its role in alternative splicing regulation. Essentially, RBM4 participates in the expression of mRNA isoforms that exhibit cell-type specificity or promote cell differentiation. RBM4 is expressed in embryonic brain during development and its deficiency impacts mRNA splicing isoform patterns. RBM4 promotes neuronal differentiation and neurite outgrowth *in vitro*. Disabled-1 (Dab1) is a key adaptor protein of the Reelin signaling pathway, which plays a critical role in neuronal migration. Dab1 exhibits various alternative splicing isoforms during brain development. Notably, exons 6 to 9 containing several tyrosine residues important for Reelin signaling undergo differential splicing during cortex layer formation; but its splicing mechanism still remains to be elucidated. In this study, we observed that *Rbm4* deficient mouse embryonic brain and MEF cells exhibited altered Dab1 splicing patterns. Using the Dab1 exon 6-9 minigene, we observed that overexpression of RBM4 promoted exon 7/8 inclusion. PTB also modulated exon 7/8 selection but in a manner opposite to RBM4. RBM4 counteracted the effect of PTB in exon 7/8 selection by competing off PTB binding to intron 7. RBM4 knockdown prevented exon 7/8 usage and reduced *in vitro* migration of HeLa cells. Such a cell migration defect was rescued by overexpression of Dab1. Using *in utero* electroporation, we demonstrated that depletion of RBM4 or overexpression of exon 7/8-skipped Dab1 impaired neuronal migration and this defective phenotype was rescued by functional Dab1 in embryonic brain. Our results suggested that RBM4 plays a role in cell and neuronal migration via modulating the expression of Dab1 splicing isoforms.

**376 Characterization of a *Zc3h14*<sup>Δex13/Δex13</sup> mouse reveals insight into the function of the Zc3h14 RNA binding protein in the brain***Stephanie K Jones<sup>1</sup>, Jennifer Rha<sup>2</sup>, Sara W Leung<sup>1</sup>, Jonathan Fidler<sup>3</sup>, Julia Omotade<sup>4</sup>, September Hesse<sup>3</sup>, Ayan Banerjee<sup>1</sup>, Seth M Kelly<sup>4,5</sup>, Gary J Bassell<sup>4</sup>, James Zheng<sup>4</sup>, Ken H Moberg<sup>4</sup>, Paul S Garcia<sup>3</sup>, Anita H Corbett<sup>1</sup>*<sup>1</sup>Emory University, Department of Biology, Atlanta, GA, USA; <sup>2</sup>Emory University, Department of Biochemistry, Atlanta, GA, USA; <sup>3</sup>Emory University, Department of Anesthesiology, Atlanta, GA, USA; <sup>4</sup>Emory University, Department of Cell Biology, Atlanta, GA, USA; <sup>5</sup>College of Wooster, Department of Biology, Wooster, OH, USA

*ZC3H14* is the first gene encoding a polyadenosine RNA binding protein (Zinc finger CysCysCysHis domain-containing protein 14) associated with a form of heritable nonsyndromic autosomal recessive intellectual disability. This finding uncovers the molecular basis for disease identified thus far in two independent consanguineous families and provides strong evidence that ZC3H14 is essential for proper brain function. ZC3H14 is an evolutionarily conserved member of a novel class of tandem zinc finger (CCCH) polyadenosine (polyA) RNA binding proteins. Studies of ZC3H14 orthologs in budding yeast and *Drosophila* have provided insight into the function of this protein in post-transcriptional gene regulation, but functional characterization in a mammalian system is crucial for understanding the role of ZC3H14 in higher order brain function and to more closely recapitulate the complex molecular interactions of the human brain. Therefore, we have created a mouse model to study the functional consequences of loss of *Zc3h14*. In this model, *Zc3h14* exon 13 is removed through recombination resulting in an excision in the *Zc3h14* RNA-binding domain and introduction of multiple subsequent premature stop codons. In this mouse model (*Zc3h14*<sup>Δex13/Δex13</sup>) all *Zc3h14* isoforms are affected leading to virtual loss of all detectable *Zc3h14* protein. The homozygous mutant mouse is viable, consistent with these identified patients lacking ubiquitously expressed isoforms of ZC3H14. Preliminary analyses of the *Zc3h14*<sup>Δex13/Δex13</sup> mouse brain indicate extended polyA tails in P0 hippocampi, as well as morphological changes within adult mouse lateral ventricles and cultured primary hippocampal neurons. A water radial arm maze was used to assess learning and memory of the *Zc3h14*<sup>Δex13/Δex13</sup> mice compared to control mice. Our studies reveal that *Zc3h14*<sup>Δex13/Δex13</sup> mice are not impaired in learning but show significant defects in working memory. These data are remarkably consistent with *Drosophila* studies where flies depleted of the ZC3H14 orthologue, dNab2, specifically in neurons show defects in short-term memory as well as extended polyA tails and morphological defects in the brain. The mouse model provides a critically important tool to define the role of *Zc3h14* in neurons and to understand why loss of a ubiquitously expressed RNA binding protein leads to defects specifically manifested in brain dysfunction.



### 377 Role of 3'UTR polymorphism of MMP-9 in its regulation and local translation

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Matrix metalloproteinase 9 (MMP-9) plays pivotal role in synaptic plasticity underlying both physiological and pathological processes in the brain. In neurons, MMP-9 is locally, dendritically/synaptically translated in response to synaptic stimulation in a process controlled by fragile X mental retardation protein (FMRP). We have recently shown that MMP-9 rs20544 C/T single-nucleotide polymorphism (SNP) located in the 3'UTR influences MMP-9 availability at the dendritic spines that harbor excitatory synapses. In particular, MMP-9<sub>C</sub> variant exhibits lower MMP-9 activity at the synapse than MMP-9<sub>T</sub>. Moreover, analysis of dendritic spines morphology revealed that neurons overexpressing MMP-9<sub>C</sub> variant had higher percentage of mushroom spines, harboring more efficacious synapses. To elucidate possible molecular mechanism underlying observed difference in MMP-9 levels at the synapse, we hypothesized that FMRP may differentially regulate MMP-9 mRNA, depending on the polymorphism. We have shown by means of RNA electrophoretic gel shift assay (REMSA) and filter binding assay that FMRP binds MMP-9 3'UTR and identified one of the binding sites as a G-rich sequences in MMP-9 3'UTR. Interestingly, we have shown that FMRP binding to MMP-9 mRNA is affected by studied MMP-9 3'UTR polymorphism, as MMP-9<sub>C</sub> variant was bound by FMRP with higher affinity than MMP-9<sub>T</sub> variant. Moreover, RNA structure probing by selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) analysis revealed extensive structural changes in the MMP-9 mRNA molecule that depended on the rs20544 C/T polymorphism. Of note, structural changes included also the predicted FMRP binding site (i.e., the G-rich sequence in the 3'UTR). Studying MMP-9 genetic variants can help to understand MMP-9 regulation and mechanism of action and thus to provide a deeper insight into the key biological processes in which the protein plays a role.

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### 378 Self-oligomerization of Survival Motor Neuron (SMN) regulates its stability by sequestering an SCF<sup>Slmb</sup> degron

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Spinal muscular atrophy (SMA) is caused by homozygous loss of human *SMN1* (*survival motor neuron 1*). Expression of a duplicate gene (*SMN2*) primarily results in skipping of exon 7 and production of an unstable protein, called SMN $\Delta$ 7. Although *SMN2* exon skipping is the principal contributor to SMA severity, mechanisms governing stability of SMN protein isoforms are poorly understood. We used a *Drosophila* model system and 'label-free' proteomics to identify the SCF<sup>Slmb</sup> ubiquitin E3 ligase complex as a novel SMN binding partner. We show that this interaction is conserved from fly to human, and that SCF<sup>Slmb</sup> interacts with a phospho-degron embedded within the SMN YG-box self-oligomerization domain. Substitution of a conserved serine (S270A) interferes with SCF<sup>Slmb</sup> binding and greatly stabilizes SMN $\Delta$ 7. SMA-causing missense mutations that block multimerization of full-length SMN are also stabilized in the degron mutant background. Furthermore, overexpression of SMN $\Delta$ 7<sup>S270A</sup>, but not wild-type SMN $\Delta$ 7, provides a protective effect in SMA model mice and human motor neuron cell culture systems. Our findings support a model wherein the SCF<sup>Slmb</sup> degron is largely exposed when SMN is monomeric, whereas it is sequestered when SMN forms higher-order multimers. SMN stability is thus regulated by self-oligomerization, providing an elegant mechanism for controlling functional activity.



### 379 Targeted intron retention and excision for rapid gene regulation in response to neuronal activity

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Intron retention (IR) in polyadenylated RNAs of mammalian cells has recently emerged as a widespread class of alternative splicing event that affects a large number of multi-exonic genes. IR is mainly thought to regulate overall transcript abundance through RNA degradation pathways. Moreover, a handful of publications indicate that cellular stress conditions affect splicing of a subset of retained introns in higher eukaryotes. However, it was currently unknown whether IR events are regulated under non-pathological conditions and whether they contribute to physiological processes.

In the present work, we observed IR events in transcripts that escape RNA degradation and, instead, contribute to the temporal control of the neuronal transcriptome. In primary neocortical cells, we found that a sub-population of polyA<sup>+</sup> transcripts retaining select introns are substantially expressed and stably confined in the nucleus. Upon neuronal stimulation, these transcripts undergo rapid intron excision, and the fully spliced mRNAs are exported to the cytoplasm, thus generating a readily available pool of mRNAs for translation. Notably, this novel class of intron retention events is particularly enriched in long genes whose transcription requires hours. Thus, we propose that regulated intron retention in neurons provides a novel, transcription-independent mechanism to rapidly modify the neuronal transcriptome in response to stimuli.

### 380 MicroRNA mediated regulation of ITGB3 and CHL1 is implicated in SSRI action

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Major depression disorder (MDD) is the most widespread mental disorder and among leading causes of morbidity globally. Selective serotonin reuptake inhibitor (SSRI) antidepressant drugs remain the first-line MDD treatment but are effective in <70% of patients.

Our objective was to better understand the mechanism involved in SSRI action by using global gene and microRNA expression levels.

Using genome-wide transcriptomic studies, we observed that the levels of two genes coding for the cell adhesion proteins, close homologue of L1 (CHL1) and integrin beta-3 (ITGB3), and microRNAs miR-151a-3p and miR-221/222, are implicated in the variable sensitivity and response of human lymphoblastoid cell lines (LCLs) from unrelated individuals to SSRI drugs.

Leveraging these results to patients, we observed decreased mRNA levels of ITGB3 in PBMCs from MDD patients vs. healthy controls. This suggests an association with reduced brain neuronal plasticity that may contribute to depression (and presumably corrected by chronic SSRI medication).

Our overall results link genes and their regulating microRNAs with cellular SSRI sensitivity phenotypes via direct microRNA regulation of CHL1 and ITGB3. We show that human miR-151a-3p and miR-221/222 and their targeted genes CHL1 and ITGB3, respectively, are implicated in the response of human LCLs to SSRIs. Finally, we outline a new perspective of novel tentative MDD drug targets based on transcriptomics studies. Using this approach, we identified 3 human microRNAs that may be involved in the mode of action of SSRIs. Those candidate microRNAs could serve as SSRI response biomarkers, as well as potential new antidepressant therapeutic targets following further validation in clinical trials.

### 381 Function and Regulatory Mechanism of Axonal MicroRNAs in Developing Axon of Primary Sensory Neuron

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Neurons are highly polarized cells that possess dendrites with vast, complicated spines to accept information and axons extending very distally to transmit signals. Some mRNAs are known to traffic to dendrites and axons for local protein synthesis in response to extracellular signals that are involved in neuronal differentiation, synapse formation and axon growth during neuronal development. As post-transcriptional regulators, microRNAs (miRNAs) have recently been demonstrated to regulate mRNA translation in developing and mature nervous system. However, the specific distribution, function and regulatory mechanism of axonal miRNAs in primary sensory neurons are yet to be defined. In our study, by using a microfluidic culture of embryonic dorsal root ganglion (DRG) neurons, we identified the relative enrichment of miR-181d in axons. miR-181d affected axon elongation by locally targeting the transcripts of microtubule-associated protein 1B (MAP1B) and calmodulin. Importantly, we found that *Fmr1*-encoded protein, FMRP mediates axonal delivery of miR-181d with transcripts of MAP1B and calmodulin. The downregulation of FMRP in *Fmr1*<sup>1304N</sup> mice or in the cell bodies of cultured DRG neurons impaired the axonal delivery of miR-181d with transcripts of MAP1B and calmodulin, leading to decreased protein levels of MAP1B and calmodulin in axons. Furthermore, nerve growth factor (NGF) increased the local synthesis of MAP1B and calmodulin in axons by releasing *Map1b* and *Calml* from FMRP and miR-181d-repressing granules, thereby promoting axon elongation. Thus, we reveal a mechanism for axon elongation by the FMRP-mediated axon delivery of miR-181d and its associated mRNAs and local regulation of protein synthesis. More recently, using highly sensitive RNA-sequencing technology, we firstly identified axonal profiles of miRNAs and found 292 miRNAs are localized in axons of DRG neurons, indicating comprehensive miRNA-based regulatory network during axon development. Future work of genome-wide and integrated analysis of miRNA: mRNA interaction in axons will greatly expand understanding of the local control during axon development and improve system modeling of axonal miRNA regulatory pathway.

### 382 Sequential regulatory loops as key gatekeepers for neuronal reprogramming in human cells

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Direct conversion of somatic cells into neurons holds great promise for regenerative medicine. However, neuronal conversion is relatively inefficient in human cells compared to mouse cells. It has been unclear what might be the key barriers to reprogramming in human cells. We recently elucidated an RNA program mediated by the polypyrimidine tract binding protein PTB to convert mouse embryonic fibroblasts (MEFs) into functional neurons. In human adult fibroblasts (HAFs), however, we unexpectedly found that invoking the documented PTB-REST-miR-124 loop generates only immature neurons. We now report that the functionality requires sequential inactivation of PTB and the PTB paralog nPTB in HAFs. Inactivation of nPTB triggers another self-enforcing loop essential for neuronal maturation, which comprises nPTB, the transcription factor BRN2, and miR-9. These findings suggest that two separate gatekeepers control neuronal conversion and maturation and consecutively overcoming these gatekeepers enables deterministic reprogramming of HAFs into functional neurons.

**383 RNA processing - a potent means to control self-renewal and cell fate transitions**

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The establishment and maintenance of pluripotency depend on precise coordination of gene expression but the role of RNA binding proteins in pluripotent stem cells remains less well understood. We show that serine-arginine rich splicing factor 3 (SRSF3) binds to and regulates the processing of RNAs encoding key components of the pluripotency circuitry. Operating as a multifunctional RNA regulator, SRSF3 controls not only alternative exon inclusion but also nucleo-cytoplasmic mRNA export, mRNA stability and pri-miRNA processing. We demonstrate that SRSF3 directly regulates the expression NANOG protein by enhancing the nucleo-cytoplasmic export of the Nanog mRNA. We also show that SRSF3 binds to slowly spliced introns and through this binding inhibits the inclusion of the neighbouring alternative exons which is in contrast to the classical view of SR proteins as positive regulators of exon inclusion. Overall, SRSF3 post-transcriptionally coordinates the expression of a cohort of transcription factors, chromatin modifiers, splicing factors and cell cycle regulators with established roles in pluripotency. At the cellular level, SRSF3 is required to maintain self-renewal of pluripotent stem cells and it facilitates the activation of the pluripotency program during reprogramming. Our investigation places SRSF3 high upstream in the pluripotency hierarchy and uncovers how SRSF3-mediated RNA processing events are critical during reprogramming and in stem cell self-renewal. Furthermore, the determination of SRSF3 activities in self-renewing cells now provides insights into the essential functions of SRSF3 during early development.

**384 The RNA-binding landscape of the human exosome-associated exonuclease RRP6**

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The exosome complex is the major 3'→5' RNA decay machinery in eukaryotes. In humans, the nuclear form of the exosome is composed of nine different inactive subunits, which form a ring structure and associate to two different catalytic subunits DIS3 and RRP6 [1]. The activity of the exosome is governed by binding to other cofactors and adapter complexes, which provide specificity for recruiting this complex to specific RNA substrates. However, until recently, the identification of the RNA substrates for the alternative ribonucleases of the exosome still remains unknown on a global scale in higher eukaryotes.

We have performed iCLIP on endogenous and overexpressed RRP6 (wild-type and a catalytically inactive form) to identify the cellular RNA targets of the human exosome. The use of a catalytically inactive form of RRP6 stabilized important in vivo interactions that are highly dynamic and transient, and also highlighted the role of RRP6-mediated trimming of 3'flanks of immature non-coding RNAs such as snoRNAs and 5.8S rRNA. We will present a global view of the RNA-binding capacity of the RRP6-form of the exosome.

1. Tomecki, R. et al. The human core exosome interacts with differentially localized processive RNases: hDIS3 and hDIS3L. EMBO J. 29, 2342-57 (2010).

### 385 How the DIS3 proteins shape the human transcriptome

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The final step of eukaryotic mRNA degradation proceeds in either a 5'-3' direction, catalyzed by XRN1, or in a 3'-5' direction catalyzed by DIS3, DIS3L1 (the catalytic subunits of the exosome) and/or DIS3L2 (exosome-independent). Important findings over the last years have shed a new light onto the mechanistic details of RNA degradation by these exoribonucleases. In addition, it has been shown that they are involved in growth, mitotic control and important human diseases, including cancer. With the aim of analyzing how DIS3, DIS3L1 and DIS3L2 regulate the human transcriptome, each one of these nucleases was depleted by RNA interference in HeLa cells and levels of several reporter mRNAs was monitored by RT-qPCR. Our results show that these exoribonucleases are target specific and not directly involved in a particular mRNA surveillance mechanism. In parallel, our bioinformatics analysis of available transcriptomic data from cells depleted of DIS3L1, DIS3L2, XRN1, or UPF1 (which has a central role in nonsense-mediated mRNA decay) has shown some, but not full, redundancy among the transcripts regulated by these nucleases, which supports our experimental data. Presently, we are exploring the molecular mechanisms underlying our observations.

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### 386 Withdrawn

### 387 A method for purification and unbiased proteomic analysis of mouse U7-dependent processing complexes

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In animal cells, U7 snRNP is a multi-subunit endonuclease that cleaves replication-dependent histone pre-mRNAs at the 3' end, generating non-polyadenylated histone mRNAs. U7 snRNP consists of a ~60-nucleotide U7 snRNA and a heptameric Sm ring with two U7-specific proteins: Lsm10 and Lsm11. The N-terminus of Lsm11 interacts with FLASH and these two proteins function as a docking platform for the Histone pre-mRNA Cleavage Complex (HCC), which contains several polyadenylation factors, including the CPSF73 endonuclease. The 5' end of U7 snRNA base pairs with a purine-rich Histone Downstream Element (HDE) located 3' of the cleavage site, bringing U7 snRNP to histone pre-mRNA for processing. An additional mechanism for the recruitment of U7 snRNP is provided by Stem-Loop Binding Protein (SLBP), which interacts with a conserved structure located upstream of the cleavage site and stabilizes the duplex between U7 snRNA and the HDE, likely by contacting a subunit(s) of the U7 snRNP. The exact composition of the mammalian U7 snRNP and the identity of its SLBP-interacting component(s) have not been determined. Here, we used a highly active mouse nuclear extract and a synthetic histone pre-mRNA to assemble large amounts of mouse processing complexes *in vitro* and purified them by a new approach to further analyze for the presence of unknown proteins and/or RNA species. Our results demonstrate that mouse U7 snRNP associates with symplekin, CstF64 and multiple CPSF subunits but contains no CFI<sub>m</sub> 68, ZFP100 or Y1/Y3 noncoding RNAs that were previously implicated in 3' end processing of histone pre-mRNAs in mammalian cells. We also show that the SLBP-mediated recruitment of the U7 snRNP to histone pre-mRNA depends on FLASH but not on the polyadenylation factors of the HCC. Finally, we identified two main sequence elements in mammalian SLBP that are essential for the recruitment of the U7 snRNP: helix B of the RNA Binding Domain (RBD) and a short C-terminal region that directly follows the RBD. These requirements and the overall composition of the mammalian U7 snRNP mirror our findings from *Drosophila* studies, indicating that the mechanism of the U7-dependent 3' end processing is highly conserved between vertebrates and invertebrates.

### 388 Mutations of EXOSC3/Rrp40p associated with neurological diseases impact ribosomal RNA processing functions of the exosome in *S. cerevisiae*

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The RNA exosome is a conserved multiprotein complex involved in a large number of processive and degradative functions in eukaryotic cells. Recently, mutations that result in pontocerebellar hypoplasia, a neurodegenerative disorder characterized by cerebellar and spinal motor neuron degeneration in human patients, have been mapped to one of the subunits of the exosome, EXOSC3 (yeast Rrp40p). However, the molecular impact of these mutations in the pathology of these diseases is not well understood. To investigate the molecular consequences of mutations in EXOSC3 that lead to neurological diseases, we analyzed the effect of three of the mutations that affect conserved residues of EXOSC3/Rrp40p (G31A, G191C, and W238R; G8A, G148C, and W195R, respectively, in human and yeast) in *S. cerevisiae*. We show that the severity of the phenotypes of these mutations in yeast correlates with that of the disease in human patients, with the W195R mutant showing the strongest growth and RNA processing phenotypes. Furthermore, we show that these mutations affect pre-ribosomal RNA processing functions of the exosome more severely than other nuclear processing or surveillance functions. These results suggest that delayed or defective pre-rRNA processing might be the primary defect responsible for the pathologies detected in patients with mutations affecting EXOSC3 function in residues conserved throughout eukaryotes.



### 389 Integrator complex and 3'-end snRNA processing

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The processing machinery involved in 3'-end formation of poly(A) mRNA is well characterized containing members of the polyadenylation specificity factor (CPSF) and cleavage stimulation factor (CstF). The 3'-end formation of other types of RNAs such as replication-dependent histone mRNAs, whose lack the poly (A) tail, or small nuclear RNAs (snRNAs) is not well known.

snRNAs are a group of highly abundant non-polyadenylated, short (60-200 nucleotides), non-coding transcripts. It is comprised of U1, U2, U4, U5, U7 (transcribed by RNA pol II) and U6 (transcribed by RNAPol III). snRNAs are components of the spliceosome, that is involved in the removal of introns, with the exception of U7 that is involved in the 3' end processing of the replication- dependent histone mRNA.

In 2005 Baillat et al., identified a novel multiprotein complex named Integrator composed for at least twelve subunits that were associated with the CTD of the largest subunit (Rpb1) of RNAPII and is involved in snRNA 3'-end formation. Proteomic analyses confirmed its composition and identified new subunits and a genome wide RNAi screen confirmed new genes required for snRNA 3'-end formation. The Integrator Complex, composed at least by 14 subunits in humans, appears to be broadly conserved through evolution; nearly all its subunits have homologs in metazoans.

Here, we define *C. elegans* Integrator Complex members by affinity purification and function in 3'-end snRNA processing. Moreover, we suggest that *C. elegans* Integrator Complex has additional roles and it might be acting beyond specific conditions producing a transcriptional response.

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### 390 Development of an *in vitro* technique to identify RNA sequences of RNA-binding proteins

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RNA-protein interactions are important in controlling many aspects of gene regulation and it is important to understand their various biological functions. Although many techniques to discover RNA-protein interactions have been developed, noteworthy challenges such as discovering the RNA sequences of RNA-binding proteins (RBPs) remain unsolved. Here, we developed a novel technique wherein a 4-thio-uridine-incorporated RNA pool is used to identify the RBP-consensus sequences of RBPs produced by *in vitro* transcription and translation. To confirm the fidelity of the technique, it was used to determine the consensus sequence of RBFOX2. The obtained consensus sequence of RBFOX2, UGC(A/U)(A/U)NU, is very similar to the known RBFOX2 binding sequence, UGCAUG. Using this technique, consensus sequences were discovered for three RBPs, namely FUS (FUS RNA binding protein), SFPQ (splicing factor proline and glutamine rich), and SAM68 (Src-Associated substrate in Mitosis 68 kDa). Our study thus reports a novel technique to predict the target RNA sequences of RBPs for understanding RBP-mediated gene regulation.

### 391 Characterization of the 3'-end cleavage in pre-16S rRNA processing by two archaeal proteins, *Pf-Nob1* and *Pf-Dim2*

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In ribosome biogenesis, stepwise processing of pre-rRNAs by ribonucleases is one of fundamental mechanisms to produce each functional rRNA. It has been reported that Nob1 is an endoribonuclease responsible for generating the mature 3'-end of pre-18S rRNA in eukaryote, and its co-factor Dim2/PNO1 is required for the maturation. Meanwhile, in archaea, the molecular mechanism of pre-16S rRNA processing by the homologous proteins is poorly understood. In the current study, to clarify the biochemical role of archaeal Nob1 and Dim2, we first identified two proteins, PF1205 (named *Pf-Nob1*) and PF1580 (named *Pf-Dim2*) in the genome of a hyperthermophilic archaeon, *Pyrococcus furiosus* using a bioinformatics approach. Then, we constructed an *in vitro* reconstitution system for archaeal pre-16S rRNA processing using purified recombinant proteins.

*Pf-Nob1* showed an endoribonuclease activity at 60°C in the presence of Mn<sup>2+</sup> ion. Primer extension as well as 5' RACE analyses confirmed that *Pf-Nob1* mainly cleaved at 3'-end of pre-16S rRNA. A single-point mutant (D6N) of the *Pf-Nob1* did not show such an activity. A series of deletion mutant analysis of the pre-16S rRNA substrate suggested that the Helix 44, the specific RNA secondary structure located at 3'-end of 16S rRNA, is required for the efficient cleavage by *Pf-Nob1*. While, the cleavage position by *Pf-Nob1* is changed in a *Pf-Dim2* dose-dependent manner. 5' RACE analysis showed that the newly detected cleavage sites are mapped onto the upstream regions of 3'-end of mature 16S rRNA. The data suggested that this cleavage resulted in producing the 16S rRNA without an anti-SD sequence, which is the key sequence for the binding interactions between 16S rRNA and mRNA. Furthermore, preliminary mapping of 3'-end of *Pf-Dim2*-binding region by primer extension analysis suggested that *Pf-Dim2* bound close to the *Pf-Nob1* cleavage site (the 3'-end of 16S rRNA). This may because *Pf-Nob1* could not cleave the 3'-end of 16S rRNA in the presence of *Pf-Dim2* but cleaved the new sites. These results showed that cooperative regulation by archaeal *Pf-Nob1* and *Pf-Dim2* might be involved in the regulation of translational repression through the 3'-end cleavage in pre-16S rRNA processing.

### 392 Lariat capping as a tool to manipulate the 5' end of individual yeast mRNA species in vivo

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The 5' cap structure of eukaryotic mRNA is critical for its processing, transport, translation, and stability. The many functions of the cap and the fact that most, if not all, mRNA carries the same type of cap makes it difficult to analyze cap function in vivo at individual steps of gene expression. We have used the lariat capping ribozyme (LCrz) from the myxomycete *Didymium* to replace the mRNA m7G cap of a single reporter mRNA species with a tiny lariat in which the first and the third nucleotide are joined by a 2', 5' phosphodiester bond. We show that the ribozyme functions in vivo in the budding yeast *Saccharomyces cerevisiae* presumably without cofactors and that lariat capping occurs co-transcriptionally. The lariat-capped reporter mRNA is efficiently exported to the cytoplasm where it is found to be oligoadenylated and evenly distributed. Both the oligoadenylated form and a lariat-capped mRNA with a templated poly(A) tail translates poorly, underlining the critical importance of the m7G cap in translation. Finally, the lariat-capped RNA exhibits a threefold longer half-life compared to its m7G-capped counterpart, consistent with a key role for the m7G cap in mRNA turnover. Our study emphasizes important activities of the m7G cap and suggests new utilities of lariat capping as a molecular tool in vivo. The expression of stable, lariat capped mRNA with very low translation competence offers at least two applications. First, these mRNAs could be used to screen for sequence elements that stimulate translation in a cap-independent fashion upon insertion into the 5' UTR of the mRNA. Second, lariat-capped mRNAs could be used as competitors for binding of factors to endogenous mRNAs. Here, it is an advantage, that sequence-identical and lariat-capped versions of any mRNA can readily be designed and expressed. We envisage that such LC-mRNAs could act as decoy targets with minimal effects on other mRNAs and thereby provide important information about the effects of interference with individual mRNA species.

### 393 Quality control of mRNA capping in plants: biochemical properties and function of AtDXO from *Arabidopsis thaliana*

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Co-transcriptional 5'-end capping facilitates correct processing, function and turnover of transcripts produced by the RNA polymerase II. Cap acquisition undergoes surveillance by RAI/DXO proteins that, due to their decapping and 5'-3' exoribonuclease activities, perform the removal of potentially deleterious improperly capped RNA molecules. In this work, we combined the biochemical and functional approaches to show that *Arabidopsis thaliana* RAI/DXO homolog, AtDXO (AT4G17620), removes both incomplete and correct cap structures to further proceed with the 5'-3' transcript degradation.

Members of the plant RAI/DXO family contain features distinctive from their yeast and mammalian counterparts, namely a plant-specific point modification within the enzyme active site and an extended N-terminal region, both of which exert pronounced effects on the enzyme *in vitro* activity. Knockdown of *Arabidopsis* AtDXO causes severe morphological and molecular abnormalities, including the accumulation of aberrantly capped mRNAs that trigger the RNA quality control siRNAs biogenesis, as revealed upon the comparison of *dxo* whole transcriptome and small RNA sequencing results. Analysis of these high-throughput data allowed us for the experimental identification of several potential *in vivo* AtDXO decapping mRNA targets. Surprisingly, catalytic activities associated to cap surveillance are only partially responsible for the observed *dxo* morphological and molecular phenotype, while the involvement of plant-specific N-terminal extension appears to be imperative in this regard. This N-terminal fragment contains predicted chloroplast transit peptide therefore expanding AtDXO possible function to this plant cellular compartment, especially that chloroplast rRNA precursors strongly accumulate in *dxo* mutant lines. Altogether, our results underline the functional significance of AtDXO in mRNA cap surveillance, but also imply additional plant-specific roles of this decapping nuclease.

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### 394 Polyadenosine RNA binding protein, ZC3H14, interacts with EJC component SRm160 in modulating post transcriptional RNA processing

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ZC3H14/Nab2 is an evolutionarily conserved, nuclear, zinc finger poly(A) RNA binding protein (PABP) that plays key roles in post-transcriptional RNA processing, including regulation of poly(A) tail length. Mutations in the human *ZC3H14* gene cause a non-syndromic form of autosomal recessive intellectual disability, highlighting the importance of ZC3H14 in proper brain function. In addition, depletion of *Drosophila* dNab2 in flies causes defects in locomotor function and neuronal patterning in the fly brain. Notably, ZC3H14 localizes to nuclear speckles in cultured rat hippocampal neurons and HeLa cells. To identify proteins that interact with ZC3H14 and shed light on the function of ZC3H14, we performed a yeast two-hybrid screen with ZC3H14. We found that ZC3H14 interacts with several splicing factors, including SRm160, an exon junction complex (EJC) component that functions in splicing and 3'-end processing. ZC3H14 directly interacts with SRm160 and colocalizes with SRm160 in nuclear speckles. In addition, knockdown of ZC3H14 and SRm160 in mammalian cells alters the steady-state levels of several RNA transcripts and causes defects in RNA processing. To explore the functional link between ZC3H14 and SRm160, we depleted dNab2 and SRm160 in *Drosophila* and examined the phenotypes of the mutant flies. Taken together, these studies provide insight into molecular defects that impact brain function in intellectual disability patients with mutations in *ZC3H14*.

### 395 Substitutions in Conserved Regions Preceding and Within the Tether Affect Activity and Flexibility of tRNase Z<sup>L</sup>, the Long Form of tRNase Z

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The enzyme tRNase Z, a member of the metallo- $\beta$ -lactamase family, endonucleolytically removes the 3' trailer from precursor tRNA (pre-tRNA), preparing it for CCA addition and aminoacylation. The short form of tRNase Z, tRNase Z<sup>S</sup>, functions as a homodimer and is found in all prokaryotes and some eukaryotes. The long form, tRNase Z<sup>L</sup>, apparently evolved from a tandem duplication of tRNase Z<sup>S</sup> and is found only in eukaryotes. tRNase Z<sup>L</sup> consists of diverged amino and carboxy domains linked by a flexible tether<sup>[1][2]</sup>, functions as a monomer and possesses ~2000-fold greater catalytic efficiency than tRNase Z<sup>S</sup><sup>[3]</sup>. The carboxy domain of tRNase Z<sup>L</sup> retained the active site, while the amino domain retained the flexible arm responsible for substrate binding. Tether flexibility may contribute to the higher catalytic efficiency observed in tRNase Z<sup>L</sup> by enabling greater mobility of the functionally diverged amino and carboxy domains relative to tRNase Z<sup>S</sup>. This hypothesis was explored by Ala-scanning through two conserved regions of *D. melanogaster* tRNase Z: N<sub>dom</sub>T<sub>prox</sub>, located at the carboxy end of the amino domain proximal to the tether (H<sub>315</sub> - G<sub>333</sub>), and T<sub>flex</sub> (M<sub>376</sub> - R<sub>384</sub>), the most flexible region in tether. Substitutions in a hydrophobic patch (V<sub>328</sub> - L<sub>331</sub>) in N<sub>dom</sub>T<sub>prox</sub> show ~130 - 300-fold impairment relative to wild type, accompanied by reduced flexibility inside the N<sub>dom</sub>-tether boundary. The alanine substitution at R<sub>382</sub> in the T<sub>flex</sub> region shows a ~50-fold decrease in catalytic efficiency, while the Ala substitution for N<sub>378</sub> has ~8-fold higher catalytic efficiency than wild type and locally decreased flexibility. These changes in pre-tRNA catalysis and protein flexibility highlight importance of the tether that flexibly links the amino and carboxy domains of tRNase Z<sup>L</sup>.

<sup>[1]</sup>Levinger, L. et al. (2009) Effect of changes in the flexible arm on tRNase Z processing kinetics. *Journal of Biological Chemistry*, 284(23), 15685

<sup>[2]</sup>Wilson, C., et al. (2013) Tethered domains and flexible regions in tRNase Z<sup>L</sup>, the long form of tRNase Z. *PLoS ONE*, 8(7)

<sup>[3]</sup>Yan, H., et al. (2006) Naturally occurring mutations in human mitochondrial pre-tRNA<sup>Ser(UCN)</sup> can affect the tRNase Z cleavage site, processing kinetics and substrate secondary structure. *J. Biol. Chem.* **281**: 3926

### 396 RNA adenylation during oocyte-to-embryo transition in mice

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All animal embryos undergo a transition during which development is handed from maternal to the zygotic control. In mouse, the oocyte-to-embryo transition is a highly coordinated process largely relying on post-transcriptional mechanisms because of transcriptional quiescence between the fully-grown oocyte stage and the activation of gene expression in a cleaving zygote. This makes mechanisms of post-transcriptional control of gene expression primary candidates for essential control elements of the maternal to zygotic transition. One of such mechanisms is covalent modification of RNA 3' termini by a family of RNA-specific ribonucleotidyl transferases, which add A or U ribonucleotides at the 3' terminus. Covalent modifications at 3' termini plays a role in processing of eukaryotic messenger RNA precursors, control of translation, and RNA quality control. Here, we report our analysis of RNA adenylation during oocyte-to-embryo transition in mice where the maternal transcriptome undergoes distinct waves of RNA adenylation and deadenylation.



### 397 The fission yeast MTREC and EJC orthologs ensure the maturation of meiotic transcripts during meiosis

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Meiosis is a highly regulated process by which genetic information is transmitted through sexual reproduction. It encompasses unique mechanisms that do not occur in vegetative cells, producing a distinct, well-regulated meiotic transcriptome. During vegetative growth, many meiotic genes are constitutively transcribed, but most of the resulting mRNAs are rapidly eliminated by the Mmi1-MTREC (Mtl1-Red1 core) complex. While Mmi1-MTREC targets premature meiotic RNAs for degradation by the nuclear 3'-5' exoribonuclease exosome during mitotic growth, its role in meiotic gene expression during meiosis is not known. Here, we report that Red5, an essential MTREC component, interacts with pFal1, an ortholog of eukaryotic translation initiation factor eIF4aIII in the fission yeast *Schizosaccharomyces pombe*. In mammals, together with MAGO (Mnh1), Rnps1, and Y14, eIF4AIII (pFal1) forms the core of the exon junction complex (EJC), which is essential for transcriptional surveillance and localization of mature mRNAs. In fission yeast, two EJC orthologs, pFal1 and Mnh1, are functionally connected with MTREC, specifically in the process of meiotic gene expression during meiosis. Although pFal1 interacts with Mnh1, Y14, and Rnps1, its association with Mnh1 is not disrupted upon loss of Y14 or Rnps1. Mutations of Red1, Red5, pFal1, or Mnh1 produce severe meiotic defects; the abundance of meiotic transcripts during meiosis decreases; and mRNA maturation processes such as splicing are impaired. Since studying meiosis in mammalian germline cells is difficult, our findings in fission yeast may help to define the general mechanisms involved in accurate meiotic gene expression in higher eukaryotes.

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### 398 RNA lariat debranching enzyme acts on retroviral RNA to promote reverse transcription

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RNA debranching enzymes are 2'-5' phosphodiesterases found in all eukaryotes. Their main role is the cleavage of intron RNA lariat branch points during intron RNA turn over. Consistent with this role, cells with reduced RNA debranching enzyme activity accumulate intron RNA lariats. The *Saccharomyces cerevisiae* RNA debranching enzyme Dbr1p is also a host factor for the yeast retrovirus-like element Ty1, a well-established model for many aspects of retroviral replication. Likewise, the human RNA debranching enzyme hDbr1 is a host factor for HIV. The yeast and human RNA debranching enzymes promote reverse transcription, the distinctive retroviral replication step that creates a cDNA copy of the retroviral RNA genome. In this work we show that Dbr1p must be active as a 2'-5' phosphodiesterase to function as a Ty1 host factor. We also show that Dbr1p acts on a branched Ty1 RNA substrate. Although efficient production of Ty1 cDNA requires Dbr1p, we show that the earliest steps of reverse transcription occur at normal levels in the absence of Dbr1 enzyme. Therefore, we hypothesize that the creation and removal of the Ty1 RNA branch occur at the minus strand transfer step of reverse transcription. Since the Ty1 genome lacks introns, Ty1 RNA branch formation may occur by a novel mechanism. To exploit the potential of the human Dbr1 enzyme as an anti-HIV drug target, we performed a high-throughput screen to identify Dbr1 enzyme inhibitors. Validated hits from the screen cause cells to accumulate RNA lariats and we are proceeding with preclinical studies to develop new anti-HIV drugs.



### 399 RNA binding protein, RBM10 controls Star-PAP 3'-UTR processing of cardiac mRNAs: A novel anti-hypertrophy mechanism in the heart

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mRNA processing at the 3'-untranslated region (3'-UTR) is an essential step in eukaryotic gene expression that involves two tightly coupled steps: endonucleolytic cleavage followed by addition of a poly(A) tail (polyadenylation). There are two major poly(A) polymerases (PAPs) that polyadenylates nuclear pre-mRNAs: canonical PAP $\alpha/\gamma$  and Star-PAP. Star-PAP (Speckle targeted PIPKI $\alpha$  regulated PAP) is a recently identified non-canonical PAP that selects pre-mRNA targets for polyadenylation. A large set of Star-PAP target mRNAs encode factors that plays crucial roles in heart function and diseases including cardiac hypertrophy (CH), a major risk factor for heart failure and arrhythmia.

We hypothesised that Star-PAP regulates CH through unique associated factors. We identified RNA binding protein motif 10, RBM10 as a unique Star-PAP co-regulator that controls expression of key anti-CH regulators in the heart. RBM10 is enriched in the heart and stimulates Star-PAP polyadenylation activity. RBM10 binds target mRNA and guides Star-PAP complex to specifically process cardiac mRNAs. Since most overlapped mRNA targets of both Star-PAP and RBM10 are involved in CH, we extended the study to physiological relevant models of CH, cellular (rat cardiomyoblast, H9C2), and animal (Wistar rat) models for CH (chemical induced and pressure overload). We observed downregulation of both RBM10 and Star-PAP resulting in reduced expression of target anti-hypertrophic genes. In H9C2 cell line, RBM10 depletion resulted in the generation of molecular events of hypertrophic response, and ectopic re-expression of RBM10 rescued the induced-hypertrophy. Our results show a novel anti-hypertrophy mechanism mediated through Star-PAP-RBM10 complex and reveals its role in pathological CH and putatively in cardiac growth/development.

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### 400 Cancer associated mutant splicing factor U2AF35 S34F confers altered DNA damage response, enhanced cell viability and activation of SASP in human bronchial epithelial cells

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In blood and lung cancers, the heterozygous somatic mutation S34F in the U2AF35 subunit of the splicing factor U2AF results in the preferential recognition of the nucleotides CAG in the 3'-splice site of an alternatively spliced exon by the mutant protein (UAG by the WT protein) leading to altered splicing outcomes *in vivo*. To understand the role of the mutant protein in the transition from being a splicing factor to oncogenic function, we have developed isogenic human bronchial epithelial cell lines (by TALEN mediated gene editing) carrying the heterozygous S34F mutation of U2AF35. Although mutant cells have similar growth characteristics as wild type cells, widespread altered splicing outcomes over a wide variety of genes was observed in mutant cells. However, RNA-seq data indicated only a modest (1.5-fold) change in gene expression for most genes. Gene ontology analysis showed an enrichment for genes associated with DNA replication and repair pathways. Consistent with this finding, mutant cells exhibited altered DNA damage response when subjected to X-ray irradiation (20Gy), with ~20% of cells showing weak DNA damage response although there was no significant change in damage itself. In addition, the mutation conferred enhanced cell viability post irradiation. The mutation enhances chemokine expression in mutant cells resulting in an elevated level of the chemokine IL-8 (~15-20 fold) at steady state; and post irradiation, the cells entered a prolonged senescent state for over 30 days (wild type cells died off after 15 days) with sustained secretion of IL-8. Interestingly, there was no expression of the interleukins IL-1 $\beta$ , -2, -4, -6, -10, the inflammatory factor IFN  $\gamma$  or the cytokine TNF  $\alpha$ . These altered responses of the mutant cells can be rescued by a frameshift knock-out mutation of the mutant allele. Conditioned medium collected from mutant cells induced epithelial to mesenchymal transition of MCF-7 cells as shown by the loss of E-cadherin and expression of Fibronectin in these cells. Taken together, our results suggest that this mutation could elicit Senescence Associated Secretory Phenotype (SASP) and may enhance or influence the growth and proliferation of surrounding cells in a non-cell autonomous fashion.

## 401 Large-scale molecular evolutionary analysis of the Clp1 polynucleotide kinase family that is involved in pre-tRNA splicing

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In many eukaryotes and archaea, transfer RNA (tRNA) genes often possess an intron in their anticodon loop region, and therefore exact pre-tRNA splicing is required to produce a mature and functional tRNA. The pre-tRNA splicing occurs in two distinct steps: (i) pre-tRNA intron removal by a tRNA splicing endonuclease and (ii) the ligation of tRNA halves by a tRNA ligase. In the latter step, it has been reported that Clp1 polynucleotide kinase is involved in the phosphorylation at the 5' end of the 3' half fragment. Thus, Clp1 is one of the important enzymes playing a crucial role in vivo, however knowledge of the molecular evolution of Clp1 and its phylogenetic distribution is still limited. In this study, we conducted the large-scale molecular evolutionary analysis of Clp1 to clarify the structural diversification of this enzyme.

The HMMER web server was used to search the UniprotKB database for homologous proteins of human Clp1 (UniProtKB: Q92989). HMMER is designed to sensitively detect remote homologous proteins. As a result, Clp1 homologous proteins were found in almost eukaryotic and archaeal species. However, in bacteria, the limited number of Clp1 homologous proteins was presented in distantly related phyla. Since the amino acid sequence and domain structure of the bacterial Clp1 is similar to those of archaeal Clp1, it is suggested that bacterial Clp1 may have been acquired through horizontal gene transfer. In eukaryotes, it has also been reported that Clp1 is composed of three domains: a polynucleotide kinase domain, a N-terminal domain, and a C-terminal domain. Meanwhile, archaeal and bacterial Clp1 is mainly composed of the polynucleotide kinase domain, suggesting that both the N- and C-terminal domains may have been acquired in the eukaryotic ancestor. Finally, the phylogenetic distribution of Clp1-related proteins involved in pre-rRNA processing (Nol9 and Grc3) and in pre-mRNA 3'-end formation (Clp5) was examined. Our results suggest that Clp1 gene duplication and functional diversification have occurred in certain lineages of eukaryotes.

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## 402 The human pre-mRNA 3' processing factor Cleavage Factor II (CFII<sub>m</sub>) consists of two subunits and binds to RNA with high affinity

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In eukaryotes, pre-mRNA undergoes cleavage downstream of a specific polyadenylation signal (AAUAAA in animal cells) and the addition of a polyA tail by a multimeric protein complex. Four heteroligomeric protein complexes contributing to 3' processing have been identified: the cleavage and polyadenylation specificity factor (CPSF), the cleavage stimulation factor (CstF) and the cleavage factors I and II (CFI<sub>m</sub>, CFII<sub>m</sub>). We find that CFII<sub>m</sub> is active in 3' end cleavage can be reconstituted from two previously described subunits, hPcf11 and hClp1 (de Vries et al., EMBO journal 2000). The hClp1 protein is highly similar to its yeast and *c. elegans* homologs and had the anticipated 5'-polynucleotide kinase activity (Weitzer et al., Nature 2007). The kinase activity was dispensable for pre-mRNA cleavage. Human Pcf11 is similar to its yeast homolog with respect to an N-terminal CID and two Zinc-fingers (Yang et al., RNA 2017) flanking the Clp1 interaction domain in the C-terminal region. It was previously known that Pcf11 is required for 3' cleavage and termination of transcription. We now find that CF II also contributes to recognition of the RNA substrate, binding with high affinity to SV40 late and L3 3' processing substrates. A specific G-rich region within SV40 late RNA located downstream of the cleavage site, previously shown to be important for the processing reaction, was identified as the preferred CFII<sub>m</sub> binding site. Human Pcf11 is the CFII<sub>m</sub> subunit primarily responsible for RNA binding.

**403 In vitro reconstitution of mammalian pre-mRNA 3'-processing***Lars Schönemann, Uwe Kühn, Peter Schäfer, Elmar Wahle***Martin-Luther-University Halle-Wittenberg, Halle, Germany**

We have previously reconstituted the second step of pre-mRNA 3'-processing, the polyadenylation reaction, from recombinant proteins and identified the minimum of proteins required for this step of mRNA maturation [Schönemann et al., 2014, GenesDev].

Our current goal is to also reconstitute the first 3'-processing step, the defined endonucleolytic cleavage of nascent mRNAs. For this purpose we produced the known mammalian 3'-processing factors, mostly in the baculovirus-system, and purified them. We assayed poly(A) polymerase, CPSF, CstF, CF I, CF II, as well as several other putative 3'-processing factors for their individual activities. However, attempts to reconstitute the cleavage reaction have so far been without success.

We are currently testing the functionality of our recombinant protein preparations in the cleavage reaction by complementation with partially fractionated nuclear extract from HeLa cells. So far, we have shown that our recombinant poly(A) polymerase, CstF and CF II are active in the cleavage reaction.

**404 DNA damage - dependent regulation of pre-mRNA 3'-end processing***Rym Sfaxi<sup>1,3</sup>, Michelle Newman<sup>1,3</sup>, Galina Boldina<sup>1,2</sup>, Mandy Cadix<sup>1,4</sup>, Martin Dutertre<sup>1,3</sup>, Stephan Vagner<sup>1,2</sup>*

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DNA lesions regulate gene expression including the 3'-end processing of pre-mRNAs, a nuclear process through which almost all eukaryotic pre-mRNAs generated by RNA polymerase II (RNAPII) are cleaved at their 3'-ends and acquire a poly(A) tail either in a co- or post-transcriptional manner. Following DNA-damage, pre-mRNA 3' end processing is strongly but transiently inhibited. However, transcripts of genes implicated in the DNA-Damage Response (DDR) should be properly processed to maintain the expression of genes involved in the DNA damage response (DDR). This suggests that specific compensatory mechanisms must exist to ensure the expression of DDR genes, such as the tumor suppressor p53. We have previously shown that the 3'-end processing of the p53 pre-mRNA is maintained following DNA damage (1). The underlying mechanism involves a G-quadruplex structure (G4) located downstream of the p53 polyadenylation site and the hnRNP H/F RNA binding proteins

We show here that the G4 resolvase and RNA helicase DHX36 is involved in the mechanism by which the p53 pre-mRNA is able to escape from the inhibition of 3'-end processing imposed by DNA damage (2). DHX36 binds to the p53 pre-mRNA G4 thereby potentially leading to G4 unwinding and increased interaction of hnRNP H/F with the G-rich region. In addition, we provide evidence that the p53 pre-mRNA is processed in a post-transcriptional manner, *i.e.* when the pre-mRNA is released from the chromatin. Finally, using dedicated RNA-seq approaches and hnRNP H/F CLIP-Seq experiments, we show that the pre-mRNA 3'-end processing of several other transcripts is regulated by hnRNP H/F following DNA damage.

(1) Decorsière et al., *Genes & Dev* 2011. (2) Newman et al., *JMB* 2016

#### **405 A novel method of NGS library preparation to identify new PARN substrates**

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The poly(A) specific ribonuclease (PARN) is a poly(A)-specific 3'-exoribonuclease that is stimulated by the cap structure. This led to the conclusion that PARN has a function in mRNA decay. However, PARN is localized in nucleoli in somatic cells and is involved in cycles of adenylation by PAPD5 and deadenylation during the maturation of H/ACA box snoRNAs (Berndt *et al.* 2012, *RNA*). Similar roles in the processing of other small RNAs have been reported. In order to identify PARN substrates globally, we developed a new method of NGS library preparation that theoretically can detect changes in abundance as well as in 3' end sequences of steady state and newly synthesized RNAs. Our first analyses confirmed that the method detects PARN's previously described role in H/ACA box snoRNA trimming.

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#### **406 In search of novel U7 snRNP function on the ground of its subcellular localization during the cell cycle**

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The well-known function of U7 snRNP is related to the 3' end processing of replication-dependent histone pre-mRNAs. The higher efficiency of this process during the G1/S phase transition and the activation of histone gene transcription during S phase by NPAT (Nuclear Protein, Ataxia-Telangiectasia locus) are crucial to facilitate accurate DNA replication. Since the expression of replication-dependent histone genes is strictly linked to S phase, the abundance and/or activity of few proteins essential for this process is also cell cycle-regulated. In contrast, the level of all three unique components of U7 snRNP: U7 snRNA and two proteins: Lsm10 and Lsm11, remains stable during the entire cell cycle. Thus, the question arises: what is the role of U7 snRNP and its components out of the S phase of the cell cycle?

It has been reported that U7 snRNP can repress histone gene expression under cell cycle-arrested conditions in complex with heterogeneous ribonucleoprotein (hnRNP) UL1. These data correlates with our results. We found that hnRNP UL1 is bound by FUS - the protein that activates histone gene expression in complex with U7 snRNP. Interestingly, while the FUS/U7 snRNA complex was mainly formed in S phase and then maintained in G2 phase, the FUS/hnRNP UL1 association was particularly enriched in G2 and G1 phases. Thus, FUS may mediate U7 snRNP/hnRNP UL1 complex assembly.

To identify and understand additional roles of U7 snRNP and its components in the mammalian cell we have focused on the localization of the complex during the cell cycle. We applied FISH, IF and PLA techniques to investigate whether U7 snRNP may be re-localized within the cell during the cell cycle and/or exchange the protein partners to play additional roles in the cell. The localization and interactions of U7 snRNP with NPAT, hnRNP UL1 and FUS in cells synchronized to G1 or S phase will be presented.

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## 407 Multifaceted Roles of LAMMER-related Kinases in Gene Expression

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The LAMMER-related kinases are involved in cell proliferation, differentiation, development, and apoptosis, performing fundamental functions in eukaryotic cells. Dsk1 and Kic1/Lkh1 are members of the family in the fission yeast *Schizosaccharomyces pombe*, and are the functional homolog of human SR protein-specific kinase1 (SRPK1) and mammalian Clk1/Sty1, respectively. In addition to their role in cell cycle and pre-mRNA splicing, we previously reported the nuclear accumulation of poly(A)<sup>+</sup> RNA in  $\Delta dsk1$  and  $\Delta kic1$  mutant cells, as well as the association of Dsk1 with Pabp/Pab1, a poly(A)-binding protein functioning in mRNA export. These results led us to further study the kinase involvement in nuclear export of mRNA. To examine cellular distribution of mRNA from a single gene, we employed the “green RNA” system for imaging the localization and trafficking of specific transcripts in living cells. We designed the experiments to dissect the steps of splicing and export to assess the involvement of the kinases in mRNA export process. The results confirmed the mRNA export defect rendered by deletion of *dsk1* or *kic1*. The studies provide the first evidence for a role of Dsk1 and Kic1 in mRNA export distinct from their functions in splicing.

We also explored the involvement of LAMMER-related kinases in centromere dynamics based on the reported role of Dsk1 in the sister-chromatid segregation at the metaphase/anaphase transition, the similar cellular distribution pattern between Kic1 and Swi6/HP1, and the association of Dsk1 with the catalytic subunit of CK2. We genetically tested the functional involvement of the Dsk1 and Kic1 in gene silencing at centromeres in *S. pombe*. The results not only confirm a role of Dsk1 in centromeric gene silencing, but also demonstrate the requirement of its kinase activity for the function in the nucleus. Our preliminary biochemical data support the notion that the Dsk1 and Kic1 kinases participate in the regulation of Swi6/HP1 activity possibly through a phosphorylation cascade. Our studies revealed for the first time the novel functions of Dsk1 and Kic1 in multiple steps of eukaryotic gene expression: nuclear export of mRNA and transcriptional gene silencing (TGS) at centromeres.

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## 408 Genome-wide Identification of Enzymatic Targets of a DEAD-box RNA Helicase Reveals Roles for Secondary Structure Remodeling During Pre-mRNA Processing

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Proper formation of messenger RNA-protein complexes (mRNPs) is critical for accurate gene expression and requires temporal regulation of mRNA structures. Given the physical properties of RNA, RNA molecules tend to form stable secondary structures that are long-lived and require large amounts of energy to unfold and refold to alternative conformations. Enzymes, such as RNA helicases, are therefore required to accelerate RNA structural conversions inside the cell.

RNA helicases play fundamental roles in modulating RNA structures and facilitating RNA-protein (RNP) complex assembly *in vivo*. Our lab has shown that the DEAD-box RNA helicase Dbp2 in *S. cerevisiae* is a *bona fide* RNA helicase *in vitro* that is required for proper transcription termination and assembly of RNA-binding proteins onto poly(A)<sup>+</sup> RNA *in vivo*. This suggests that Dbp2 remodels RNA structures in cells to promote RNP assembly. To test this, we employed iCLIP and Structure-seq to identify RNA-binding targets of Dbp2 and sites of Dbp2-dependent secondary structure remodeling across the transcriptome. We then used the intersection of the two data sets to cross-reference these *in vivo* targets of Dbp2 helicase activity with misprocessed transcripts in *dbp2Δ* cells. Results of these studies indicate that DEAD-box RNA helicases modulate RNA secondary structure *in vivo*, in line with *in vitro* biochemical studies, and that this activity is essential for pre-mRNA processing.



#### 409 Small-angle X-ray scattering studies of the dimer structure of the SERRATE<sub>194-579</sub> protein in solution – a key player in the process of miRNA biogenesis in plants

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The SERRATE (SE) protein is a crucial component of the molecular machinery processing miRNA precursors in *Arabidopsis thaliana*. The SE protein was found to greatly improve the accuracy of miRNA excision from its precursor. The fragment covering amino acid residues 194-579 of the protein (SE<sub>194-579</sub>) is sufficient to restore the wild type-like levels of miRNAs in SE-deficient *Arabidopsis* plants. SE<sub>194-579</sub> is also able to bind the precursor of miRNA-164c (pre-miRNA). Crystallographic studies of the shorter fragment (SE<sub>194-543</sub>) revealed that it adopts a walking man-like shape, with the C-terminal zinc finger and N-terminal domains standing for the „legs” attached to the middle domain.

The structure of recombinant SE<sub>194-579</sub> protein in solution was examined by the small-angle X-ray scattering (SAXS). Based on the SAXS data obtained, a molecular mass of SE<sub>194-579</sub> protein was estimated, indicating that the protein existed mainly in a dimeric state. In the next step, modeling of the structure on the basis of SAXS data was performed. The crystal structure of SE<sub>194-543</sub> was used as a constraint. First, the loops and the C-terminal tail, missing from the crystal structure, were modelled using the Modeller software. Next, the AllosMod tool was used to generate low-energy structures by a molecular dynamics simulation. All of them were separately provided to the FoXSDock tool. FoXSDock models the dimer structure, simultaneously fitting it against the experimental SAXS data, taking into account a contribution from the monomer. The dimer characterized by the best score, together with the monomer, was subjected to analysis by the OLIGOMER algorithm. The assessed fraction of the dimer in a mixture was about 64%.

The best dimer model has an asymmetric structure. The main intermolecular contacts are provided by the N-terminal domain of one monomer and the first loop of the middle domain of another monomer. The putative RNA-binding zinc finger domains and the C-terminal tails are located far from each other.

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#### 410 Regulated Intron Retention and RNA Stability during CD4 T Cell Activation

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Intron retention (IR) is a form of alternative splicing broadly involved in cell fate determination. Our previous study demonstrated that intron retention is prevalent in resting CD4 T cells and is dramatically decreased upon cell activation. We obtained initial evidence that intron retention is associated with transcript instability, serving as an important mechanism for posttranslational gene regulation. In this study, we performed BruChase-Seq to experimentally monitor the expression dynamics of nascent transcripts in resting and activated CD4 T cells, and applied computational modeling to investigate the impact of IR on the kinetics of mRNA degradation. As expected, intron retained transcripts are considerably less stable than spliced transcripts. In addition, the decrease in the steady-state IR level in activated CD4 T cells are in part due to increased splicing efficiency and further stabilization of spliced transcripts. We propose that coordination between splicing regulation and mRNA stability may provide a novel paradigm to achieve spatiotemporal control of gene expression during T cell activation.

**411 Comparative structural analysis of group I introns from different subgroups***Astha<sup>1</sup>, Deepak Kumar<sup>2</sup>, Catarina Almeida<sup>1</sup>, Janusz Bujnicki<sup>1,2</sup>*<sup>1</sup>International Institute of Molecular and Cell Biology in Warsaw, Trojdena 4, 02-109 Warsaw, Poland;<sup>2</sup>Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland

Group I introns belong to a family of widespread non-coding ribozyme RNAs, known for their self-splicing from the host precursor RNA. Commonly, these introns are classified into 15 subgroups based on conserved core sequences and peripheral structures. So far, only three group I introns (Azoarcus, Tetrahymena, and Twort) from three subgroups (IC3, IC1, IA2) have their high-resolution structures determined by X-ray crystallography, while other intron subgroups remain structurally uncharacterized.

There are a high sequence and length variability between introns representing different subgroups, in particular for insertions that are unique to individual groups. Understanding the rules that govern the evolution of sequence-structure relationships of all group I introns subgroups requires the knowledge of their molecular structures.

Crystallization of RNAs remains a formidable experimental challenge and the number of RNA crystal structures still remains scarce. Therefore, in addition to X-ray crystallography, we have used low-resolution structural probing methods such as selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) and hydroxyl radical footprinting (HRF), for evaluation and modification of our theoretical models. These methods combined with theoretical structure predictions have been shown to be good alternatives for X-ray crystallographic structure determination.

In this work, we have used a homology modeling approach, together with de novo RNA structure modeling approach for the peripheral elements, to built models of one representative from each group I intron subgroups. Moreover, SHAPE and HRF experiments were used to further validate and modify our theoretical models. We expect that the results of this study will provide a perspective for studying group I introns and for interpretation of their sequence evolution in a structural context.

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**412 Facing the challenge of solving the solution structure of the cytoplasmic element binding protein 3 ribozyme by NMR spectroscopy***Kenneth Adea<sup>1</sup>, Miriam Skilandat<sup>1</sup>, Daniela Donghi<sup>1</sup>, Magdalena Rowsinka-Zyrek<sup>1,2</sup>, Silke Johannsen<sup>1</sup>, Roland K. O. Sigel<sup>1</sup>*<sup>1</sup>University of Zurich, Zurich, Switzerland; <sup>2</sup>University of Wroclaw, Wroclaw, Poland

Ribozymes are RNA molecules capable of catalyzing specific chemical reactions. Several small ribozymes were recently discovered in mammals<sup>1</sup>. The cytoplasmic element binding protein 3 (CPEB3) ribozyme is a self-cleaving ribozyme that was found to be highly conserved in the mammalian genome<sup>1</sup>. Characterization of the CPEB3 ribozyme showed that it has similar properties with the hepatitis delta virus (HDV) ribozyme, including the fold and catalytic mechanism<sup>1,2,3</sup>. Unlike some self-cleaving ribozymes such as the hammerhead, hairpin and VS ribozymes where high concentrations of monovalent metal ions can promote catalysis, the CPEB3 and HDV

ribozymes Mg<sup>2+</sup> is required<sup>4</sup>. Both ribozymes fold in a sophisticated nested double pseudoknot motif. According to the work previously done in our group<sup>3</sup>, the CPEB3 ribozyme is significantly compacted with monovalent ions alone but the active fold is only achieved after addition of millimolar amounts of Mg<sup>2+</sup>. NMR studies showed the formation of a GU wobble pair with addition of Mg<sup>2+</sup>, which may correspond to the GU wobble that is an essential part of the active site in the HDV ribozyme<sup>5</sup>.

In this project, we aim to solve the solution structure of the CPEB3 ribozyme through NMR spectroscopy. The assignment of the <sup>1</sup>H-<sup>1</sup>H NOESY spectra of the full length ribozyme is a challenge due to the limited building blocks of RNA as well as the length of the CPEB3 ribozyme at 67 residues. In order to alleviate the spectral overlap, various labelled nucleotides were incorporated through in vitro transcription including partially deuterated, fully deuterated and <sup>13</sup>C, <sup>15</sup>N-labeled nucleotides. Furthermore, truncated constructs were also investigated.

### 413 Nuclease resistant non-coding RNAs in Zika virus

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The outbreak of Zika virus (ZIKV) and associated fetal microcephaly mandates efforts to understand the molecular processes of infection by this emerging global pathogen. Related flaviviruses, including Dengue and West Nile virus, produce unique non-coding RNAs known as subgenomic flaviviral RNAs (sfRNAs). sfRNAs are highly conserved and have been linked to the pathology of the virus in cell culture and mouse model systems. sfRNAs are formed by co-opting Xrn1, the cell's predominant 5'→3' exonuclease. The viral RNA genome contains highly structured Xrn1-resistant RNAs (xrRNAs), which halt the enzyme leaving sfRNAs behind. While the biology of sfRNAs has been explored in other flaviviruses, the existence of sfRNAs or xrRNAs during ZIKV infection has not been reported. Here, we demonstrate that ZIKV infection in multiple cell types results in distinct sfRNA patterns. We solved the structure of a ZIKV xrRNA responsible for sfRNA production in these cell types. The complete ZIKV xrRNA structure reveals how xrRNAs resist the helicase activity of Xrn1, using two intertwined RNA pseudoknots which form a molecular "slipknot." Furthermore, the ZIKV xrRNA structure provides a model for understanding Xrn1-xrRNA interactions, and identifies conserved features that modulate function in diverse pathogenic flaviviruses.

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### 414 Mechanistic insight into intrinsic and extrinsic factors stabilizing the MALAT1 triple helix

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A 3' terminal 93-nt triple helical protection element confers persistent evasion of RNA turnover processes to the ~8-kb oncogenic MALAT1 long non-coding RNA. Together with the crystal structure, previous mutational analyses monitored by Northern blots have demonstrated the functional importance of several sequence and structural elements within this compact RNA motif. We employ UV, fluorescence, and FRET measurements to investigate the mechanisms of folding and stability for this protection element. We design two- and three-strand constructs to investigate the interplay between standard duplex regions and the unusually long triplex region. UV melt assays demonstrate the critical role of the Hoogsteen strand in triple helix formation. A 15-nt linker region also confers significant stability to the RNA. These results inform the design of fluorescent labeling schemes for FRET experiments. We develop a new method to efficiently attach fluorophores to RNA 3' ends, following templated addition of a modified nucleotide by the Klenow fragment. Both synthetic and *in vitro* transcribed RNAs are labeled using this method. We use a multiplexed experimental FRET approach to investigate the folding and stability of this RNA in response to temperature, monovalent cations, and Mg<sup>2+</sup>. Results from two distinct pairs of labeling positions within the RNA indicate that the 3' end unfolds first. Interestingly, an increase in monovalent cation concentrations disrupts the triple helix regardless of Mg<sup>2+</sup> concentration. Future work will focus on mutation and small molecule studies to evaluate stability "hot spots" for potential therapeutic intervention.

**415 Stabilization of RNA hairpins using non-nucleotide linkers and circularization**Leszek Blaszczyk, Agnieszka Kiliszek, Ryszard Kierzek, Wojciech Rypniewski**Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland**

An RNA hairpin is an essential structural element of RNA. Hairpins play crucial roles in gene expression and intermolecular recognition but are also involved in the pathogenesis of some congenital diseases. Structural studies of the hairpin motifs are impeded by their thermodynamic instability, as they tend to unfold to form duplexes, especially at high concentrations required for crystallography or NMR spectroscopy. We have elaborated techniques to stabilize the RNA hairpins by linking the free ends of the RNA strand at the base of the hairpin stem. One method involves stilbene diether or hexaethylene glycol linkers and circularization by T4 RNA ligase. Another method uses click chemistry to stitch the RNA ends with a triazole linker. Both techniques are efficient and easy to perform. They should be useful in making stable, biologically relevant RNA constructs for structural studies.

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**416 A macromolecular crowding study of RNA folding and activity: polymer pore size matters!**Richard Börner<sup>1</sup>, Erica Fiorini<sup>1</sup>, Bishnu Paudel<sup>2</sup>, Michael Kovermann<sup>3</sup>, David Rueda<sup>2</sup>, Roland KO Sigel<sup>1</sup>**<sup>1</sup>University Zurich, Zurich, Switzerland; <sup>2</sup>Imperial College London, London, UK; <sup>3</sup>University Konstanz, Konstanz, Germany**

Ribozymes are catalytic active RNAs requiring a high magnesium(II) concentration to show folding and function *in vitro* (1,2). In contrast, *in vivo* conditions are characterized by a highly crowded cellular environment and much lower ion concentration. Molecular crowding agents are used to mimic the cellular environment. However, particular physical/chemical properties explaining the co-solutes influence on folding and function of RNAs are poorly understood. In this study, we gain new insights on how polymer properties like viscosity, pore size etc. influence the activity and folding of a group IIB intron ribozyme (3,4). We combined bulk activity assays, smFRET experiments and NMR screening PEG volume fraction (%) and molecular weight (MW) and unveiled an optimal pore size in terms of the catalytic activity.

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## 417 Mapping the Universe of RNA Tetraloop Folds

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We present a comprehensive analysis of all RNA tetraloops available in the entire PDB database. Tetraloops with similar structures are clustered together and, as expected, the two largest clusters are the canonical GNRA and UNCG folds. We identify other clusters corresponding to known tetraloop folds such as U-turns, GGUG, RNYA, AGNN and CUUG, as well as previously unreported folds.

The analysis allowed us to investigate the non-trivial relationship between three-dimensional fold and sequence. We find a significant number of non-GNRA and non-UNCG sequences adopting the canonical GNRA and UNCG folds. Conversely, we find a significant number of GNRA and UNCG sequences adopting non-GNRA and non-UNCG folds. Our analysis demonstrates that there is no simple one-to-one, but rather a many-to-many mapping between tetraloop sequence and tetraloop fold.

Our method can be easily applied to any nucleic acid three-dimensional motif and serves as a general tool for describing and classifying known as well of future, newly discovered RNA three-dimensional folds.

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## 418 Refining molecular dynamics simulations of RNA using solution NMR data

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RNA structure and dynamics play a fundamental role in non-coding RNAs and significantly affect functions such as gene expression inhibition, splicing and catalysis. Molecular dynamics is a computational tool that can be in principle used to investigate RNA structure and dynamics at atomistic resolution. However, its capability to predict and explain experimental data is limited by the accuracy of the employed potential energy functions, also known as force fields. Recent works have shown that state-of-the-art force fields could predict unphysical conformations that are not in agreement with experiments. The emerging strategy to overcome these limitations is to complement molecular dynamics with experimental data included as restraints. Solution NMR data are particularly useful since they provide averages over the conformations explored on the experimental time scale and ultimately give access to RNA dynamics. We here propose a scheme based on the maximum entropy principle to combine bulk experiments with molecular dynamics simulations explicitly taking into account experimental errors. The method is applied to a set of nucleosides and dinucleotides in a chemically-consistent manner and suggests a new paradigm for force field refinement.



## 419 Occurrence and Stability of Lone Pair- $\pi$ Stacking Interactions between Ribose and Nucleobases in Functional RNAs

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The specific folding pattern and function of RNA molecules lies in various weak interactions, in addition to the strong base-base pairing and stacking. One of these relatively weak interactions, characterized by the stacking of the O4' atom of a ribose on top of the heterocycle ring of a nucleobase, has been known to occur but has largely been ignored in the description of RNA structures. We identified 2015 ribose-base stacking interactions in a high-resolution set of nonredundant RNA crystal structures. They are widespread in structured RNA molecules and are located in structural motifs other than regular stems. Nearly 50% of them involve an adenine, as we found ribose-adenine contacts to be recurring elements in A-minor motifs. Fewer than 50% of the interactions involve a ribose and a base of neighboring residues, while approximately 30% of them involve a ribose and a nucleobase at least four residues apart. Some of them establish interdomain or inter-molecular contacts and often implicate functionally relevant nucleotides. Accurate evaluation of the ribose-base stacking interaction energy indicates that it contributes 2-3 kcal/mol to the RNA stability. Finally, we found that lone pair- $\pi$  stacking interactions also occur between ribose and aromatic amino acids in RNA-protein complexes.

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## 420 Role of Structure and Flexibility on RNA-Cation Binding

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RNA folding stands upon a delicate balance between base-base interaction and electrostatic repulsion arising from the phosphate moieties in the RNA backbone. Mg<sup>2+</sup> cations are undoubtedly important to modulate RNA structure and function by compensating the negative charge repulsion and allowing RNA compaction and folding. We introduce a molecular dynamics method to compute RNA-Mg<sup>2+</sup> affinities with high statistical accuracy so as to assess the effects of ion competition, RNA hybridization and flexibility (Cunha and Bussi, 2017). We find that flexibility plays a non-trivial role mainly due to entropic effects. We then apply a similar approach to predict and characterize the Mg<sup>2+</sup> binding sites in a set of motifs including duplexes with different sequences and length as well as hairpins. Results are in agreement with NMR experiments (Bonneau and Legault 2013) and statistical analysis of crystallographic structures (Zheng et. al., 2015).

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Cunha, Richard A., and Giovanni Bussi. "Unravelling Mg<sup>2+</sup>-RNA binding with atomistic molecular dynamics." *RNA* (2017): Published in Advance.

Zheng, Heping, et al. "Magnesium-binding architectures in RNA crystal structures: validation, binding preferences, classification and motif detection." *Nucleic acids research* 43.7 (2015): 3789-3801.

## 421 Automated Design of Three-Dimensional RNA Structures at Near-Atomic Accuracy and RNA Structure Refinement for Geometric Paragons

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The emerging field of RNA nanotechnology seeks to generate nano-scale machines through the repurposing of natural RNA modules. Unlike other areas of material and molecular engineering, there is no automated tool for 3D structural RNA design, and successes so far have been limited to symmetric assemblies repeating a single non-canonical motif. Here we describe RNAMake, a suite that enables design of non-symmetric 3D structures by automated combination of RNA 3D motifs. To validate RNAMake, we generated 16 distinct 'miniTTRs', asymmetric RNAs that contain the two segments of the tetraloop/tetraloop-receptor and aligns their translation and orientation so that they are poised to form this high affinity tertiary contact. For each RNA, we synthesized an RNAMake-designed sequence and performed nucleotide-resolution chemical mapping, Mg<sup>2+</sup> titrations, and native gel electrophoresis. For 12 of the 16 designs, we obtained experimental results consistent with our models. For two designs with highest stabilities, solution x-ray scattering data provided independent support for well-defined monomeric folds. Lastly, we obtained an x-ray crystallographic structure of one design at a diffraction resolution of 2.55 (I/σ=1.0) Å, which conformed to the design fold with an average backbone RMSD of 4.13 Å and with large sections achieving near-atomic accuracy (< 2.00 Å).

In addition, we used a crystal structure of one of the failed miniTTR constructs to improve RNA structure refinement. RNA crystal structure determination comprises refining a model against the observed diffraction data and geometric restraints to minimize R/R-free. MolProbity clash score indicate structures often have errors we sought to improve with ERRASER, position refinement program aimed to produce paragon RNA models. We demonstrate ERRASER's abilities to correct common refinement program errors with a novel 300+ nucleotide RNA structure of a miniTTR tetramer resulted in a MolProbity clash score under 2 without geometric errors.

## 422 RNA Structural Characterization by Small Angle X-ray Scattering

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Small-angle X-ray scattering (SAXS) is a powerful tool for structural characterization of macromolecules including RNA. It allows studies of the global structures of macromolecules and their complexes in solution in near physiological environments. SAXS is particularly well suited for RNA because the contrast between RNA and buffer is high due to the electron-rich phosphate backbone in RNA. The large RNA can be difficult to crystallize and NMR characterization is challenging due to spectral overlap and fast relaxation. SAXS is appropriate for RNAs from 25 to hundreds of nucleotides in size. SAXS data provide information about the size and global RNA structural information that outlines backbone topologies and molecular envelopes. The low-resolution models generated from SAXS data can be compared with homologous crystal or NMR structures. All-atom models can also be filtered and refined against SAXS data. SAXS can also be used to study structure-function relationship and conformation space of RNA. The SAXS Core Facility of Center for Cancer Research (CCR) of National Cancer Institute opens to all intramural and extramural research communities free of charge. The mission of the SAXS Core Facility is to provide support to Core user community including expertise in experimental design, data collection, processing, analysis and interpretation. This presentation gives introduction to the SAXS Core Facility of CCR and highlights recent scientific achievements in RNA structure characterization produced by the SAXS Core users.

SAXS Core website: <https://ccr-staging.ncifcrf.gov/resources/sbl/Saxs/default.aspx>

## 423 Extensive Dynamic Sampling of the Hairpin Ribozyme Loop A Suggests Hybrid Binding Mechanism

*Michael Feig, Patrick Ochieng, Neil White, Charles Hoogstraten*

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A series of microsecond molecular dynamics simulations of loop A of the hairpin ribozyme are presented that show extensive dynamic sampling on a complex energy landscape. Markov state analysis revealed several major conformations distinguished by different base pairing/stacking arrangements within the loop region along with numerous minor conformations indicating a very rich conformational landscape for RNAs with internal loops. As loop A interacts with loop B to form the catalytically active structure, further analysis focused on the question of whether binding to loop B followed a conformational selection or induced-fit paradigm. As the simulations revealed partially binding-competent states, a hybrid binding mechanism is postulated where initial conformational selection is followed by induced-fit adjustment facilitated by the significant plasticity of the RNA.

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## 424 eBroccoli: Understanding and Optimizing the Structure and Function of the Broccoli RNA Aptamer for Better Fluorescence

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The Spinach family of aptamers are RNA mimics of green fluorescent protein (GFP) that have previously been designed to address the challenges of imaging RNA inside living cells. However, relatively low levels of free intracellular magnesium limit the practical use of these aptamers. New cell-based selections identified the Broccoli RNA aptamer, which required less magnesium for fluorescence. However, the structure of Broccoli and the basis for lower magnesium dependence were unknown. Here we find that Broccoli RNA shares the same core quadruplex structure as Spinach and is nearly identical to the structure of a truncated version, Baby Spinach. Differences in stability and metal ion preferences between these two aptamers, and among Broccoli mutants tested, are primarily associated with the sequence, structure and stability of predicted quadruplex-flanking stem and stem-loop structures. Mutation of purine-purine pairs in Broccoli at the terminal stem-to-quadruplex transition caused reversion of Broccoli to a higher magnesium dependence. Unique duplex-to-quadruplex transitions in GFP-mimic RNAs likely explain their sensitivity to magnesium and certain other metal ions. Thus, optimizations designed to improve aptamers should pay careful attention to the role of transitions between distinct or independently folding RNA structural motifs. Systematic mutagenesis and comparative structure-function analyses have allowed us to rationally design an enhanced Broccoli aptamer, called eBroccoli, that exhibits better folding and higher stability than the original Broccoli aptamer.

**425 Triangulating Nucleic Acid Conformations Using Multicolor Surface Energy Transfer***Nancy Greenbaum<sup>1</sup>, Ryan Riskowski<sup>2</sup>, Rachel Armstrong<sup>2</sup>, Geoffrey Strouse<sup>2</sup>*<sup>1</sup>Hunter College and The Graduate Center of The City University of New York, New York, NY, USA;<sup>2</sup>Florida State University, Tallahassee, FL, USA

Optical ruler methods employing multiple fluorescent labels offer great potential for correlating distances among several sites, but are generally limited to inter-label distances under 10 nm; additionally, these methods often suffer from complications due to spectral overlap. Here we demonstrate a multicolor surface energy transfer (mcSET) technique able to triangulate multiple points on a biopolymer, allowing for analysis of global structure in complex biomolecules. McSET couples the competitive energy transfer pathways of Förster Resonance Energy Transfer (FRET) with gold-nanoparticle mediated Surface Energy Transfer (SET) in order to correlate systematically labeled points on the biomolecule at distances greater than 10 nm and with decreased spectral overlap. To demonstrate the mcSET method, the structures of a linear B-DNA and a folded RNA ribozyme were analyzed within the mcSET mathematical framework. The improved multicolor optical ruler method takes advantage of the broad spectral range and distances achievable when using a gold nanoparticle as the lowest energy acceptor. The ability to report distance information simultaneously across multiple length scales, including short range (10-50 Å), mid-range (50-150 Å) and long-range (150-350 Å), distinguishes this approach from other multicolor energy transfer methods.

**426 RNA Folding and Protein Binding: rRNA GTPase Center and L11***Robb Welty<sup>1</sup>, Ben Hudson<sup>2</sup>, Michael Rau<sup>1</sup>, Hani Zaher<sup>2</sup>, Kathleen Hall<sup>1</sup>*<sup>1</sup>Washington University School of Medicine, Saint Louis, MO, USA; <sup>2</sup>Washington University, Saint Louis, MO, USA

The 60 nucleotide GAC site in 23S rRNA is a control center for hydrolysis of GTP during translation. In structures of the ribosome and the 50S subunit, the GAC is exposed on the surface of the particle, where L11 can sometimes be observed bound to it; in some structures, other protein factors appear associated. When GAC is observed in these contexts, it has a conserved tertiary structure.

All GAC secondary structures have two hairpins and a stem connected by a 3-way junction. The tertiary structure is formed by nucleobase stacking and triples that juxtapose and anchor the two hairpins together, while a triloop is formed in the junction. Notably, there is no solution or crystal structure of the GAC alone; our knowledge of its tertiary structure comes from cocrystals of GAC:L11. Previous experiments from the Draper lab showed that prokaryotic L11 would not bind to a GAC that had no tertiary structure.

We have shown by stopped-flow fluorescence experiments that GAC tertiary folding, triggered by addition of divalent ions, is a multi-step process (Welty & Hall, 2016). Folding takes place on timescales from < 1 ms to ~25 s at 25° C. In vivo, as transcription progresses, the GAC environment would include Mg<sup>2+</sup> ions which are needed for its folding, but the L11 protein could also be available. Here, we ask if and how L11 affects GAC folding using stopped-flow fluorescence measurements.

In X-ray crystal structures and cryo-EM structures of ribosomes and large subunits, the GAC/L11 region of the particles is sometimes obscure. Presumably, its lack of definition is due to flexibility of this region, perhaps as the RNA structure changes or the protein factors exchange. To explore the role of GAC structure on protein association, we have incorporated mutant GAC sequences into E coli 23S rRNA. These mutants lack specific interactions that are found in the tertiary structure, to address the question of how the GAC tertiary fold contributes to its function in the ribosome.

## 427 A structured RNA ribosome sensor: How a double-pseudoknotted 3'UTR can regulate translation

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Eukaryotic messenger RNAs (mRNAs) contain cis-acting signals that enhance the rate at which they are translated. Although the most common examples of such translation enhancement are the 5' cap and 3' poly(A) tail signal, there is growing evidence that other signals exist within mRNAs to regulate their translation. This includes signals based on unique structured RNA elements within a message's untranslated region (UTR). Despite this growing realization of these elements, there is little understanding of their mechanism of action. We seek to understand the processes of translation regulation by structured 3' UTRs using a powerful model system from the turnip yellow mosaic virus (TYMV). The TYMV viral RNAs are capped at the 5' end and terminate in a highly structured 3' UTR that enhances both translation and the stability of the viral genome. The TYMV 3'UTR folds into a structure with two domains: an upstream pseudoknot domain (UPD) and a tRNA-like structure (TLS). The structures drive aminoacylation of the 3' end of the viral genome, an event important for its function. Previous results show that the two TYMV 3'UTR domains structurally interact and influence one another in a multi-domained architecture that is not fully understood. This architecture appears to be structurally plastic and this ability to switch conformations is likely involved in controlling translation, replication, and stability activities. These features make the TYMV 3'UTR an intriguing model for learning fundamental rules for how a multi-domained, conformationally dynamic, and structured RNA can regulate translation, potentially acting as a sensor of the ribosome through programmed conformational changes. Here we use new multi-dimensional chemical mapping, biochemical, and biophysical experiments to address how a double-pseudoknotted 3'UTR can manipulate the process of translation from the 5' end.

## 428 MD Simulations of HIV - 1 DIS Kissing-Loop Complexes Predict Bulged-In Conformation of the Bulged Bases

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RNA kissing loop complex located in HIV-1 dimerization initiation site plays a key role in dimerization of retroviral genome that enables formation of new virions. Despite many structural investigations by X-ray, NMR, and MD techniques, the position of the bulged purines of the kissing complex has not been unambiguously resolved. The X-ray structures consistently show bulged-out positions of the unpaired bases, while several NMR studies show bulged-in conformations. The NMR studies are, however, mutually inconsistent regarding the exact orientations of the bases. The earlier simulation studies predicted the bulged-out conformation; however, this finding could have been biased by the short simulation time scales. Our extensive set of explicit solvent molecular dynamics (MD) simulations (~25  $\mu$ s of accumulated simulation time) reveals that all unpaired bases of the kissing-loop complex stay preferably in the interior of the kissing-loop complex. The MD results are discussed in the context of the available experimental data and we suggest that both conformations are biochemically relevant. We also show that MD provides a quite satisfactory description of this RNA system, contrasting recent reports of unsatisfactory performance of the RNA force fields for smaller systems such as tetranucleotides and tetraloops. We explain this by the fact that the kissing complex is primarily stabilized by an extensive network of Watson-Crick interactions which are rather well described by the force fields. We tested several different sets of water/ion parameters but they all lead to consistent results. However, we demonstrate that a recently suggested modification of van der Waals interactions of the Cornell et al. force field deteriorates the description of the kissing complex by the loss of key stacking interactions stabilizing the interhelical junction and excessive hydrogen-bonding interactions.



## 429 Conformational dynamics of a Tetracycline-binding RNA aptamer and its dependence on divalent metal ions investigated by pulsed EPR spectroscopy

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In recent years, Tetracycline-binding RNA aptamers have attracted a lot of attention, mainly because the aptamers are known to function as an artificial riboswitch even in living cells [1]. X-ray data and initial EPR experiments show that the RNA binds the antibiotic Tetracycline via a divalent  $Mg^{2+}$  or  $Mn^{2+}$  ion. Although a crystal structure has been published for the aptamer in the TC-bound state [2], there is still a need for further investigation of the conformational dynamics of the aptamer with other spectroscopic methods. [3]

In this study, we used two methods of pulsed Electron Paramagnetic Resonance spectroscopy to study structural dynamics of the TC-aptamer and how these dynamics are affected by  $M^{2+}$  concentration.

Pulsed Electron-Electron Double Resonance (PELDOR) [4] was used to obtain long-distance constraints of a doubly nitroxide-labeled TC-aptamer. The effect of different  $Mg^{2+}$  concentrations on the distance profile of the PELDOR experiment in the presence or absence of TC was evaluated and compared to data from the crystal structure. Our PELDOR measurements show that TC is required at intermediate  $Mg^{2+}$  concentrations in order to stabilize the native structure of the aptamer. At high  $Mg^{2+}$  concentrations, however, the aptamer is present in its native form, irrespective of the presence or absence of TC.

ELDOR-detected NMR (EDNMR) [5] was applied to study the binding of paramagnetic  $Mn^{2+}$  to NMR-active nuclei such as  $^{31}P$  and  $^{13}C$ . More specifically,  $^{13}C$ -enriched Tetracycline was used to monitor the binding of TC to the RNA via  $Mn^{2+}$ . EDNMR revealed that binding of TC to the RNA competes with the formation of a free  $Mn^{2+}$ -TC complex. To show that  $^{31}P$  signals of the RNA-backbone and  $^{13}C$  signals of  $^{13}C$ -TC interact with the same  $Mn^{2+}$  ion, 2D-EDNMR correlation experiment were performed [6].

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## 430 Cryo-EM structure of the 80S *C. elegans* ribosome

*Maja Holch Nielsen*<sup>1</sup>, *Rune Kidmose*<sup>1</sup>, *Alexander Myasnikov*<sup>2</sup>, *Bruno Klaholz*<sup>2</sup>, *Lasse Jenner*<sup>1</sup>

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The nematode *Caenorhabditis elegans* (*C. elegans*) has for many years been the model organism of choice to study molecular and cellular biology. Therefore its genetics and cellular and neuronal development has been extensively studied and are now well understood. One of the major challenges in molecular biology today is to understand how protein synthesis is controlled at the level of translation to regulate different cellular processes, including neuronal development.

In order to provide a structural platform linking cellular and developmental studies with regulation of translation, we set out to determine the structure of the 80S *C. elegans* ribosome.

Furthermore, a structure of the *C. elegans* ribosome could be a useful tool in the development of novel drugs against parasitic nematodes such as Hookworms, Ascariasis, and Trichuriasis. The World Health Organization (WHO) estimates that these parasitic nematodes infects between 2.0 and 2.7 billion people world wide, primarily in developing countries where sanitary conditions are poor. The nematode ribosome could be a good drug target in the treatment of such parasitic infections, just as the bacterial ribosome is the drug target of several antibiotics.

We will present the structure of the 80S *C. elegans* ribosome determined by cryo electron microscopy to a resolution of 4 Å. This relatively high-resolution structure shows the detailed architecture of the *C. elegans* ribosomes and allows us to identify differences and similarities with its yeast and mammalian counterparts.

**431 Genomic influenza RNA – secondary structure motifs and global folding**

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The outbreak of epidemics and pandemics of influenza virus is a constant threat for human health and even human life. Every year, around billion people suffer from seasonal flu, and three to five million undergo severe infections with a death toll of up to half million individuals. The influenza virus has a segmented RNA genome, consisting of eight segments of negative polarity. The segmented genome allows for undergoing genetic reassortments which give rise to dangerous pandemics. There is a need for gaining wider knowledge about the genomic RNA of influenza virus (vRNA). One important aspect to be studied further is its secondary structure.

Here, we present secondary structures of two genomic RNA segments for type A influenza. The structure models of vRNA7 and vRNA8 segments were proposed on the basis of chemical mapping, and then validated with the use of microarray mapping, RNase H cleavage and comparative sequence analysis. Possible structure rearrangements, which permit or exclude long-range RNA interactions for vRNA7, are also suggested. Several structural motifs of vRNA8 and vRNA7 are highly thermodynamically stable, overlapping with vRNA genome packaging signals, which could be connected with the regulation of virion assembly. Other structural motifs may be involved in blocking the host immunity, regulation of the replication and/or transcription, and more. Oligonucleotide probing with microarray mapping also provides a comprehensive view of the oligonucleotide-accessible regions in the genomic RNA of influenza virus, leading to new, oligonucleotide-based drugs, targeting vRNA.

**432 Study of Fox-1 and SRSF1 RRM - the molecular recognition and hydration**

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RNA Recognition Motif (RRM) proteins represent an abundant class of proteins which are widely conserved in eukaryotic genome. We use molecular dynamics (MD) simulations in conjunction with NMR spectroscopy to interpret and expand the available data on the Fox-1 and SRSF1 RRM which are implicated in several diseases affecting cell development. We show that several segments of the protein-RNA interface may involve competition between dynamical local substates rather than firmly formed interactions, which is indirectly consistent with the primary NMR data. We also describe a previously unobserved protein/RNA interaction in the SRSF1 RRM system.<sup>1</sup>

In second part of our work, we utilize X-ray and NMR structures of the Fox-1 RRM to study structured hydration. The MD excellently reproduces the most occupied hydration sites. Simulations of the protein/RNA complex show hydration consistent with the isolated protein complemented by hydration sites specific to the protein/RNA interface. We then characterize two of them using NMR spectroscopy and RNA binding with switchSENSE. Both hydration sites are experimentally confirmed and their abolishment reduces the binding free energy. Significantly, one of the hydration sites is evolutionarily conserved within the RRM domains genome. The MD is an effective tool for predicting and interpreting the hydration patterns of protein/RNA complexes, which are not easily detectable in experiments but which affect stability of protein/RNA complexes.

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### 433 Cooperative Folding of tRNA in Cellular Conditions Arises from Both Stabilization of Tertiary Structure and Destabilization of Secondary Structure

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RNA folding occurs in a largely hierarchical manner, where secondary structure forms before tertiary structure. The RNA folding process and the structures that RNA forms in vivo are not well understood, however. Several recent studies, including genome-wide efforts from our lab [1], have shown that certain classes of RNAs adopt very different structures in vivo than predicted in vitro or in silico. The conditions typically used to study RNA folding in vitro are highly non-physiological: dilute conditions with high salt concentrations often at 1 M Na<sup>+</sup> and/or 10 mM Mg<sup>2+</sup>. The conditions of the cell are very different. The predominant monovalent ion is 140 mM K<sup>+</sup>, there is only 0.5-1.0 mM Mg<sup>2+</sup> (eukaryotes) and 1.5-2.0 mM Mg<sup>2+</sup> (prokaryotes), and there is an estimated 20%-40% molecular crowding. In the work to be presented, the molecular mechanism behind tRNA<sup>phe</sup> folding in conditions that mimic the cellular environment is investigated using biophysical, structural, and RNA mapping methods on full length RNA and model oligonucleotides that represent secondary structure elements. Our results show that under dilute in vitro conditions tRNA folds in a non-cooperative manner, but under cellular ionic and crowding conditions it folds in a two-state, cooperative manner, much like small proteins. Strong secondary structure has been suggested to drive RNA folding, but surprisingly we observe that cellular conditions destabilize RNA secondary structures, contributing to cooperative RNA folding. Overall, two-state RNA folding in cellular conditions is achieved by a combination of stabilization of tertiary structure and destabilization of secondary structure.

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### 434 Data-directed RNA ensemble analysis

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RNA is a versatile molecule that plays a central role in diverse cellular functions, such as gene expression, gene regulation, catalysis, antiviral defense, and environmental sensing. Its broad functional spectrum often critically depends on its ability to fold into specific structures and/or interconvert between structures. Determining an RNA's three-dimensional structure is the ultimate goal in understanding its biological function and predicting its secondary structure is typically a first step in such endeavors. Experimental methods to analyze structure, such as crystallography and nuclear magnetic resonance, are reliable but expensive, time-consuming, and limited in their applicability. As alternative approach, computational methods have advanced our ability to predict secondary structures. Yet, their performance is still generally poor when using sequence information alone. Additional structural information can also be gleaned with structure probing, from which computational approaches can greatly benefit through its proper interpretation and use. Nowadays, data-directed structure prediction approaches are providing valuable insights via combining computation and structure probing data. However, these methods are limited in that they predict a single structure and do not account for more complex dynamics where multiple co-existing structures are relevant to function. Many RNAs, such as ribozymes and riboswitches, rely on transitions between several states for their function, and in fact, these are special hallmarks of biological function rather than an intrinsic feature of generic RNA sequences.

The objective of our work is to develop a framework for analyzing RNA structural dynamics, which has the potential to enhance our understanding of RNA biology and advance us towards efficient discovery and predictable design of RNAs for therapeutic and biotechnology applications. Our approach utilizes statistical modeling to predict multiple dominant RNA secondary folds, taking advantage of chemical probing data. One key feature of our framework is that it is flexible to incorporate many recent types of chemical probing data, for example, SHAPE-Seq, SHAPE-MaP, DMS-Seq and DMS-MaP. Furthermore, our method accommodates pseudoknoted folds and could be extended to predict three-dimensional folds. We demonstrate our approach using simulations.

**435 Human DDX21 binds and remodels RNA guanine quadruplexes**Ewan McRae<sup>1</sup>, Evan Booy<sup>1</sup>, Aniel Moya-Torres<sup>1</sup>, Jörg Stetefeld<sup>1,2</sup>, Sean McKenna<sup>1,2</sup><sup>1</sup>University of Manitoba Department of Chemistry, Winnipeg, Manitoba, Canada; <sup>2</sup>Department of Biochemistry & Medical Genetics, Manitoba Institute for Materials, Winnipeg, Manitoba, Canada

Guanine quadruplexes (G4s) are an important structure of nucleic acids (DNA and RNA) with roles in several cellular processes. RNA G4s require specialized remodeling enzymes, of which only two have been previously identified. We describe the results of a simple and specific mass spectrometry guided method used to screen HEK293T cell lysate for G4 binding proteins. From these results, we validated the RNA helicase protein DDX21. DDX21 is an established RNA helicase, but has not yet been validated as a G4 binding protein. Through biochemical techniques, we confirm that DDX21-quadruplex RNA interactions are direct and mediated via a site of interaction at the C-terminus of the protein. Furthermore, we show that DDX21 can remodel RNA G4 through monitoring changes in nuclease sensitivity. Finally, as proof of principle, we demonstrate the ability of DDX21 to suppress the expression of a protein with G4s in the 3' UTR of its mRNA.

**436 Modeling RNA Interactions with SHAPE Reagents**Vojtech Mlynsky, Richard A. Cunha, Giovanni Bussi

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Chemical probing methods are routinely used in combination with nearest neighbor models to determine RNA secondary structure. In particular, selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE) is receiving significant attention due to its capability to probe all nucleotides [1] and to the possibility to be used *in vivo* [2,3]. The usual presumption is that unpaired nucleotides should be considered as flexible, and thus reactive. We here use molecular dynamics simulations and enhanced sampling techniques [4] to analyze the accessibility of paired and unpaired nucleotides to SHAPE reagents. RNA flexibility in several structural motifs is taken into account and it is shown to affect the affinity for reagents. Results are consistent with experimental SHAPE data measured on different stem-loop structures. Our protocol is able to distinguish between nucleotides based on their structural predispositions towards the acylation reaction. The method can be used for interpretation of previously unexplained reactivity patterns and provides the initial step for fast and cheap classification of alternating RNA structures.

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**437 Determination of the HTLV-1 *pro-pol* Frameshift Site Secondary Structure**

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An estimated ten million people are infected with human T-cell lymphotropic virus type I (HTLV-I). Approximately 5% of these infections are associated with adult T-cell leukemia/lymphoma, an aggressive form of leukemia/lymphoma. Establishment of new HTLV-I infections is dependent upon the reverse transcription of the viral positive sense RNA genome and the integration of the resulting DNA into the host genome. Both processes require viral enzymes that are expressed by way of two independent one nucleotide (-1) programmed ribosomal frameshifts (PRF). These events occur at the *gag-pro* and *pro-pol* open reading frames junctions and are stimulated by *cis*-acting RNA elements within the viral transcript. Each frameshift site includes a heptanucleotide slippery sequence followed by a downstream structure. While -1 PRF and the slippery sequence of the *pro-pol* frameshift site has been established in HTLV-I, its structure has not been experimentally determined. Here, we report a preliminary secondary structure of the HTLV-1 *pro-pol* frameshift site RNA. Selective 2'-hydroxyl acylation experiments analyzed by primer extension were used to measure the HTLV-I *pro-pol* frameshift site nucleotide reactivities. This data was combined with RNAstructure to generate a preliminary secondary structure, which is consistent with the pseudoknot structure previously predicted using solely computational methods. Experiments to evaluate the importance of the pseudoknot structure to frameshift site function are underway. At this time, we report an *in vitro* frameshift efficiency of 14(±1)% for the *pro-pol* frameshift site. Interestingly, a six- to ten-fold decrease in frameshifting is observed when the putative pseudoknot structure is disrupted. The reduction in frameshift efficiency observed suggests that a more complex downstream structure, likely a pseudoknot, is required to achieve optimal levels of frameshifting.

**438 Structural studies of the T box riboswitch and its tRNA ligands**

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Over the past decade, RNA-mediated regulation has emerged as a central theme in gene expression. A common control mechanism of Gram-positive bacteria is the riboswitch. In riboswitches, a sensor domain is mechanistically coupled to a regulatory domain composed of mutually exclusive terminator/anti-terminator RNA structure elements that either attenuate or increase translation/transcription. A particularly widespread type of riboswitch is the “T box”, a tRNA-actuated switch whose default state is to attenuate transcription of essential genes encoding aminoacyl-tRNA synthetases, amino acid biosynthetic enzymes, and amino acid transport machinery. High ratios of charged-to-uncharged tRNA maintain the switch in the off state and suppress gene expression. The performance of the riboswitch is subject to modulation by multiple factors, including nucleotide sequence variations around conserved structure elements and tRNA base modification, and is crucial to the fitness of the cell.

We are using a variety of biophysical methods (SAXS, NMR, ITC) to identify the global conformations and structural details of model tRNA-riboswitch complexes and to develop a binding model of the T box riboswitch mechanism that includes variables such as tRNA modification and tRNA aminoacylation state. Progress in these studies will be presented along with results from cellular studies examining the functional role of nucleotide base modification in T box riboswitch function.



### 439 rnaCARD - software for identification of conformational rearrangements in alternative RNA secondary structures

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Current high-throughput methods of RNA probing enables transcriptome-wide analysis of RNA secondary structures in various experimental conditions (*e.g. in vivo, in vitro* or in temperature gradient) (Spitale *et al.* 2015; Wan *et al.* 2012). Conformational flexibility of RNA molecules allows condition-dependent dynamic rearrangement of secondary and tertiary structures. This feature is exploited by different regulatory RNAs, *e.g.* riboswitches, whose mechanism of action is solely based on structural alterations. Thus, identification of condition-dependent structural changes of the RNA molecules could reveal novel RNA regulatory mechanisms.

Here, we present a new software, rnaCARD, aimed at identification of local RNA domains, which undergo condition-specific conformational rearrangements. This goal is achieved by analysis of alternative structures of RNA obtained *e.g.* by structure probing experiments. In order to enable focus on changes in general shape of RNA domains, excluding minor structural variations, rnaCARD utilize the abstract shapes notation of RNA structure (Giegerich *et al.*, 2004). The predicted structural rearrangements of RNA domains are validated using the provided chemical probing data. The result is a comprehensive lists of structural RNA domains, which reveal switching potential across analyzed conditions. It is also possible to obtain a list of domains for which the structure is condition-independent. rnaCARD is applicable to any set of alternative RNA foldings, derived either from probing experiments, crystal structures or other computational predictions.

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### 440 Combining molecular dynamics and NMR spectroscopy to characterize structure and dynamics of an RNA hairpin from an inverted SINEB2

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An inverted SINEB2 (invSB2) element embedded in a non-coding transcript has been identified as crucial for the enhancement of mRNA translation under cellular stress [1]. The transcript contains a sequence complementary to the target mRNA at its 5' end, and an invSB2 element at its 3' end, which performs the translation-enhancing function. A 29 nt RNA hairpin within invSB2 has been identified to be important for this process. To reveal the tertiary structure of this element, NMR experiments have been performed and 125 NOE signals have been recorded, which were translated into maximal average distances between hydrogen pairs throughout the molecule [2].

Based on these data, a set of three-dimensional structures was refined which was then used as a starting point for molecular dynamics (MD) simulations. In a replica exchange MD with 20 replicas, the Hamiltonian (*i.e.* electrostatic, van-der-Waals, and dihedral potentials) of the hairpin was gradually scaled down to enhance sampling, while adaptive restraints according to the maximum entropy principle were applied to satisfy the NOE distances as an average over the unbiased simulation, *i.e.* the replica with unscaled Hamiltonian [3]. The resulting ensemble had an improved agreement with the NOE restraints, compared with the initially refined set of structures. A clustering analysis based on the  $\epsilon$ RMSD - a metric which measures structural similarity by analyzing base-pairing and stacking interactions [4], rather than only absolute atomic coordinates like the RMSD - yielded several conformational states, some of which had shifted base pairs with respect to the initial set of structures. Averaging over the structures within one conformational cluster, it became evident that some NOEs are satisfied in specific clusters, while not in others, leading to the conclusion that the NOE signal represents an average over different conformations exchanging rapidly on a timescale below what can be resolved by NMR. Using our approach, the population of these excited states could be quantified, and the single conformations can be studied towards their structure-function relationship.

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#### 441 The effect of 5-methyl cytosine on RNA duplex stability

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Thermodynamic parameters for 5-Methylcytosine-guanosine (5MeC-G) pairs are important for predicting the secondary structures of RNA and for finding genomic sequences that code for structured RNA. Optical melting curves were measured for RNA duplexes with 5MeC-G pairs in all possible nearest neighbor environments to improve the prediction of stability of helices containing 5MeC. Duplexes where a 5MeC-G is substituted for a C-G base pair are on average slightly less stable, by 0.2 kcal/mol, on average. Nearest neighbor parameters for 5MeC-G base pairs derived from the data display a wider range of values (0.6-5.0 kcal/mol) than are observed for C-G base pairs (2.11-3.26 kcal/mol). On average, the nearest neighbor values for 5MeC-G base pairs 5' of a Watson-Crick base pair are 1.6 kcal/mol less stable; while a 3' 5MeC-G Watson-Crick nearest neighbor pair is 1.3 kcal/mole more stable than the corresponding C-G Watson-Crick nearest neighbor pair. A 5'MeC-G terminal base pair destabilizes a helix by 0.9 kcal/mol while a 3'MeC-G terminal base pair stabilizes a helix by 1.4 kcal/mol. These results should improve predictions of secondary structure for RNAs with modified base pairs.

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#### 442 Mapping recurring RNA structures to navigate the uncharted genome

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Understanding how the non-protein coding genome functions is essential to improve our understanding of normal biology and disease, as exemplified by the 4:1 ratio of disease-associated mutations arising in non-coding vs. protein coding regions in the human genome. Despite their ever increasing prevalence in reference transcriptomes, relatively few lncRNAs have been functionally characterised to date. All ncRNAs form higher-order structures via complementary base-pairing. We have previously identified the evolutionary hallmarks of functional RNAs in over 18% of the human genome by leveraging the mutational flexibility of structured RNAs. This approach has since been combined to *in vivo* RNA-RNA interaction data to enable the full length characterisation of lncRNA structures transcriptome-wide, helping resolve the structural topology of the well-studied lncRNA XIST and novel insights into its molecular function, amongst others.

In order to assign function to RNA structure predictions, we have refined a pipeline for the discovery of recurring structures in RNA-protein binding data. At its core lies DotAligner, a new lightweight RNA base-pairing probability matrix alignment heuristic that can classify RFAM sequences with exceptional precision and speed. DotAligner's performance enables large-scale and computationally tractable clustering of thousands of RNA structures through all-vs-all pairwise comparisons. We applied DotAligner to public ENCODE eCLIP data for 44 RNA-binding proteins and the evolutionary conserved structure predictions mentioned above. The resulting clusters of structural motifs can then be used to generate covariance models and scan the genome for homology.

Our results reveal thousands of new hits, increasing the genomic coverage of the original queries by 150x and supporting the modularity of lncRNA exon structure. We expect that this work will facilitate the attribution of specific biological functions to lncRNAs in a systematic manner.

**443 Structures of riboswitch RNA reaction states by mix-and-inject XFEL serial crystallography**

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Riboswitches are RNA structural elements generally located in the 5' untranslated region (3'UTR) of mRNA. In the genetic regulation, ligand binding to the aptamer domain of a riboswitch triggers a signal to the downstream expression platform. A complete understanding of the structural basis for this mechanism requires the ability to study structural changes over time. We apply femtosecond X-ray free electron laser (XFEL) pulses to obtain structural measurements from crystals so small that diffusion of a ligand can be timed to initiate a reaction prior to diffraction. We demonstrate this approach by determining four structures of the adenine riboswitch aptamer domain during the course of a reaction involving two apo, one ligand-bound intermediate, and the final bound states. These structures support a reaction mechanism model with at least four states and illustrate the structural basis for signal transmission. The two apo conformers differ significantly in the three-way junction and the P1 switch helix relative to the ligand-bound conformation. Our time-resolved crystallographic measurements with a 10-second delay captured the structure of an intermediate with changes in the binding pocket that accommodate the ligand. With a >10-minute delay, the RNA molecules were fully converted to the bound state, in which the substantial conformational changes resulted in conversion of the space group. Such drastic changes *in crystallo* highlight the important opportunities that micro/nanocrystals may offer in these and similar time-resolved diffraction studies. These results all together demonstrate the potential of "mix-and-inject" time-resolved serial crystallography to study biochemically important interactions between biomacromolecules and ligands, including those involving large conformational changes.

**444 CryoEM on small RNAs**

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RNAs remain underexplored by single particle electron cryo-microscopy (cryoEM), mostly due to their intrinsic conformational variability for relative large RNAs, and low contrast and orientation misalignment for more rigid but smaller RNAs. Here we present our recent progress of cryoEM studies approaching nanometer resolution on a variety of small RNAs ranging from 30 to 80 kDa with highest resolution at 9 Angstrom. Our studies demonstrate the potential of cryoEM towards determining high resolution structures of small RNAs, as well as providing structural information that is otherwise not accessible by NMR and X-ray crystallography.

#### 445 Structural dynamics of Kaposi sarcoma herpesvirus polyadenylated long noncoding RNA in living cells and virions

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Polyadenylated nuclear (PAN) noncoding RNA of Kaposi's sarcoma-associated herpesvirus (KSHV) facilitates lytic infection and modulates the cellular immune response. Although a number nucleoprotein interactions involving PAN RNA have been shown to play a role in KSHV replication, binding partners and binding sites in these complexes, as well as the structure of PAN itself, remain poorly understood. We used selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) together with complementary methods of data analysis to probe PAN RNA in nuclear and cytoplasmic compartments of living cells and within viral particles. We have thus characterized and put into context discrete RNA structural elements, including the *cis*-acting Mta responsive element (MRE) and expression and nuclear retention element (ENE).

PAN RNA was exposed to the acylating reagent in its native nuclear, cytoplasmic or viral compartment (*in cellulo*, *in virio*) or following cell/virion lysis and removal of proteins by organic extraction (*ex vivo*, *ex virio*). By comparing the mutational profiles from these respective sets of experiments, we identified sites on PAN RNA either protected by protein binding or characterized by a loss of structure mediated by transient protein interactions or the intra-compartmental ionic environment. Some of the protein binding sites were localized to only one or two of these compartments while others appear to be occupied in all three. For example, the MRE sequence was found to be occupied exclusively in PAN isolated from the nucleus, suggesting a compartment-specific role for the ORF57 protein-MRE.

Individual binding sites of select KSHV gene products on PAN RNA were also identified using purified recombinant KSHV proteins that were incubated with *in vitro* transcribed, polyadenylated PAN RNA and subjected to SHAPE-MaP analysis. Results indicate that some segments of PAN RNA serve as shared docking sites for several KSHV proteins, while others are bound by only one or a few. This work constitutes the most extensive structural characterization of a viral lncRNA and its interactions with various protein partners in discrete cellular and viral compartments, and as such creates a broad framework for understanding the functions of PAN RNA in KSHV infection at a sub-molecular level.

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#### 446 Size of long RNA molecule in dilute solution is determined by the branching pattern of secondary structure

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Long RNA molecules are involved in gene expression and regulation on multiple levels, as well as serving as genetic material in a large class of ssRNA viruses. In contrast to their shorter counterparts, e.g., ribozymes, few studies have addressed the basic physical properties of long RNAs. Long RNA sequences (with heterologous, non-repeating subsequences) usually adopt highly ramified secondary structures and, accordingly, it is useful to describe them as effectively branched polymers. In order to test whether a simple branched polymer model can estimate the overall size of large RNA we employed fluorescence correlation spectroscopy to examine the dilute-solution sizes of a broad spectrum of biologically important RNAs, ranging from viral RNA genomes to long non-coding regulatory RNAs. The sizes of long RNAs measured at low ionic strength conditions correspond well to those predicted by two theoretical approaches accounting for the effective branching of these molecules. The first approach estimates the RNA size from the maximum ladder distance, mapping each branched structure onto an effective linear polymer, while the second employs Kramers' theorem to calculate directly the radius of gyration. Divalent and trivalent cations cause further RNA compaction in proportion to the original size, suggesting that in the absence of binding proteins long RNA molecules adopt highly branched configurations with their relative size dictated by the branching pattern of secondary structure.

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**447 Agarose binding RNA aptamer***So Umekage<sup>1</sup>, Yo Kikuchi<sup>2</sup>*<sup>1</sup>Toyohashi Tech., Toyohashi, Japan; <sup>2</sup>Waseda Univ., Tokyo, Japan

Our research group succeeded in obtaining an 82 nt of agarose binding RNA aptamer by using the systematic evolution of ligands by exponential enrichment (SELEX) method. This aptamer bound to both sol state and gel state agarose. Also, this aptamer showed affinity for some agarose related compounds such as low-melting agarose, Sepharose CL-4B and carrageenan. Estimated pseudoknot structure by the VS fold program was confirmed by both nuclease mapping analysis and mutational analysis. In addition, the predicted pseudoknot structure was protected against nuclease attacks in the presence of agarose. Therefore, we conclude that pseudoknot structure of the aptamer is essential for agarose binding. Furthermore, we also showed that the agarose aptamer could be separated by using agarose beads column from a tRNA contaminated mixture.

**448 Structure of CUG triplet repeat expanded RNA in myotonic dystrophy type 1***Remco T.P. van Cruchten, Bé Wieringa, Derick G. Wansink*

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Myotonic dystrophy type 1 (DM1) is a neuromuscular disorder affecting approximately 1 in 8,000 people. Muscular, neurological and endocrine symptoms in DM1 are caused by expansion of a CTG repeat in the 3' UTR of the DMPK gene. Repeat lengths >50 triplets are considered pathogenic whereas the general population carries 5-37 CTGs. The structurally altered expanded DMPK mRNA has toxic effects, caused by formation of a CUG hairpin which abnormally binds RNA-binding proteins and may also have other anomalous effects. Of specific interest is a potential G-quadruplex, a structure commonly associated with protein binding and regulation of translation, which is located near the repeat.

Our goal is to determine what effect repeat length has on the folding and topology of the DMPK transcript, and ultimately to know how altered RNA structure contributes to DM1 pathology.

We applied Selective 2' Hydroxyl Acylation analyzed by Primer Extension (SHAPE) on in vitro transcribed DMPK RNA with variable CUG repeat lengths, and performed structure probing using NAI, which modifies flexible, non-basepairing nucleotides. Besides, we measured G-quadruplex formation by the induction of reverse transcription stops under influence of K<sup>+</sup> and pyridostatin, a G-quadruplex stabilizing ligand.

By probing the 3' UTR region of DMPK transcripts (~1 kb) containing 5, 38, 69 or 147 CUG triplets we confirmed that a segment containing 5 CUGs does not form a hairpin. A hairpin is formed in all transcripts carrying 38 or more CUG triplets. Moreover distinct other structural features in adjacent segments occurred only in the transcript carrying 147 CUG triplets. We have also shown that a G-quadruplex structure is formed in the DMPK transcript and that this structure is moderately influenced by length of the repeat.

Our in vitro data suggest that the folding of the DMPK transcript may be an important parameter in the disease etiology of DM1. More in depth knowledge will give valuable leads for development of therapeutics such as antisense oligonucleotides. Currently we are expanding our view by probing the DMPK RNA structure in DM1 patient-derived muscle cells



## 449 Inducible Pseudouridylation Modulates the Structural Equilibrium of Stem II of the Yeast U2 snRNA

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During pre-mRNA splicing, the U2 snRNA transitions between multiple conformations and inhibition of these transitions can cause changes in splicing. We are particularly interested in two, mutually-exclusive U2 structures formed by nucleotides immediately downstream of U2's branchpoint recognition region, stem IIa and stem IIc. Stem IIa is essential for spliceosome assembly and rearrangement of the spliceosome active site, while stem IIc formation is important for the catalytic steps in splicing. Stress-induced pseudouridylation occurs in the stem II region and has been shown to result from either heat stress or nutrient deprivation. Utilizing single molecule FRET, we show that the pseudouridylation of specific nucleotide positions in stem II affects the *in vitro* equilibrium between stem IIc and a set of conformations consistent with stem IIa formation. The impacts of the induced pseudouridines are not identical.  $\Psi 56$  is primarily induced by heat stress and favors stem IIc formation.  $\Psi 93$ , which along with  $\Psi 56$  is induced by nutrient deprivation, favors stem IIa conformations when compared with the non-pseudouridylated RNA. Observations from single molecule experiments agree with RNA structural probing of stem II with and without pseudouridines at these positions.

In addition, these pseudouridine modifications also impact how the stem II region switches conformation in response to  $Mg^{2+}$  or the U2 protein, Cus2. With the addition of Cus2 or  $Mg^{2+}$  during smFRET experiments, wild type stem II transitions out of the stem IIc conformation. In comparison, the response of stem II modified by  $\Psi 56$  is less robust, suggesting that spliceosome assembly may occur less efficiently during heat stress. Stem IIa consistent conformations are still observed in the presence of Cus2 or  $Mg^{2+}$  in  $\Psi 93$ -containing RNAs. These results suggest that post-transcriptional modifications impact both structural equilibrium of U2 stem II as well as its interactions with both proteins and metal ions and potentially provide a mechanism for precisely modulating splicing activity of the cell under certain growth conditions.

## 450 The Structure of a GTP-bound Aptamer Reveals a Complex RNA-Fold and a Stably Protonated Adenine

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The GTP class II aptamer is one of 11 structurally and sequentially diverse GTP-binding RNA aptamers. Despite its small size (34 nt) it binds GTP with a  $K_D$  in the nanomolar range<sup>[1]</sup>. We initiated structural studies of the aptamer GTP-complex by solution NMR in order to gain insight into the structural diversity of GTP recognition by different aptamers<sup>[2]</sup>.

We solved the structure of the RNA-GTP complex by solution NMR with a low overall rmsd (0.5 Å). GTP binds to the apical loop of the aptamer and is recognized in a base triplet containing an intermolecular Watson-Crick base-pair and a sugar edge interaction with an adenine. The base of the ligand binding site consists of an intramolecular base triplet with a similar architecture. A base quartet is located adjacent to this intramolecular base triplet, where a guanosine imino group forms a hydrogen bond to a phosphate group oxygen. Moreover, an adenosine is stably protonated at its N1 position with a NMR-observable imino proton resonance. The  $pK_a$  for the protonation is  $\sim 9.0$  and thus shifted by more than five pH units compared to free adenosine (3.7). Interestingly, the free RNA exists as a partially pre-folded conformational ensemble already containing the protonated adenine and the base quartet at pH 5.3 in the presence of  $Mg^{2+}$ . This suggests that the ligand selects the prefolded, protonated state of the aptamer for binding and stabilizes its structure even further. Structural and thermodynamic studies of several mutants and ligand analogs show the role of individual residues for the intricate structure and the interplay of hydrogen bonding interactions of the RNA-ligand complex, respectively.

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**451 The analysis of structures of small RNAs bound by *Escherichia coli* ProQ protein***Zuzanna Wroblewska, Ewa Stein, Mikolaj Olejniczak***Adam Mickiewicz University, Poznan, Poland**

Bacterial small non-coding RNAs (sRNAs) participate in cell adaptation to changing environmental conditions, maintenance of cellular homeostasis and regulation of DNA metabolism. They affect gene expression by pairing with sequences of partial (*trans*-encoded sRNAs) or perfect (*cis*-encoded sRNAs) complementarity in regulated mRNAs. The outcomes of sRNA pairing to 5'-UTR or the coding sequence are changes in mRNA translation or stability. The regulation by *trans*-encoded sRNAs is often dependent on a matchmaker protein Hfq. Until recently, this homohexameric ring-shaped protein was considered as the main chaperone affecting sRNA interactions with target mRNAs in *Escherichia coli* and *Salmonella enterica*. However, recent studies showed that an abundant and constitutively expressed FinO-domain protein ProQ binds a wide spectrum of RNA molecules, including many *cis*-encoded sRNAs. It was proposed that ProQ-bound sRNAs are more structured than those recognized by Hfq.

Here, the secondary structures of three ProQ-recognized *Escherichia coli* sRNAs, SibA, SraL, and IsrB, were analyzed to better understand the structural differences between sRNAs bound by ProQ and by Hfq. For *in vitro* structure probing single-strand specific and double-strand specific RNases were used. The results showed that these three sRNAs formed extensively base-paired structures. Further studies are planned to compare other ProQ-dependent sRNAs and to analyze the effects of ProQ binding on their structures.

**452 Following RNA splicing in vitro by single-molecule FRET***Susann Zelger-Paulus, Mélodie Hadzic, Besim Fazliji, Richard Börner, Roland Sigel***University of Zurich, Zurich, Switzerland**

Self-splicing group II introns are very interesting examples of catalytically active RNAs where structure and function are inextricably linked with each other.

We are investigating the complex RNA folding of the mitochondrial group II intron ai5y from *Saccharomyces cerevisiae* and monitor its self-cleavage reaction by single-molecule Förster Resonance Energy Transfer (smFRET). For this purpose, we are fluorescently labelling the RNA at various positions; within the intron, at its flanking exons and cross-wise.[1] While RNA splicing in vivo is promoted by proteins, self-excision in vitro on the other hand requires higher concentrations of salt and elevated temperature. We are encapsulating the group II intron into surface-immobilized vesicles and initiate the splicing process by increasing the temperature in situ on the microscope. In this way, we can follow the transition of an inactive partially folded ribozyme towards its cleavage competent fold on a single-molecule level.[2]

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**453 Recent Force Fields for RNA Simulations - Performance, Comparison and Challenges**Marie Zgarbova<sup>1</sup>, Petr Jurecka<sup>1</sup>, Pavel Banas<sup>1</sup>, Marek Havrila<sup>2</sup>, Jiri Sponer<sup>1,2</sup>, Michal Otyepka<sup>1</sup><sup>1</sup>Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Palacky University Olomouc, Olomouc, Czech Republic; <sup>2</sup>Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

The sugar-phosphate backbone of RNA can exist in diverse rotameric substates, giving RNA molecules enormous conformational variability. For molecular modeling of biomolecules, it is important to know how well various RNA conformations are described by the available molecular mechanics RNA force fields.

Here, rotameric conformational substates including noncanonical  $\alpha/\gamma$  backbone dihedral angle combinations were studied in several prototypical RNA structures, such as canonical and mismatched RNA duplexes, UUCG and GAGA tetraloops, Loop E, the sarcin-ricin loop, a parallel guanine quadruplex, and a viral pseudoknot. Molecular dynamics simulations of the selected NMR structures and well-resolved X-ray structures were performed using several force fields, including the currently recommended ff99bsc0 $\chi$ OL3 and a combination of  $\chi$ OL3 with our recent modifications for DNA,  $\chi$ OL3 +  $\epsilon\zeta$ BOL1. Although some substates were stable with seemingly well-described equilibria, many were unstable in our simulations. Possible reasons for this instability are discussed. Our work reveals a potentially important artifact in RNA force fields and highlights a need for further force field refinement.

**454 Structural mechanisms of amino acid sensing on the tRNA by the T-box riboswitches and Gcn2 kinase**Shuang Li<sup>1</sup>, Zhaoming Su<sup>4</sup>, Soheila Bahmanjah<sup>1</sup>, Lixin Fan<sup>3</sup>, Alan Hinnebusch<sup>2</sup>, Wah Chiu<sup>4</sup>, Jinwei Zhang<sup>1</sup><sup>1</sup>NIDDK, National Institutes of Health, Bethesda, USA; <sup>2</sup>NICHD, National Institutes of Health, Bethesda, USA; <sup>3</sup>National Cancer Institute, Frederick, USA; <sup>4</sup>Baylor College of Medicine, Houston, USA

Amino acids are the most ubiquitous nutrients in life, whose levels are monitored and maintained by diverse mechanisms. In contrast to protein (e.g. Sestrin2, TRAP) and RNA (e.g. riboswitches) devices that sense free amino acids, amino acid availability can also be sensed in the form of aminoacyl-tRNAs, as exemplified by the T-box riboswitches in Gram-positive bacteria and the Gcn2 kinase in eukaryotes. T-boxes are noncoding RNAs in the 5' untranslated regions that regulate downstream amino-acid metabolic genes [1]. T-boxes share a phylogenetically conserved architecture comprised of two domains — a 5' Stem I and 3' antiterminator domain [2,3]. Stem I provides a flexible platform that selectively docks a cognate tRNA via sequence- and structure-specific interactions [4-6]. The antiterminator domain interacts with the 3' region of the docked tRNA, and probes the molecular volume of its 3' end to sense aminoacylation [7]. The presence or absence of aminoacylation dictates the formation of either an intrinsic transcription terminator or antiterminator, respectively. The overall architecture of a full T-box complex and interactions between the tRNA 3' region and the antiterminator remain poorly defined.

Using phylogenetics, Small-angle X-ray Scattering (SAXS), single-particle cryo-electron microscopy (cryo-EM), we propose a structural model of a complete T-box riboswitch in complex with its cognate, uncharged tRNA. These analyses support the notion that the intermolecular coaxial stacking between the tRNA and an antiterminator helix is responsible for antiterminator stabilization and transcription readthrough.

In eukaryotes, Gcn2 employs a sensory domain that shares sequence homology with Histidyl-tRNA synthetases (HisRS) to evaluate tRNA aminoacylation and govern the starvation response [8]. We determined the molecular envelope of the HisRS-like and CTD domains of Gcn2. This shows that the tRNA-sensory domain of Gcn2 structurally resemble authentic HisRS enzymes.

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## 455 Genome-scale characterization of RNA tertiary structures and their functional impact by RNA solvent accessibility prediction

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As most RNA structures are elusive to structure determination, obtaining solvent accessible surface areas (ASA) of nucleotides in an RNA structure is an important first step to characterize potential functional sites and core structural regions. Here, we developed RNAsnap, the first machine-learning method trained on protein-bound RNA structures for solvent accessibility prediction. Built on sequence profiles from multiple sequence alignment (RNAsnap-prof), the method provided robust prediction in five-fold cross-validation and an independent test (Pearson correlation coefficients,  $r$ , between predicted and actual ASA values are 0.66 and 0.63, respectively). Application of the method to 6178 mRNAs revealed its positive correlation to mRNA accessibility by dimethyl sulphate (DMS) experimentally measured *in vivo* ( $r=0.37$ ) but not *in vitro* ( $r=0.07$ ) despite the lack of training on mRNAs and the fact that DMS accessibility is only an approximation to solvent accessibility. We further found strong association across coding and noncoding regions between predicted solvent accessibility of the mutation site of a single nucleotide variant (SNV) and the frequency of that variant in the population for 2.2 million SNVs obtained in the 1000 Genomes Project. Moreover, mapping solvent accessibility of RNAs to the human genome indicated that introns, 5' cap of 5' and 3' cap of 3' untranslated regions are more solvent accessible, consistent with their respective functional roles. These results support conformational selections as the mechanism for the formation of RNA-protein complexes and highlight the utility of genome-scale characterization of RNA tertiary structures by RNAsnap. The server and its standalone downloadable version are available at <http://sparks-lab.org>.

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## 456 Biogenesis and biotechnology of circular RNAs generated by tRNA splicing

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Mature tRNAs are generated by multiple post-transcriptional processing steps, which can include removal of introns. Recently, our lab discovered a new class of circular RNAs formed by intramolecular ligation of excised tRNA introns in animal cells. We term these molecules tRNA intronic circular (tric)RNAs. We have found tricRNAs to be stable, highly abundant, and evolutionarily conserved.

To investigate the *cis*-acting elements and *trans*-acting factors required for tricRNA biogenesis, we generated reporters that contain aptamer-tagged insertions within the introns of *Drosophila* and human tRNA genes. Using the Broccoli fluorescent RNA aptamer tag, we found that a stem of at least eight nucleotides is necessary for efficient splicing and intron circularization. We also found that point mutations disrupting the helix portion of the bulge-helix-bulge motif, which is a conserved feature of pre-tRNAs, result in reduced tricRNA production. Additionally, we show that several known tRNA processing factors, such as the RtcB ligase and components of the TSEN endonuclease complex, are also involved in tricRNA biogenesis. Depletion of these factors reduces both mature tRNA and tricRNA levels.

Thus, human cells possess the machinery to make tricRNAs, enabling the possibility of developing tricRNAs as an *in vivo* circular RNA expression system. Using external RNA polymerase III promoters to optimize engineered tricRNA expression, we can express functional proteins from tricRNAs using an internal ribosome entry site (IRES). Indeed, using a Gaussia luciferase reporter construct, we observed strong induction of luciferase activity. Mutations that affect tricRNA splicing (but not pre-tRNA stability) resulted in dramatically reduced luciferase levels. These results indicate that most, if not all, of the translation is derived from the circle rather than the pre-tRNA. Additionally, mutations in sequences that affect IRES-dependent translation will be discussed. These and other results establish tRNA-based vectors as a method for robust circular RNA expression with a wide variety of potential uses.



**457 Identifying the factors required for local translation of pheromone "a" in mating yeast***Stella Aronov, Polina Geva, Einav Halperin***Ariel University, Ariel, Israel**

Polarization of certain cell types in response to external signals is essential for the reproduction, development and viability of eukaryotic organisms. The mating yeast, *Saccharomyces cerevisiae*, is an excellent model system for studying polarized growth. Mating occurs when two haploid cells with develop in an asymmetric fashion in response to opposite phenotypes MAT $\alpha$  and MAT $a$  to " $\alpha$ " and " $a$ " pheromones ( $\alpha$ F and  $a$ F, respectively). Mating efficiency depends on expression of high pheromone levels. The biogenesis and secretion of  $\alpha$ F is through the "classic" secretory pathway encoded by two redundant genes MFA1 and MFA2, while the mechanism of " $a$ " pheromone ( $a$ F) translation remains unclear. We recently showed that one of the two redundant genes encoding  $a$ F, MFA2, uses a mRNA transport mechanism. Its transcript is asymmetrically delivered to the yeast cell's growth site – the shmoo projection – where it is locally translated, providing local high expression levels of  $a$ F. We also showed that MFA2 mRNA transport to the shmoo tip requires processing bodies (PBs), cytoplasmic structures responsible for storage and sorting of translationally-repressed mRNAs.

Here we show that MFA2 mRNA localization and stability require cis-regulatory elements that are present in the 3' untranslated region (3'UTR) of the transcript. We also found that deleting some of the RNA binding proteins (RBPs) that are known to play a role in RNA biogenesis of budding yeast, namely, Sec3, Loc1 and Scp160, also significantly reduced the localization of MFA2 mRNA as well as the efficiency of mating. In addition, we showed that MFA2 mRNA localization to the shmoo requires an intact actin cytoskeleton and endoplasmic reticulum (ER) to play the functional role in MFA2 mRNA biogenesis. Our work is innovative because it shows, for the first time, the role of regulatory factors in MFA2 mRNA localization, local protein synthesis and the mating process.

**458 Sharing the load: the ability of mRNA exporter Mex67-Mtr2 to function in tRNA nuclear export***Kunal Chatterjee<sup>1</sup>, Shubhra Majumder<sup>1</sup>, Vijay Shah<sup>1</sup>, Yao Wan<sup>1</sup>, Jingyan Wu<sup>2</sup>, Anita K. Hopper<sup>1</sup>***<sup>1</sup>The Ohio State University, Columbus, Ohio, USA; <sup>2</sup>Stanford University, Stanford, California, USA**

tRNAs perform essential role of delivering amino acids to the cytoplasmic protein synthesis machinery. To execute this role in protein production, eukaryotic tRNAs have to be escorted out of the nucleus, their site of synthesis, to the cytoplasm, their site of function. By the primary nuclear export, newly transcribed, end-matured, partially modified tRNAs are shuttled to the cytoplasm, where, in yeast, but not vertebrate cells, the tRNA splicing machinery is located. Genetic and in vivo biochemical data in yeast have shown two members of  $\beta$ -importin family, Los1 (Exportin-t in vertebrates) and Msn5 (Exportin 5), to serve overlapping but distinct functions in tRNA export. Although Los1 and Msn5 both participate in tRNA nuclear export, they cannot be the only nuclear exporters for tRNAs in yeast as *los1 $\Delta$  msn5 $\Delta$*  double mutant cells are viable. A comprehensive screening representing ~90% of the total yeast proteome conducted in our laboratory uncovered novel genes involved in tRNA subcellular localization. Two such proteins Mex67 and Mtr2 when inactivated, rapidly accumulate end-matured unspliced tRNA<sup>Ile</sup><sub>UAU</sub> and tRNA<sup>Tyr</sup><sub>GUA</sub> in the nucleus. Surprisingly, only subset of the ten intron-containing pre-tRNA families were found to accumulate in *mex67-5* and *mtr2* cells at 37°C. These nuclear tRNA accumulation defects were rectified by gene complementation. Remarkably, over-expression of Mex67-Mtr2 heterodimer completely suppressed tRNA splicing defect of *los1 $\Delta$*  cells. Moreover, tRNA nuclear accumulation observed in *los1 $\Delta$*  cells can be also suppressed by Mex67-Mtr2 over-expression. Finally, in vivo co-immunoprecipitation assay of tRNA nuclear export complexes showed that the Mex67-Mtr2 heterodimer can bind both intron-containing and mature tRNA. Thus, several lines of genetic, cytological and biochemical data suggest that bulk mRNA exporter Mex67-Mtr2 heterodimer also participates in tRNA nuclear export in yeast.



**459 The role of Loc1p in the formation of the nuclear *ASH1* transport complex***Crystal Fagan, Adrian Ferré-D'Amaré***National Heart, Lung, and Blood Institute, Bethesda, MD, USA**

The motor-dependent localization of mRNAs in the eukaryotic cell requires specific recognition of a *cis*-acting localization elements (LE) most often found in the 3'UTR of the localized mRNA by *trans*-acting RNA-binding proteins (RBPs). The best understood example of mRNA transport is the *ASH1* mRNA localization system in *Saccharomyces cerevisiae*. During mitosis, the *ASH1* mRNA is co-transcriptionally recognized by She2p and Loc1p to form a nuclear *ASH1* transport complex. Upon export from the nucleus, Loc1p is replaced by She3p which tethers the cytoplasmic transport complex to the myosin 4 protein motor for transport along the actin cytoskeleton. Even though the mRNA sequence of the LEs and the protein factors are known, it remains unclear how these mRNA transcripts are identified and specifically bound to form the nuclear transport complex. She2p alone forms only transient interactions with the LEs and requires Loc1p to form a stable complex. In fact, in *loc1* knockout cells, the *ASH1* mRNA is distributed throughout the cytoplasm indicating the cells are unable to form the transport complex. Loc1p is primarily disordered with only a small structured central domain, making it prone to aggregation and precipitation when mixed with other components of the nuclear transport complex. Using bio-layer interferometry and immobilizing Loc1p to a sensor, we have been able to overcome the inherent instability of Loc1p and biochemically characterize the binding of Loc1p to both She2p and one of the *ASH1* mRNA LEs. While the N and C-terminal regions of the protein appear to contribute little to the binding of either component, the structured central domain is not sufficient for binding the E3 localization element nor the She2p. Furthermore, we can show that Loc1p uses a similar hooking motif found in She3p to bind to She2p helping to explain how She3p is able to compete off binding of Loc1p.

**460 Competing exon definition events control the choice between nuclear retention and cytoplasmic export of U11/U12-65K mRNA***Jens Verbeeren<sup>1</sup>, Bhupendra Verma<sup>1</sup>, Elina Niemelä<sup>1</sup>, Karen Yap<sup>2</sup>, Eugene Makeyev<sup>2</sup>, Mikko Frilander<sup>1</sup>***<sup>1</sup>University of Helsinki, Institute of Biotechnology, Helsinki, Finland; <sup>2</sup>King's College London, Centre for Developmental Neurobiology, London, UK**

Cellular homeostasis of the minor spliceosome is regulated by a negative feed-back loop that targets U11-48K and U11/U12-65K mRNAs encoding essential components of the U12-type intron-specific U11/U12 di-snRNP. This involves interaction of the U11 snRNP with an evolutionarily conserved splicing enhancer giving rise to unproductive mRNA isoforms (1). In the case of U11/U12-65K, this mechanism controls the length of the 3' untranslated region (3'UTR). We provide evidence that this process is dynamically regulated in developing neurons and some other cell types and involves a binary switch between translation-competent mRNAs with a short 3'UTR to non-productive isoforms with a long 3'UTR that are retained in the nucleus or/and spliced to the downstream amylase locus. Importantly, the choice between these alternatives is determined by competing terminal exon definition events regulated by conserved U12- and U2-type 5' splice sites as well as sequence signals used for pre-mRNA cleavage and polyadenylation. We additionally show that U11 snRNP binding to the U11/U12-65K mRNA species with a long 3'UTR is required for their nuclear retention. Together, our studies uncover an intricate molecular circuitry regulating the abundance of a key spliceosomal protein and shed new light on the mechanisms limiting the export of non-productively spliced mRNAs from the nucleus to the cytoplasm.

## 461 Selective Recruitment of ER-Localized Transcripts to Stress Granules During the Unfolded Protein Response

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The unfolded protein response (UPR) functions to restore endoplasmic reticulum (ER) homeostasis during stress. The UPR effector proteins - ATF6, PERK and IRE1 - achieve this task by concurrently increasing ER folding ability through enhanced transcription of ER chaperones and membrane expansion while also decreasing ER protein folding load by the suppression of general translation and activation of ERAD. Recent work in our lab has shown ER protein folding load during UPR is also decreased by selective release into the cytosol of polysomes containing ER-protein encoding mRNAs (Reid DW et al 2014 Cell 158:1362-74). Additionally, cytosol-localized mRNAs can be sequestered in stress granules (SG) during cytotoxic stress presumably in response to attenuated global translation. We sought to test whether ER protein encoding mRNAs once released into the cytosol during the UPR would localize to stress granules while upregulated, ER-localized, chaperone encoding mRNAs would not. Surprisingly, results obtained from IF-FISH studies show that  $\beta$ 2-microglobulin mRNA, an mRNA released into the cytosol during the UPR, does not co-localize with SG. However, selective recruitment into SG of ER chaperone GRP94, and to a lesser degree, BiP mRNAs was seen during the UPR and this recruitment is dependent on both active transcription and translation within the cell. Polysome gradient and cell fractionation analysis further reveals GRP94 to be predominantly ribosome- and ER-bound during stress induction and recovery implying 1) stress granules serve as a staging area to prepare these RNAs for translation and 2) UPR-induced SG localize to the ER. In summary, selective recruitment of ER-localized mRNAs into stress granules may serve as a mechanism to protect and differentially regulate newly synthesized, stress-induced mRNAs as the cells recover from ER stress. (Supported by a grant from the NIH, GM101533 (CVN).

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## 462 tRNA splicing and subcellular dynamics

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tRNA biogenesis and subcellular trafficking are essential for decoding the genome and regulating the proteome. Hundreds of gene products function in tRNA biology and mutations of numerous of them cause human diseases. To address the information gap in tRNA biology, we conducted a screen of nearly the entire yeast proteome and identified 162 novel proteins (Wu et al., 2015). We are focusing on those that function in tRNA nuclear export of intron-containing pre-tRNAs to the cytoplasm and splicing on the mitochondrial surface. Surprisingly, we identified mRNA nuclear export machinery, Mex67 (TAP)/Mtr2 (p15), and the protein nuclear exporter, Crm1 (Exportin-1), as tRNA nuclear exporters. Four lines of evidence support this: (1) Northern analyses demonstrate that *mex67-5*, *mtr2-ts*, and *crm1-1* mutants accumulate unspliced tRNAs at the non-permissive temperature; (2) FISH studies show that the mutations cause increased tRNA nuclear pools; (3) genetic studies demonstrate synthetic growth defects for *los1Δ* and *crm1-1* and Mex67 can completely substitute for Los1 when it is over-expressed; (4) *in vivo* pull-down biochemical studies document that Mex67 co-purifies with pre-tRNAs. Thus, there are at least 3 parallel pathways to export tRNAs from their nuclear site of synthesis to their cytoplasmic site of function, underscoring redundancy for this essential cellular process. We also identified Tom70 and Sam37, mitochondrial outer membrane proteins functioning in mitochondrial protein import and tethering of other proteins to the mitochondrial surface. Three lines of evidence support the model that Tom70 and Sam37 are required for efficient assembly of the heterotetrameric splicing endonuclease on the mitochondrial surface: (1) Northern analyses document that *tom70Δ* and *sam37Δ* cause defective pre-tRNA splicing; (2) FISH analyses show that unspliced pre-tRNAs accumulate in the cytoplasm; (3) microscopy and subcellular fractioning studies document that Sen subunits are depleted from mitochondria. It appears that only 1 of the 4 Sen subunits possesses mitochondrial targeting information, supporting the notion that 1 subunit interacts with mitochondria via Tom70/Sam37 and the 3 remaining subunits assemble via protein-protein interactions. We are addressing how and why the mitochondrial surface functions as a warehouse for RNA processing steps from pre-tRNA splicing in yeast to piRNA end-processing in metazoans.

### 463 Sm proteins interact with polyadenylated mRNAs within cytoplasmic bodies - RIP-seq and *in situ* analysis

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Posttranscriptional regulation of gene expression in Eukaryota is a complex and multistep process, comprising a cascade of events taking place in both nucleus and cytoplasm. A number of the cytoplasmic posttranscriptional mRNA processing steps take place in highly specialized, evolutionary conserved microdomains, referred as to cytoplasmic bodies. Cytoplasmic bodies function as specific centers of balance coordination between mRNA translation and degradation, which results in gene expression on proper level.

Our research revealed cyclic occurrence of large, spherical cytoplasmic bodies enriched in Sm proteins and polyadenylated RNA (poly(A)RNA) in larch microsporocytes. Sm proteins play a fundamental role in pre-mRNA splicing as being core component of snRNPs. To date, however, the literature lacks information concerning interaction between mRNA and Sm proteins in processes other than splicing in plants. Given this, we find our observations particularly interesting, since larch Sm-rich accumulations lack other elements of spliceosomal units (UsnRNAs, U2B", m3G cap), which indicates a novel role of Sm in mRNA cell cycle in plants.

The aim of our work was to investigate the cytoplasmic interaction of Sm proteins with polyadenylated mRNAs. In order to identify which mRNAs are accumulated within Sm-rich cytoplasmic bodies, we applied RIP-seq approach of Sm-RNA complexes from cytoplasmic fraction of larch anthers. Transcriptomic analysis revealed 473 unique RNAs, of which 365 were next identified as specific mRNAs by homolog searching with NCBI's nr database. The identified transcripts could be divided into 3 major classes: those encoding ribosome/translation-related proteins, as well as mitochondrial and chloroplastic proteins. Finally, *in situ* localization of selected transcripts confirmed its accumulation within Sm-rich cytoplasmic bodies.

Based on our results we propose that evolutionary conserved Sm proteins have been adapted to perform several functions related to posttranscriptional regulation of gene expression in Eukaryota, which presumably enabled them to coordinate the interdependent processes of splicing and further mRNA maturation. Our observations are the first to report the participation of Sm proteins in posttranscriptional regulation of gene expression in the cytoplasm in plants.

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### 464 Role of post transcriptional regulation of SIR2 in aging in *Saccharomyces cerevisiae*

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The rate of aging is an actively regulated process, controlled by both environmental and genetic factors. SIR2 (Silent information regulator 2- human homolog SIRT1) is one of the key genes identified to control aging. Sir2p is an NAD<sup>+</sup> dependent protein deacetylase that conserved from bacteria to human. This protein was originally identified as genetic silencing factor and was later found to prolong lifespan in yeast. Despite the indispensable role of Sir2p in cellular senescence and in the aging-related diseases, studies related to regulation of SIR2 mRNA itself remains elusive. In our study, we fluorescently labelled SIR2 mRNA using U1A-GFP system and its protein labelled using mCherry protein in living yeast cells. Using fluorescent microscopy analysis, we found that most of SIR2 mRNA formed granules and localized to the perinuclear endoplasmic reticulum and few granules were observed in cytoplasmic area. The Sir2p was translated on ER and was delivered to nucleus. In addition, during early stage of cell cycle, SIR2 mRNA granules reached daughter cell, particularly to bud tip. The delivery of SIR2 mRNA granules to the daughter cells was dependent on SHE1-5 family proteins, where the deletion of one of these RNA binding proteins significantly altered the transport of SIR2 mRNA granules to the daughter cell. Thus, there were two localized SIR2 mRNA pools observed in budding yeast. One pool was localized to perinuclear ER, whose translated product reaches nucleus to control aging by acting as a silencing factor in nucleus of mother cells. The second pool of SIR2 mRNA was delivered to the daughter cell during early stage of cell cycle via control of SHE1-5 family proteins. Recent study by B Liu et al., showed that Sir2p translation is required in daughter cell for regulation of the retrograde transport of damaged proteins from daughter cell to mother cell. For this, delivery and possible local translation of Sir2p in daughter cells may require. The SIR2 mRNA post-transcriptional regulation is important for local protein expression and function, and dysfunction of any of two mRNA localization processes can affect aging of both the mother and daughter cells.

## 465 A dual protein/mRNA localisation screen reveals locally translated mRNAs in human cells

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While most mRNAs are randomly distributed in the cytoplasm, some are localised in particular subcellular areas. mRNA localisation can be involved in mRNA storage/processing, or it can lead to local translation, a process involved in many biological functions, such as cell polarisation, asymmetric cell division, cell migration, and neuronal functions. In order to obtain a broad view of mRNA localisation in human cells, we performed a screen in which we simultaneously analysed the localisation of mRNAs with their encoded protein. This was achieved by performing single mRNA detection (smFISH) with anti-GFP oligonucleotide probes, on a collection of HeLa cell lines expressing GFP-tagged genes from bacterial artificial chromosomes (BACs). The analysis of more than 500 mRNAs showed that around 8% of them displayed non-random localisation patterns, with several novel patterns and subcellular structures found in both interphase and mitotic cells. Interestingly, only a fraction of the localised mRNAs colocalised with their corresponding proteins, suggesting that mRNA localisation can be involved in other functions than localising the mature protein. To better understand these cases, we used an improved SunTag system to directly label translation sites. In one case where the mRNA was localised in cytoplasmic aggregates from which the mature protein was absent, we could show that the mRNA aggregates function as specialised translation factories. Our study highlights the diversity of functions of mRNA localisation in human cells.

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## 466 Single-molecule quantification of translation-dependent association of mRNAs with the endoplasmic reticulum

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It is well established that mRNAs encoding secretory or membrane-bound proteins are translated on the surface of the endoplasmic reticulum (ER). To what extent mRNAs that encode cytosolic proteins, however, associate with the ER remains controversial. To address this question, we quantified the number of cytosolic protein encoding mRNAs that co-localize with the ER using single-molecule RNA imaging in fixed and living cells. We find a small, but significant, number of mRNAs that encode cytosolic proteins associate with the ER and show that this interaction is translation-dependent. Furthermore, we demonstrate that cytosolic protein encoding transcripts can remain on the ER with dwell times consistent with multiple rounds of translation. These results advance our understanding of the diversity and dynamics of localized translation on the ER.



## 467 A role of the crosstalk between the NEXT complex and SERRATE in degradation of miRNA precursor fragments

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The *Arabidopsis thaliana* SERRATE protein (SE), which is a homologue of the human ARS2 protein, is involved in two important pathways of plant RNA metabolism: miRNA biogenesis and pre-mRNA splicing. Originally, SE was characterized as a protein involved in miRNA biogenesis, where together with DCL1 (Dicer Like 1) and HYL1 (HYPONASTIC LEAVES 1) form a core of the plant microprocessor. In this complex SE influences the accuracy of pri-miRNA cleavages catalyzed by DCL1. The *Arabidopsis* *se* null mutants are embryonic lethal that proves a key role of SE in plant development and growth. SE together with another factor involved in miRNA biogenesis, the nuclear cap-binding protein complex (CBC), have been also ascribed to splicing of pre-mRNA. We have shown that SE interacts with CBC, and both these factors influence alternative splicing. In order to understand the roles of SE and CBC in different pathways of RNA metabolism, we decided to search for novel proteins interacting with SE. To this end, we carried out co-immunoprecipitation of the FLAG:SERRATE fusion protein that were expressed in the *se-1* mutant genetic background. The SE-bound proteins were identified by mass spectrometry, and the putative protein interactions were confirmed by the yeast two hybrid system and pull-down experiments. Additionally, we confirmed these interactions in living cells using FLIM-FRET technique in *Arabidopsis* protoplasts. Our results have clearly demonstrated that SE contacts directly the Nuclear Exosome Targeting complex (NEXT). In our studies we characterized the interactions between SE and the subunits of NEXT. Moreover, we have shown that the NEXT complex is necessary for proper degradation of 5' pri-miRNA fragments after excision of miRNA by DCL1. We suggest that molecular interactions between CBC, SE and the NEXT complex is important for the quality control of miRNA precursors and degradation by the nuclear exosome 5' pri-miRNA fragments produced during miRNA biogenesis in the plant cell nucleus.

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## 468 Deconvoluting the LSm14 interaction network in translational repression and mRNA decay

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mRNA degradation and translational repression are central processes in post-transcriptional regulation of eukaryotic gene expression. Shortening of the 3' poly(A) tail of eukaryotic mRNAs by the CCR4-NOT deadenylase complex leads to translational repression and mediates the recruitment of the decapping machinery including the LSm1-7, DCP1-DCP2 complex and the 5'-3' exonuclease XRN1. This in turn results in the removal of the 5'-terminal cap structure and ultimately leads to the degradation of the mRNA body.

Tight control of this critical process is orchestrated by a complex and dynamic network of post-transcriptional regulators including Edc3, Pat1, LSm14, 4E-T and the DEAD-box helicase DDX6. Recent studies indicated that LSm14 functions as an interaction partner for several translational repressors and decapping activators. To shed light on the molecular details and the cellular functions of these interactions, we have employed X-ray crystallography combined with biochemical and cellular assays. We have determined the crystal structures of the DDX6-LSm14 and LSm14-4E-T protein complexes, identifying conserved sequence and structural motifs mediating protein-protein contacts. Our studies reveal the presence of both mutually exclusive and simultaneous interactions within the translational and decapping networks and highlight the importance of evolutionarily conserved short linear sequence motifs in LSm14 and its interacting partners.



**469 Mining for new *cis*-regulatory elements in the mRNA***Johannes Braun*<sup>1</sup>, *Oliver Binas*<sup>2</sup>, *Harald Schwalbe*<sup>2</sup>, *David Mathews*<sup>3</sup>, *Julia Weigand*<sup>1</sup><sup>1</sup>Department of Biology Technical University Darmstadt, Darmstadt, Germany; <sup>2</sup>Center for Biomolecular Magnetic Resonance Johann Wolfgang Goethe- University Frankfurt, Frankfurt, Germany; <sup>3</sup>Department of Biochemistry & Biophysics University of Rochester Medical Center, Rochester, USA

Many posttranscriptional regulatory pathways involve sequence and/or structural elements within the untranslated regions (UTRs) of the mRNA. Structural elements in UTRs have been found to guide alternative splicing, to regulate the accessibility of miRNA binding sites and are themselves often recognized by proteins. Yet, little is known how widespread the involvement of structural elements in posttranscriptional regulation is. In this study structurally conserved RNA elements in UTRs were assessed by genome wide comparison of all human and mouse UTRs using the folding algorithm *Dynalign*. Putative elements were functionally characterized by reporter gene assays and qRT-PCR. Using this approach a new functional element in the 3'UTR of *uncoupling protein 3 (UCP3)* was identified. UCP3 is a mitochondrial membrane protein associated with obesity and diabetes, which functions in the energy metabolism of the cell. Despite great interest, to date the physiological and biochemical function of UCP3 is not known.

The regulatory element significantly reduces gene expression in several cell lines. It comprises two AU-rich elements (AREs) as well as binding sites for miR-152 and the RNA binding protein (RBP) HuR. RNA affinity chromatography revealed that HuR binds adjacent to the miR-152 seed. HuR knock down and miR-152 overexpression, negatively regulate gene expression compared to non-overexpressing cells suggesting a cross regulation. Independent of this, the regulatory element contains two AREs (ARE I and ARE II), which differ in sequence and structure, but act in concert in regulating mRNA stability. Mutation of either of the AREs abolishes regulation. NMR spectroscopy of ARE II shows that it folds into a stem loop structure. Structure formation is mandatory for gene regulation. I identified several canonical RBPs as well as moonlighting enzymes binding to the element *in vitro* and currently evaluate their biological function. Interestingly known ARE binding proteins do not bind to the UCP3 regulatory element.

**470 Structural and functional characterization of an adaptor protein for a major endoribonuclease in *Escherichia coli****Svetlana Durica-Mitic*<sup>1</sup>, *Grecia M. Gonzales*<sup>2</sup>, *Ben Luisi*<sup>2</sup>, *Boris Görke*<sup>1</sup><sup>1</sup>Department of Microbiology, Immunobiology and Genetics, Max F. Perutz Laboratories (MFPL), University of Vienna, Vienna Biocenter (VBC), Vienna, Austria; <sup>2</sup>Department of Biochemistry, University of Cambridge, Cambridge, UK

In diverse bacteria, RNase E represents one of the central components of RNA decay machinery, as it is involved in turnover of a plethora of substrates. The first adaptor protein identified for this major endoribonuclease is RapZ, a conserved protein that promotes RNase E mediated cleavage of a small RNA GlmZ. This processing event renders GlmZ inactive and unable to up-regulate expression of its target - metabolic enzyme GlmS. When activity of GlmZ is needed in the cell, its degradation is counteracted by GlmY, a homologous small RNA which binds and sequesters RapZ. This complex interplay exemplifies a novel mode of regulating activity of global RNases, which can be diverted from bulk RNA turnover towards specific targets under particular physiological conditions. RapZ has been shown to interact with the catalytic domain of RNase E and to bind GlmZ/Y with remarkable specificity, but the exact mechanism through which it stimulates GlmZ cleavage remains to be elucidated. To answer this, we have sought out to achieve molecular characterization of this adaptor protein.

In this study, we present the structure of RapZ and show that it forms a tetramer. By mutational analysis, we could confirm the structural prediction and identify residues crucial for maintenance of its different interaction surfaces. Furthermore, we demonstrate the importance of tetrameric assembly for RapZ function *in vivo*, presumably due to its role in RNA binding. We identify the C-terminal domain (CTD) dimer of RapZ as the minimal unit needed to bind GlmZ/Y *in vivo* and *in vitro* and show that exchanges that abolish dimerization of CTD also impair its RNA binding function. We provide evidence that RapZ-CTD cannot efficiently promote GlmZ cleavage *in vitro*, suggesting that NTD is necessary for this activity. Moreover, we show that a RapZ variant impaired in RNA binding but still able to interact with RNase E can stimulate GlmZ processing at increased concentrations. Therefore, we suggest a novel model of regulating RNase E, through its allosteric activation by a protein co-factor.

## 471 Determining the role of the TORC1 signaling pathway in regulation of mRNA stability during a stress response

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Regulation of gene expression is a crucial means by which cells adapt to changes in their environment. In yeast, a dramatic decrease in ribosome biosynthesis (Ribi) and ribosomal protein (RP) mRNAs is seen during the response to osmotic stress. This decrease depends upon the Target of Rapamycin Complex 1 (TORC1) signaling pathway, which current models suggest acts at the level of transcription. However, transcriptional shut off alone cannot explain completely the decrease in levels seen for these mRNAs. In addition, other studies suggest Ribi mRNAs are destabilized during osmotic stress. We therefore hypothesize that TORC1 regulates not only transcription of mRNA but also mRNA decay. Furthermore, TORC1-mediated reduction of Ribi mRNA levels during osmotic stress is also perturbed by deletion of RNA binding proteins which we hypothesize function downstream of TORC1. Specifically, deletion of Puf4 ranked near the top of all genes that, when deleted, impaired Ribi mRNA reduction during osmotic stress. Notably, Puf4 specifically binds Ribi mRNAs and promotes mRNA decay through recruitment of the Ccr4-Not deadenylase complex. By regulating both mRNA synthesis and decay, the TORC1 pathway may help cells be able to more quickly and efficiently adapt to osmotic stress, as well as other changes in cellular environment

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## 472 A Functional Analysis of Neurological Disease Mutations Identified in RNA Exosome Genes

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The RNA exosome is an evolutionarily conserved, ribonuclease complex that processes/degrades numerous classes of non-coding RNA. The 10-subunit core exosome forms a ring-like structure composed of an upper ring of three S1/KH cap subunits (Rrp40/EXOSC3, Rrp4/EXOSC2, Csl4/EXOSC1), a lower ring of six PH-like subunits, and an active 3'-5' ribonuclease subunit, Rrp44/DIS3, at the base. The nuclear form of the RNA exosome contains an eleventh, cap-associated ribonuclease subunit, Rrp6/EXOSC10. RNAs are targeted directly to Rrp6 or threaded through the central channel of the exosome to Rrp44 for processing. Recently, mutations in two different exosome cap subunit genes, *EXOSC3/RRP40* and *EXOSC2/RRP4*, have been linked to two separate neurological diseases. Mutations in *EXOSC3* cause Pontocerebellar Hypoplasia Type 1b (PCH1b), an autosomal recessive disorder characterized by cerebellar hypoplasia and neuronal degeneration that leads to early mortality. Mutations in *EXOSC2* cause a novel syndrome characterized by retinitis pigmentosa, hearing loss, premature aging, and mild intellectual disability. To gain insight into the functional consequences of the mutations in *EXOSC3* and *EXOSC2* identified in patients, we generated the corresponding mutations in the *S. cerevisiae* genes, *RRP40* and *RRP4*, respectively, and examined their function in budding yeast. We find that *rrp40* and *rrp4* variants cause differential effects on cell growth and RNA exosome function, which could shed light on why impairments in two similarly located exosome cap subunits give rise to distinct diseases with tissue specific manifestations. An *rrp40* protein variant corresponding to the severe *EXOSC3*-W238R variant in PCH1b patients is unstable and does not associate efficiently with the RNA exosome in the presence of wildtype Rrp40, suggesting cells possess a mechanism to discriminate between wildtype and variant exosome subunits. To extend our functional studies of *EXOSC3* and *EXOSC2* mutations to mammalian cells, we have performed a proteomic analysis comparing *EXOSC3* and *EXOSC2* variant and wildtype proteins expressed in neuronal cells. These data suggest the altered protein-protein interactions of *EXOSC3* and *EXOSC2* variants could contribute to disease phenotypes by altering RNA exosome function.

### 473 Role of the upstream open reading frame ( $\mu$ ORF) in the human inducible nitric oxide synthase (iNOS) mRNA and the influence of the nonsense mediated mRNA decay (NMD)

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After induction of inducible nitric oxide synthase (iNOS) expression by cytokines the enzyme produces high amounts of NO which can have beneficial microbicidal, antiparasital, antiviral and antitumoral effects. In contrast, aberrant iNOS induction seems to be part of the pathology of septic shock and many autoimmune diseases. Therefore, it is important to examine the regulation of iNOS expression.

Analyses of the human iNOS gene structure revealed the existence of an upstream open reading frame ( $\mu$ ORF) in exon 1 with a stop codon located about 50 bp in front of the first intron which indicates a putative involvement of the nonsense mediated decay (NMD) mechanism in the regulation of iNOS expression.

The function of the  $\mu$ ORF was analyzed by cloning different EGFP and luciferase reporter constructs and transfecting them into human DLD1 cells. Transfection of  $\mu$ ORF-EGFP fusion constructs showed the translatability of the  $\mu$ ORF. Blocking the cap-dependent translation by cloning a stem loop structure in front of the iNOS 5'UTR within a luciferase reporter plasmid led to a remarkable loss of luciferase production. Thus, the expression of iNOS seems to be cap-dependent. Furthermore, transfection of constructs coding for a bicistronic renilla-firefly luciferase mRNA ruled out an IRES activity in the iNOS 5'UTR.

However, transfection of constructs containing the first two exons and the first intron of the iNOS (with or without a mutated  $\mu$ ORF) in front of a luciferase gene showed a twofold enhancement of luciferase activity in cells transfected with the mutated  $\mu$ ORF in comparison to the wild-type  $\mu$ ORF. siRNA-mediated downregulation of the essential NMD factor Upf1 resulted in enhanced cytokine-induced iNOS mRNA and protein expression as well as increased luciferase mRNA expression in cells with wild-type  $\mu$ ORF constructs.

Taken together, the iNOS expression seems to be cap-dependent and without influence of an IRES, while the  $\mu$ ORF is translatable. Additionally, the  $\mu$ ORF reduces iNOS expression by activation of the NMD mRNA decay mechanism.

Therefore, we assume that iNOS expression is only possible due to a leaky scanning mechanism depending on the weak Kozak sequence of the  $\mu$ ORF and that NMD has a regulating influence on iNOS expression.

### 474 The role and regulation of an N6-methyladenosine-specific RNA-binding protein in pluripotency

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N(6)-methyladenosine (m6A) is an abundant post-transcriptional modification that influences association of RNA-binding proteins (RBPs) and therefore has a significant impact on gene expression. Although m6A has been implicated in establishment and maintenance of pluripotency, it remains unclear which mRNAs are affected in stem cells and to what extent. One m6A-specific RBP, YTHDF2, enhances decay of its target transcripts. Intriguingly, we find that YTHDF2 is highly expressed in induced pluripotent stem (iPS) cells, and down-regulated ~8 fold in differentiated fibroblasts. We propose that YTHDF2 coordinates decay of certain m6A-modified mRNAs in order to maintain pluripotency or alternatively to drive differentiation in response to extracellular cues. Our experiments are aimed at (i) characterizing how elements in the YTHDF2 3'UTR modulate YTHDF2 expression and (ii) identifying transcripts that are dependent on YTHDF2 for appropriate expression in iPS cells.

With respect to the first aim, we have utilized luciferase reporters to identify a proximal region in the YTHDF2 3' UTR which enhances translation. The results of experiments to pinpoint the regulatory sequence elements and identify the trans-acting factors involved will be presented.

In order to isolate the set of mRNAs most affected by YTHDF2 in iPS cells, we reduced YTHDF2 by RNAi and utilized an optimized protocol for 4-thiouridine (4sU) metabolic labeling to accurately measure decay rates via digital PCR (dPCR) and RNA-seq. By identifying differentially regulated mRNAs that are also m6A-methylated and associated with YTHDF2 we will reveal how YTHDF2 contributes to the effects of m6A on pluripotency.

**475 Structural characterization of the RNA helicase DHX34**

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We have previously shown that the RNA helicase DHX34 associates with the SURF complex, comprising UPF1, SMG1 and the translation termination factors, eRF1, 3 in the vicinity of the premature termination codon. It subsequently promotes the interaction of UPF1 with UPF2 resulting in UPF1 phosphorylation and the release of eRF3. From these studies, we concluded that DHX34 acts to remodel mRNPs and triggers the conversion from the SURF complex to the DECID (Decay-induced) complex resulting in NMD progression and mRNA degradation. Using single-particle electron microscopy, we also determined that DHX34 comprises two distinct structural units, a core that binds UPF1 and a protruding C-terminal domain (CTD) that binds the SMG1 kinase. We demonstrated the existence of a complex comprising SMG1, UPF1 and DHX34, where DHX34 functions as a scaffold for UPF1 and SMG1, which promotes UPF1 phosphorylation leading to functional NMD. The truncation of the CTD compromises DHX34 binding to SMG1, affects UPF1 phosphorylation and hence abrogates NMD.

The activity of DEAH box helicases is often regulated through a specific class of proteins, containing G-patch domains. This is an intrinsically unstructured domain consisting of a stretch of 50 aa comprising conserved glycine residues, that interacts with an auxiliary OB-fold (OB-like domain) of their cognate DEAH box helicase. We will report on our current efforts aiming to identify regulators of DHX34 such as G-patch proteins that bind to the OB-fold of DHX34 and regulate its activity. For this, we are using an Immunoprecipitation-Mass Spectrometry proteomics approach with wild-type DHX34 protein or using a DHX34 deletion mutant lacking the OB-fold domain.

**476 A specific pathway eliminates chemically damaged caps that prevent eukaryotic mRNA function**

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The eukaryotic N7-methyl guanine (m7G) mRNA cap is involved in pre-mRNA splicing, polyadenylation, mRNA nuclear export, translation and mRNA stability and thus participates to the control of posttranscriptional gene expression. Analyses of DNA damage has revealed that m7G bases generated by methylating agents are chemically reactive and easily damaged by hydroxyl ions *in vivo* to form open 2-6-diamino-4-hydroxy-5N-methylformamidopyrimidine (FAPY) groups. Consistently, we observed that mRNA caps are highly reactive and spontaneously damaged at physiological pH. We show moreover that FAPY capped RNAs are poorly translated and that damaged caps can't be eliminated by the Dcp2 and DcpS decapping enzymes but are hydrolysed by the Aph1/FHIT enzyme in yeast and human. Our data support the existence of a dedicated quality control pathway ensuring the elimination of damaged inactive mRNA cap structures.



## 477 Ribosome quality control of premature polyadenylated mRNAs by a unique E3 ubiquitin ligase and RNA-binding protein

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Cryptic polyadenylation within coding sequences or incompletely removed introns produces aberrant transcripts that lack in-frame stop codons and are subject to ribosome-associated quality control (RQC). Premature polyadenylated mRNAs trigger ribosome stalling and activation of the RQC pathway, leading to degradation of the aberrant mRNA and nascent polypeptide, ribosome disassembly and recycling of its subunits. While ribosomal subunit dissociation and nascent peptide degradation are well-understood, the molecular sensors of aberrant mRNAs and their mechanism of action, especially in mammalian cells, remain largely unknown. We show that the unique RNA-binding protein and E3 ubiquitin ligase, Zinc Finger Protein 598 (ZNF598) may function as a sensor for detection of the premature polyadenylated mRNAs. PAR-CLIP assay revealed that ZNF598 crosslinks to tRNAs, mRNAs, and rRNAs, thereby placing the protein on translating ribosomes. Crosslinked reads originating from AAA-decoding tRNA<sup>Lys</sup>(UUU) were 10-fold enriched over its cellular abundance, and poly-lysine encoded by poly(AAA), but not poly(AAG), induced RQC in a ZNF598-dependent manner. Sensing of premature polyA tails by ZNF598 triggered ubiquitination of the ribosomal proteins RPS3A, RPS10, and RPS20 requiring the E2 ubiquitin ligase UBE2D3 and thereby initiating RQC. Considering that human coding sequences are devoid of >4 repeated AAA codons, sensing of premature polyA by a specialized RNA-binding protein is a novel nucleic-acid-based surveillance mechanism for contributing to RQC.

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## 478 RNA fate is regulated by 2'3' and 3'5' cyclic adenosine monophosphates

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In stark contrast, to our understanding of the biological significance of the secondary messenger, 3'5' cyclic adenosine monophosphate (cAMP), our knowledge on its positional isomer and product of RNA degradation 2'3' cAMP remain obscure. In fact it was only in 2009, when Ren and colleagues (2009) detected 2'3' cAMP in the biological material. Since then 2'3' cAMP has been measured in animals, plants and in bacteria. The role of 2'3' cAMP, other than product of RNA turn-over, is as yet unknown. Intriguingly, in our previous work separating protein bound from free small molecules using size exclusion chromatography (SEC) (Veyel et al., 2016) we could demonstrate that 2'3' cAMP is retained in protein complexes arguing for the existence of 2'3' cAMP protein receptors. Herein, using combination of biochemical (affinity purification and thermal protein profiling) and biophysical methods (microscale thermophoresis) we could demonstrate that 2'3' cAMP, but not 3'5' cAMP, binds to the Arabidopsis and human RNA binding proteins. These are involved in maintaining the balance between RNA degradation and storage under stress conditions, which fits well 2'3' cAMP accumulation in heat, darkness and upon wounding and therefore pointing towards 2'3' cAMP as molecule involved in signal transduction. In a parallel approach and again prompted by the SEC data, we looked for yet unknown in plants, soluble receptors of 3'5' cAMP. Using similar methodological pipeline we isolated two proteins, involved in RNA editing and energy balance which showed to bind 3'5' cAMP with high specificity (K<sub>d</sub> = 18nM). Interestingly, both 2'3' and 3'5' cAMP have distinct protein partners however both molecules seem to be involved in the RNA fate regulation, and in that way affect metabolic status of the cells. To our knowledge it is the first report of soluble cAMP receptors in plants.



## 479 Functional role of NBAS in the Nonsense-mediated decay (NMD) pathway

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The Nonsense-mediated mRNA decay (NMD) pathway is a highly conserved surveillance mechanism that selectively degrades mRNAs harbouring premature termination codons (PTCs), preventing the synthesis of truncated proteins. In addition, this pathway also regulates the abundance of a large number of endogenous cellular RNAs and fine-tunes many physiological processes.

We have previously identified NBAS (Neuroblastoma Amplified Sequence) as a conserved NMD factor and demonstrated that it acts in concert with core NMD factors to co-regulate a large number of endogenous RNA targets (1,2). We have now observed that NBAS is an RNA-binding protein that interacts in an RNA-independent manner with the hypo-phosphorylated form of the core NMD factor, UPF1. Using FRET-FLIM and Proximity ligation assay (PLA) we demonstrate that NBAS and UPF1 proteins interact directly. Furthermore, we observed that overexpression of NBAS in HeLa cells leads to an increase in UPF1 phosphorylation, whereas its knockdown results in decreased UPF1 phosphorylation. Altogether, these data suggests that NBAS (directly or indirectly) modulates UPF1 phosphorylation, which is a critical step leading to NMD activation.

Interestingly, NBAS was also identified as a component of the Syntaxin 18 complex that is localized at the Endoplasmic reticulum (ER) and is required for vesicular trafficking and ER homeostasis (3). In agreement, we had previously shown that NBAS targets are enriched for ER-associated transcripts (2). We will discuss current experiments aiming to uncover whether NBAS has two separate functions as an NMD factor and as a component of the ER-Golgi tethering complex or whether these two functions are somehow linked.

1. Longman,D., Plasterk,R.H., Johnstone,I.L. and Cáceres,J.F. (2007) Mechanistic insights and identification of two novel factors in the *C. elegans* NMD pathway. *Genes Dev.*, **21**,1075-1085.

2. Longman et al. (2013) DHX34 and NBAS form part of an autoregulatory NMD circuit that regulates endogenous RNA targets in human cells, zebrafish and *C. elegans*. *Nucleic Acids Res.*, **41**, 8319-8331.

3. Aoki et al. (2009) Identification of the neuroblastoma-amplified gene product as a component of the syntaxin 18 complex implicated in Golgi-to-endoplasmic reticulum retrograde transport. *Mol. Biol. Cell.*, **20**, 2639-2649.

## 480 Molecular basis for cytoplasmic RNA surveillance by uridylation-triggered decay in *Drosophila*

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The post-transcriptional addition of nucleotides to the 3' end of RNA regulates the maturation, function, and stability of RNA species in all domains of life. Here, we show that, in flies, 3' terminal RNA uridylation triggers the processive, 3'-to-5' exoribonucleolytic decay via the RNase II/R enzyme CG16940, a homolog of the human Perlman syndrome exoribonuclease Dis3l2. Together with the TUTase Tailor, dmDis3l2 forms the cytoplasmic, terminal RNA uridylation-mediated processing (TRUMP) complex, that functionally cooperates in the degradation of structured RNA. RNA-immunoprecipitation and high-throughput sequencing reveals a variety of TRUMP complex substrates, including abundant non-coding RNA, such as 5S rRNA, tRNA, snRNA, snoRNA, and the essential RNase MRP. Based on genetic and biochemical evidence we propose a key function of the TRUMP complex in the cytoplasmic quality control of RNA polymerase III transcripts in *Drosophila*.

## 481 Nonsense-mediated mRNA decay targeting of functionally Related mRNAs in *Saccharomyces cerevisiae*

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The nonsense-mediated mRNA decay (NMD) pathway is an mRNA degradation pathway that degrades mRNAs that aberrantly terminate translation. This includes mRNAs harboring premature termination codons as well as natural mRNAs that produce fully functional proteins. In *Saccharomyces cerevisiae*, a number of features have been shown to target natural mRNAs to NMD. However, the extent to which natural mRNAs from the same functional group are regulated by NMD is not fully understood. We examined a subset of natural mRNAs involved in copper homeostasis to identify the NMD-targeting features and environmental conditions that influence the regulation of these mRNAs. Out of the seven mRNAs involved in copper homeostasis and regulated in an NMD-dependent manner, *MAC1*, *CTR2*, *COX19* and *FRE2* were found to be direct NMD targets in rich media. These mRNAs had significantly altered decay rates in wild-type yeast strains relative to NMD mutant strains. Conversely, in the same conditions, *CRS5*, *COX23* and *PCAI* mRNAs accumulated to significantly higher levels in nmd mutants, but did not show statistically significant differences in half-lives in wild-type and nmd mutants. Six of these mRNAs have atypically long 3'-UTRs. Notably, we found that the long 3'-UTRs of *CTR2*, *COX19*, and *FRE2* contribute to the regulation of these mRNAs by NMD but are not the only NMD targeting features. In addition, we found that regulation of *MAC1* and *PCAI* mRNAs by the pathway is dependent on environmental copper levels.

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## 482 The translocation event generates specificity for adenine in poly(A)-specific ribonuclease (PARN)

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The deadenylase PARN is a poly(A)-specific, processive and cap-interacting 3'-5' exoribonuclease that efficiently degrades poly(A) tails of eukaryotic RNAs. We have developed a working model for PARN action (*Crit. Rev. Biochem. Mol. Biol.*, **48**, 192-209) that defines two reaction steps, a translocation event followed by a hydrolytic event that releases AMP.

The active site of PARN consists of three binding sites for adenine base (sites “-1”, “-2” and “-3”). The hydrolytic site is located between the two sites “-1” and “-2”. We find that two translocation events are required to position the first scissile bond into the hydrolytic site. Subsequently, each translocation step will position the next phosphodiester bond into the hydrolytic site. Thus, the 3' end located adenine base of a poly(A) tail binds first to site “-3”, then translocates into site “-2” at the same time as the penultimate adenine base enters site “-3”. Subsequently, the 3' end located adenine base and the penultimate adenine base translocates from sites “-2” and “-3” into sites “-1” and “-2”, respectively, at the same time as the next adenine base enters into site “-3”. At the end of these two translocation steps the first phosphodiester bond to be cleaved has been positioned in the hydrolytic site of PARN. Our analysis demonstrates that adenosine recognition is primarily linked to the translocation event where the adenine base moves from site “-3” to “-2”.

Structural studies (*EMBO J.*, **24**, 4082-4093) identify a rotational event of the base when it moves from site “-3” to “-2”. Thus, our study suggests that coordinated rearrangements of the poly(A) substrate and the active site are critical for providing the specificity of PARN for degrading poly(A). Taken together, we conclude that the translocation event generates specificity for the adenine base in PARN.

### 483 RNA Decay-mediated Regulation of PABPN1: Insight into the Muscle-specific Basis of Oculopharyngeal Muscular Dystrophy

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RNA binding proteins are critical for ensuring proper gene expression. Although many genes encoding RNA binding proteins are ubiquitously expressed, mutations in these genes often result in tissue-specific pathology. For example, the ubiquitous poly(A) binding protein, PABPN1, plays well-characterized roles in polyadenylation including poly(A) site selection. Interestingly, a GCN expansion mutation in a single copy of PABPN1, causing a modest Alanine expansion in the N-terminus of the protein, results in the autosomal dominant, muscle-specific disease oculopharyngeal muscular dystrophy (OPMD). How this mutation leads to muscle-specific disease is unknown. Determining the unique properties of PABPN1 in muscle that predispose this tissue to OPMD pathology is critical for developing therapeutic treatments. We have discovered that muscle-specific destabilization of Pabpn1 mRNA contributes to low PABPN1 protein levels in muscle. These low PABPN1 levels may make muscle susceptible to mutations that reduce PABPN1 protein function while higher PABPN1 levels in non-muscle tissues may compensate for reduced function. We have identified cis-regulatory elements and corresponding trans-acting factors that regulate Pabpn1 via the 3'UTR in a muscle-specific manner. Multiple AU-rich elements (AREs) in the Pabpn1 3'UTR confer muscle-specific expression, suggesting that trans-acting factors such as RNA binding proteins interact with these elements to modulate Pabpn1 levels in muscle. Our data suggest that multiple trans-acting factors, including the ARE-binding protein HuR and microRNAs, regulate Pabpn1 in cultured muscle cells. We are extending this work with novel mouse models that phenocopy OPMD to understand which pathways are the most critical regulators of PABPN1 *in vivo*. These results show that tissue-specific regulation of RNA decay can underlie human disease and begin to dissect the key factors that mediate this regulation.

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### 484 Acetylation-dependent changes in the poly(A) RNA-bound proteome

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Acetylation is a post-translational modification that affects the activity, function and localization of proteins in a variety of biological processes. While acetylation is known to regulate gene expression via epigenetic and transcriptional mechanisms, there is limited understanding of how it influences gene expression post-transcriptionally. We have recently uncovered a novel role for acetylation as a fundamental regulator of global mRNA turnover. Inhibition of histone deacetylases 1 and 2 (HDAC1 and 2) was found to induce widespread degradation of poly(A)-containing RNAs in mammalian cells (Sharma et al., 2016). Acetylation-induced mRNA turnover was further shown to involve p300/CBP-mediated acetylation of the exoribonuclease CAF1a, the principal deadenylase within the evolutionarily conserved CCR4-CAF1-NOT complex (Sharma et al., 2016). Hence, reversible acetylation acts as a rheostat that controls global mRNA turnover.

In order to obtain an unbiased view of acetylation-induced differences in the association of RNA-binding proteins (RBPs) with mRNAs I used poly(A) RNA interactome capture in conjunction with quantitative mass spectrometry. Chemical crosslinking with formaldehyde stabilized *in vivo* RNA-protein interactions more efficiently than conventional UV crosslinking allowing a drastic reduction in the quantity of starting material. The proteomics dataset encompassing 1265 RNA-associated proteins is in good agreement with previously published studies. Treatment with the class I-specific HDAC inhibitor Romidepsin (RMD) caused enrichment of 17 proteins and disenrichment of 50 proteins in the poly(A) RNA-bound proteome which was further validated for several candidates. Moreover, ~60% of RBPs previously identified by poly(A) RNA interactome capture were found to be acetylated in a recently published proteome-wide acetylome analysis highlighting the importance of acetylation as a post-translational modification of RBPs. Current investigations aim at identifying site-specific acetylation of RBPs and determining the functional impact of the RNA-bound acetylome on the regulation of mRNA metabolism.

## 485 Poly(C) Binding Protein 2 regulates mRNA stability to fine tune gene expression during stem cell differentiation

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Pluripotency is a key property of stem cells defined by the ability to both replicate indefinitely as well as differentiate into alternative cell types in response to cellular signals. Differentiation requires highly coordinated changes in the rates of transcription and mRNA decay to achieve tissue specific gene expression profiles. Previous global analyses of RNA stability in human foreskin fibroblasts (HFFs) and their genetically matched induced pluripotent stem cell (iPSC) counterparts revealed altered stability of mRNAs containing C-rich elements within their 3' untranslated regions. Several of these transcripts encode key transcription factors involved in differentiation. Our current objective is to identify the factors responsible for regulating decay of mRNAs bearing C-rich elements and define their role in pluripotency. We have applied 4-thiouridine metabolic labeling to examine mRNA stability following knockdown of the Poly (C) Binding Protein 2 (PCBP2) in iPSCs. This treatment significantly increases the stability of transcripts containing C-rich elements, mirroring the increased stability observed in HFFs vs iPSCs. Cross-linking followed by RNA immunoprecipitation studies in iPSCs reveal that these C-rich transcripts are directly bound by PCBP2. Upon differentiation of iPSCs to early mesoderm cells, C-rich transcripts dramatically increase in abundance and stability. Interestingly, PCBP2 protein expression is reduced rapidly (within 4 hours) and dramatically upon changing the media to favor mesoderm differentiation. Thus, PCBP2 may destabilize C-rich containing transcripts to favor pluripotency and its down-regulation may be necessary to allow cells to achieve a mesodermal gene expression profile.

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## 486 The molecular basis for the stimulation of 5'-end-dependent RNA degradation by a metabolic enzyme

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In both eukaryotes and prokaryotes, the lifetimes of individual messages can differ by as much as two orders of magnitude, with proportionate effects on protein synthesis. However, in spite of the widespread regulatory impact of mRNA degradation, the basis for most differences in mRNA longevity has yet to be explained. In *Escherichia coli*, decay of many mRNAs proceeds through a 5'-end-dependent pathway triggered by conversion of the triphosphate initially present at the mRNA 5' end to a monophosphate. A key enzyme in this conversion is the RNA pyrophosphohydrolase RppH, which generates monophosphorylated mRNA substrates for rapid cleavage by the endonuclease RNase E.

The activity of RppH is enhanced by binding to the metabolic enzyme diaminopimelate epimerase (DapF). DapF participates in the biosynthesis of L-lysine and bacterial peptidoglycan and therefore may stimulate mRNA degradation under particular growth conditions and stages. To understand the molecular mechanism by which a metabolic enzyme can accelerate RNA degradation, we determined high-resolution X-ray structures of a quaternary complex comprising *E. coli* RppH, DapF, and their respective substrates. These structures revealed a large binding interface between RppH and DapF. Surprisingly, the structures did not reveal significant allosteric changes in the RppH conformation that would affect enzyme activity. Instead, the structure showed a continuous positively charged surface protruding from DapF toward the RNA-binding cleft of RppH.

To explain the stimulatory effect of DapF on RppH, we conducted biochemical experiments with RNA substrates of various lengths. This study has shown that DapF stimulation of RppH activity correlates with substrate length and that an abrupt increase occurs when the RNA length exceeds the apparent size of the putative RNA-binding surface on RppH. Thus, the mechanism of RppH stimulation by DapF likely involves increasing the affinity of the RppH-DapF complex for RNA by combining the RNA-binding surfaces of RppH and DapF. The insights gained from this study may have important implications for the control of mRNA decay in higher organisms due to the functional and structural similarity of the RppH-DapF complex to the protein complexes that decap mRNA in eukaryotes.



#### 487 Regulating the regulator: feedback mechanisms controlling the plant nonsense-mediated mRNA decay (NMD) pathway

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Nonsense-mediated mRNA decay (NMD) is a eukaryotic RNA surveillance mechanism that down-regulates aberrant transcripts, including mutated transcripts with pre-mature termination codons (PTCs) as well as transcripts derived from pseudogenes, transposable elements, and aberrant mRNA-like non-coding RNAs. Moreover, NMD controls the levels of many normal mRNAs with cis-elements that can be recognized as PTCs. These elements include features that are abundant in normal mRNAs, such as upstream open-reading frames (uORFs) and long 3' untranslated regions (3' UTRs). Considering its major impact on the eukaryotic transcriptome, it is of importance to understand how NMD itself is regulated. We found that the *Arabidopsis thaliana* NMD factor UPF3 is feedback regulated by NMD at multiple levels. This feedback control is mediated by the long 3' UTR of *UPF3* and by other regulatory elements of this gene. A delicate balancing of *UPF3* expression by this feedback loop plays a crucial role in NMD regulation in plants. We also found that UPF3 plays a role in plant response to salt stress. *UPF3* expression is induced by salt stress, and the balanced expression of this gene is essential for coping with this stress. These findings demonstrate the physiological significance of proper NMD balancing.

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#### 488 The human nuclear exosome displays strong functional interactions with transcription, splicing and RNA export as revealed by a genome wide siRNA

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Human DIS3 is an essential catalytic subunit of the nuclear exosome complex endowed with endo- and exoribonucleolytic activities. Its dysfunction leads to massive accumulation of various pervasive transcription products and global changes in polymerase II transcriptome. As a result nearly half of human mRNAs are deregulated in cells with abolished DIS3 activities. DIS3 is also one of the most frequently mutated genes in Multiple Myeloma (MM) patients.

We have conducted a genome-wide screen in order to analyze the functional interactions between the nuclear exosome and other cellular pathways as well as to find putative drug targets for MM with DIS3 mutations. Two cell lines were engineered, one expressing wild type DIS3 and EGFP and the other - an MM-associated mutant of DIS3 deficient in exoribonuclease activity and mCherry. These cells were co-cultured in 384-well plates and transfected with a genome-wide siRNA library (Dharmacon ON-TARGETplus siRNA SMARTpool; 18104 targets), in triplicate, and viability of green and red cells was assayed by fluorescence microscopy. Genes that affected the two cell lines differently were considered hits and significant ones were further validated in a secondary screen.

## 489 Biochemical characterisation of the human REXO2 ribonuclease

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Mitochondria are semi-autonomous organelles present in almost all eukaryotic cells, which are responsible for energy conversion and many regulatory processes. The mitochondrial genome is responsible for the mitochondrial partial autonomy. In humans, it codes for only 13 proteins, but every gene encoded in the mtDNA is essential. RNA degradation is especially important in mitochondria, as in these organelles the possibilities of regulating gene expression on transcription initiation level are very limited and thus it must be modulated by altering the mRNA half-life. Moreover, the mitochondrial polycistronic transcripts contain vast intergenic regions that must be degraded. The human mitochondrial degradosome, which is composed of polynucleotide phosphorylase (PNPase) and SUV3 helicase, is the only RNA degradation machinery with documented role in mtRNA decay. However, PNPase leaves short (4-5 nt) oligonucleotides as the final product, which have to be further degraded by another nuclease. Natural candidate to perform this function is REXO2, homolog of the bacterial oligoribonuclease - the only essential exonuclease in *E. coli*. Previously, the human enzyme was shown to be capable of efficient degradation of both RNA and DNA 5-nt single-stranded oligomers, as well as of TMP 5'-p-nitrophenyl ester. It was also reported that REXO2 is crucial for maintaining mitochondrial homeostasis, but its direct function still has to be elucidated.

We demonstrate that REXO2 is capable of degrading *in vitro* not only  $\approx 5$  nt RNA oligomers, but also significantly longer (20-80 nt) single-stranded RNA substrates, with preference for  $\approx 10$  nt fragments. However, in the case of DNase activity of REXO2, its substrate specificity is restricted to  $<10$  nt oligomers. Interestingly, the mechanism of REXO2 activity is dependent on substrate length. In contrast to some of the previous studies our results suggest that human enzyme, similarly as its bacterial homologs, exists mostly as a dimer.

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## 490 RNA Half-Life in Single T Cells

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**Introduction:** Gene expression profile is dictated by the delicate equilibrium of RNA synthesis and turnover. Accurate regulation of mRNA turnover – whether by microRNAs or other post-transcriptional mechanisms – is crucial for immune system development and disease. However, there is still lack of methods to accurately measure this dynamic aspect of gene regulation in a high throughput manner to understand post-transcriptional mechanisms that affect the immune system.

**Materials and Methods:** We sorted single T cells into 96 well plates at different time points after transcription inhibition to profile the RNA decay rates of over 300 T cell marker genes in hundreds of cells with molecular barcoding. Unlike other traditional assays, molecular barcoding allows accurate counting of originally tagged mRNA transcripts by eliminating PCR bias.

**Results and Conclusions:** From our single cell sequencing data, we calculated the half-lives of T cells marker mRNAs based on molecular barcode counts. First, we identified a class of steady transcripts that are likely important for T cell maintenance. On the other hand, we identified a class of transcripts with extremely rapid RNA turnover, suggesting that they are important in dynamic cellular responses such as cell cycle or T cell activation. Unlike traditional RNA-seq assays that measure the steady state expression of genes, RNA half life profiling in single cells uncovers the underappreciated dynamics of gene regulation in development and disease.

## 491 An Improved MS2-MCP system to follow single mRNAs from transcription to degradation in living *S. cerevisiae*

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Techniques following single mRNAs are essential tools to study temporal and spatial regulation of gene expression. Insertion of multiple MS2 binding sites (MBS) into mRNAs that are bound by the MS2 coat protein (MCP) fused to a fluorescent protein allow the visualization of single mRNA molecules to study transcription, export and mRNA translation. However, in *S. cerevisiae*, tight binding of the MBS-MCP complex protects the MBS from being efficiently degraded, triggering the accumulation of 3' decay fragments. We have engineered a novel MBS-MCP tagging system (version 6) that combines reduced affinity of the MCP-GFP complex and increased space between the MS2 stem-loops to prevent decay products from accumulating, while still detecting single mRNA molecules *in vivo*. These improvements allow the MBSV6-MCP system to follow all stages of mRNA life, from transcription to degradation. Using both single molecule FISH and live imaging we followed highly regulated cell cycle mRNAs such as *ASH1* and *CLB2* from birth to death, demonstrating that the new MBSV6-MCP system can be used to visualize when and where the mRNAs decay in the cytoplasm. In addition, we explored the role of the promoter in the regulation of the cytoplasmic degradation of cell cycle mRNAs.

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## 492 BDF2 Transcript Sensitivity to RNase III-Mediated Decay is Heavily Governed by its Transcript Localization and Vulnerability to Cleavage

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Bromodomain factors are emerging as key regulators of transcription through their influence on chromatin remodeling and transcription initiation. Due to their significant impact on the eukaryotic transcriptome, bromodomain factors are under the control of several regulatory mechanisms to ensure their appropriate levels of expression in different environmental or growth conditions. One such factor in yeast, Bromodomain Factor 2 (BDF2), has been established as a target of both RNase III-mediated decay (RMD) and spliceosome-mediated decay (SMD). Our group previously showed that each of these nuclear degradation pathways are important to control BDF2 levels during osmotic stress or DNA replication stress. Although our previous work has shown that the RMD pathway for the BDF2 transcript is hyper-activated by salt stress and other environmental conditions, the mechanism driving the enhanced cleavage of BDF2 RNA under these conditions remains poorly understood. Here, we demonstrate that inhibiting the export of the BDF2 transcript out of the nucleus can recapitulate the stress-induced RMD hyper-activation. Using mutated versions of BDF2 bearing different stem-loop structures cleaved by RNase III, we also show that the cleavage efficiency of BDF2 *in vitro* and *in vivo* further contributes to BDF2 regulation by RMD. Altogether, these results suggest that the activity of RMD is heavily governed by the localization of its target transcripts, and that the efficiency of cleavage may further contribute to the sensitivity of these transcripts to degradation.

### 493 How does human Staufen1 recognize its natural dsRNA target involved in Staufen-mediated mRNA decay? Solution structure of RNA-Staufen1 complex

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Several dsRNA binding proteins (dsRBP) are involved in posttranscriptional regulation of gene expression and function based on recognition of their dsRNA targets. In general, dsRBPs interact with their cellular dsRNA targets through a combination of structure- and sequence-specific recognition. Staufen1 is a dsRBP involved in mRNA transport and localization, regulation of mRNA stability, translational efficiency and mRNA decay by a staufen-mediated mRNA decay (SMD) pathway. Staufen1 contains multiple domains and RNA binding domains (dsRBD) 3 and 4 bind to dsRNA targets. The recognition of dsRNA targets in 3'UTRs by Staufen1 has been shown to depend on enrichment of GC-content and secondary structure. The stem-loop within the 3'UTR of human ADP-ribosylation factor1 (hARF1) is one such dsRNA target and Staufen1 binding regulates cytoplasmic ARF1 mRNA levels by the SMD pathway. However, how Staufen proteins recognizes specific mRNA targets is still unknown.

To reveal how Staufen1 binds specific dsRNA targets, we are determining the solution structure of the ARF1 dsRNA - Staufen1 complex by nuclear magnetic resonance (NMR) spectroscopy. Electrophoretic mobility shift assay and fluorescent anisotropy measurement show that the interaction of ARF1 dsRNA with dsRBD3 and 4 results in a single complex with affinity in the nanomolar range. Our preliminary structure of the ARF1 dsRNA-Staufen1 dsRBD4 complex reveals that Staufen1 is indeed a sequence-specific dsRNA binding protein which specifically binds dsRNA targets by sequence readout in the minor groove. Staufen1 dsRBD4 interacts with dsRNA by three distinct binding regions, namely helix  $\alpha 1$  which recognizes consecutive pyrimidines *via* the minor groove,  $\beta 1\beta 2$  loop which anchors the dsRBD at the end of the dsRNA in the minor groove as well as lysines in helix  $\alpha 2$  which bind to the phosphodiester backbone from the major groove side. Mutagenesis data and the biological implications will be discussed.

### 494 Functional Classification of Mammalian Deadenylases

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Deadenylation of mRNA poly(A) tail is critical for translational repression and mRNA decay. Despite the central importance of deadenylases in gene expression, however, it remains unclear whether and how the multiple deadenylases are functionally distinct from each other. In mammals, the PAN2-PAN3 complex, the CNOT (Ccr4-Not) complex, and PARN are known to play major roles in mRNA deadenylation. To investigate substrate specificity of the major deadenylases on a genomic scale, we apply TAIL-seq which directly measures poly(A) length and 3'-end modifications. TAIL-seq along with RNAi of deadenylases reveals distinct properties of the deadenylases. Depletion of the PAN2-PAN3 complex leads to the accumulation of very long poly(A) tails (> ~150 nt) without a dramatic shift in the global poly(A) distribution, implying a preference of the PAN2-PAN3 complex for long poly(A) tails. Meanwhile, poly(A) length dramatically and globally increases upon disruption of the CNOT complex, confirming its role as a major deadenylase in mammals. The CNOT complex deadenylates all poly(A)+ mRNA species except for mitochondrial genome-encoded mRNAs, presumably due to their isolated subcellular localization. Both CNOT6/6L and CNOT7/8, catalytic subunits in the CNOT complex, redundantly deadenylate mRNA poly(A) tails. In contrast, depletion of PARN does not result in noticeable change in mRNA poly(A) tails, implicating a limited role of PARN in the mRNA decay pathway. Besides poly(A) tail length, our data also unveil the link between 3'-end uridylation and deadenylases. Depletion of CNOT7/8 leads to a great reduction of 3'-end uridylation on short poly(A) tails, which indicates that the CNOT complex facilitates uridylation. Overall, our genome-wide approach provides a comprehensive understanding of the differential roles of diverse deadenylases in mammalian cells.



**495 How a dimeric DEAD-box protein unwinds RNA to promote RNA decay***Hanna S. Yuan<sup>1</sup>, Jennifer Huen<sup>1</sup>, Woei-Chyn Chu<sup>2</sup>*<sup>1</sup>Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; <sup>2</sup>Department of Biomedical Engineering, National Yang-Ming University, Taipei, Taiwan

Messenger RNA decay is executed by various enzymes, including endoribonucleases, exoribonucleases and helicases. In bacterial RNA degradosome, a DEAD-box helicase that unwinds RNA with secondary structures plays a crucial role in promoting RNA decay. Here we report the crystal structure of a unique dimeric DEAD-box helicase CshA from *Geobacillus stearothermophilus* that cooperates with endo- and exo-ribonucleases in RNA degradosomes for mRNA turnover. In the crystal structure of the dimeric CshA, the two protomers are assembled into a V-shaped conformation through the dimerization domain (DD). Using small angle X-ray scattering combined with RNA-binding assays, we further show that C-terminal domain (CTD) protrudes outward from the DD and is critical for mediating strong cooperative RNA binding with the helicase core domain. Different from typical monomeric DEAD-box helicases, RNA binding of CshA is independent of ATP due to the presence of the CTD in the dimer, as RNA remains bound with CshA during ATP hydrolysis cycles. Our results demonstrate that the dimeric conformation and the CTD of CshA are crucial for its ATP-independent RNA binding activity and for efficient ATP hydrolysis. Messenger RNAs are thus effectively unwound and degraded in a processive manner through cooperation between the exoribonucleases and CshA helicase.

**496 TDS (TUT-DIS3L2 Surveillance) targets aberrant ncRNAs and short transcripts of protein-coding genes in human cytoplasm***Dagmar Zigackova<sup>1</sup>, Zuzana Feketova<sup>1</sup>, Josef Pasulka<sup>1</sup>, Dmytro Ustianenko<sup>1</sup>, Andrea Fortova<sup>1</sup>, Lukas Bednarik<sup>1</sup>, Mihaela Zavolan<sup>2</sup>, Stepanka Vanacova<sup>1</sup>*<sup>1</sup>Central European Institute of Technology, Masaryk University, Brno, Czech Republic; <sup>2</sup>Biozentrum, University of Basel, Basel, Switzerland

The 3'-terminal RNA uridylation catalyzed by the terminal uridylyltransferases (TUTases) mediates degradation of various RNAs and processing of some ncRNAs<sup>1,2,3</sup>. DIS3L2 is mammalian oligo(U) specific exonuclease, that is involved in decay of uridylated precursors of let-7 miRNAs, tRNAs and cleaved mRNAs<sup>4,5,6</sup>. Its mutation is linked to the Perlman syndrome development and Wilms tumor progression<sup>7,8</sup>. However, the function of uridylation and the involvement of DIS3L2 in these diseases remains largely unknown. Recently, TUT-DIS3L2 pathway has been identified by several studies as widespread and conserved surveillance of aberrant uridylated transcripts<sup>9,10,11,12</sup>.

By using catalytical mutant of DIS3L2 as a bait, we have identified uridylated aberrant forms of multiple types of coding and noncoding RNAs<sup>9</sup>. We demonstrate, that extended and aberrantly processed forms of ncRNAs, such as snRNAs, rRNA, tRNAs, YRNAs, and also transcripts originating from pseudogenes are uridylated, and then bound and degraded by DIS3L2. Most interestingly, we uncovered a fraction of reads mapping to 5' termini of protein coding genes. The uridylation positions overlap with the position of stalled RNA polymerase II indicating, that these fragments originate from RNA Pol II stalling. Next, we show, that uridylated 5' fragments of mRNAs are exported to cytoplasm, where they are removed by the activity of DIS3L2.

In summary, our results demonstrate, that TUT-DIS3L2 surveillance (TDS) is a general cytoplasmic RNA mechanism assuring the removal of aberrant transcripts<sup>9</sup>.

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## 498 Tandem hnRNP A1 RNA Recognition Motifs act in concert to repress the splicing of Survival Motor Neuron exon 7

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hnRNP A1 participates in alternative splicing by specific recognition of splicing silencer elements on pre-mRNA by its two RNA recognition motifs (RRM), also called unwinding protein 1 (UP1). Although structures of nucleotide bound UP1 are available [1,2], the questions remain how hnRNP A1 can bind on pre-mRNA and how the individual RRM contribute to this process.

Here, we show that both RRM have similar affinities and sequence preferences, despite RRM1 recognising a more extended RNA motif than RRM2. Both RRM can bind simultaneously to a single bipartite motif, the intronic splicing silencer ISS-N1, which controls Survival of Motor Neuron (SMN) exon 7 splicing [3], with RRM2 binding to the upstream motif and RRM1 binding to the downstream motif. Disruption of the inter-RRM interface, and therefore the topology of UP1, impairs hnRNP A1 splicing function in cells. Moreover, RRM1 can bind with high affinity only when its binding platform is supplemented by the inter-RRM linker. SMN exon 7 splicing repression requires RNA binding by both RRM, in particular RRM2. Furthermore, both binding sites within the ISS-N1 are important for splicing repression and their contributions are cumulative rather than synergetic.

Taken together we show that hnRNP A1 splicing repression of SMN strongly depends on both RRM, which must bind RNA, and that the architecture and organisation of the two RRM of hnRNP A1 strongly influence its function.

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**499 Are RNA-driven oligomeric changes a mechanism of regulation of viral RNA chaperones?***Jack Bravo<sup>1</sup>, Alex Borodavka<sup>1,2</sup>, Roman Tuma<sup>1</sup>*<sup>1</sup>University of Leeds, Leeds, UK; <sup>2</sup>Ludwig Maximilian University, Munich, Germany

Members of the *Reoviridae* family of segmented double-stranded (ds)RNA viruses face the particularly daunting challenge of packaging 9 – 12 distinct RNAs during assembly. The molecular basis for genomic segment counting is poorly understood. All known members of this family encode non-structural proteins that may function as RNA chaperones. These viral RNA chaperones are capable of non-specifically binding ssRNA with nanomolar affinity and melting helices. They are thought to act as functional homologues despite lacking any sequence similarity.

Little is known about how these viral RNA chaperones interact with RNA and facilitate its remodelling and subsequent inter-segment RNA-RNA interactions. The possibility of a conserved mechanism of helix unwinding has also remained largely unexplored.

In order to understand how this process is mediated, we compare the mechanism of RNA-binding and stem loop unwinding by the viral RNA chaperones NSP2 (Rotavirus) and sNS (Avian Reovirus). We show that stem loop stability is a major factor that governs binding by these RNA chaperones. Using a combination of SAXS, native mass spectrometry and cryo-EM we demonstrate that sNS protein undergoes a transition from a ring-shaped hexamer to a higher order species upon binding RNA. Single-molecule fluorescence assays have provided insight into binding of larger RNAs, which recruit multiple copies of sNS or NSP2. Our results suggest that although these proteins lack sequence similarity and structural homology, varying in their oligomeric states, they adopt a conserved mechanism of RNA unwinding and annealing.

**500 Asymmetric base pair opening drives helicases unwinding dynamics***Francesco Colizzi<sup>1,2</sup>, Cibran Perez-Gonzalez<sup>4</sup>, Yaakov Levy<sup>3</sup>, J. Carlos Penedo<sup>4</sup>, Giovanni Bussi<sup>1</sup>*<sup>1</sup>Scuola Internazionale Superiore di Studi Avanzati, Trieste, Italy; <sup>2</sup>Institute for Research in Biomedicine, Barcelona, Spain; <sup>3</sup>Weizmann Institute of Science, Rehovot, Israel; <sup>4</sup>School of Physics and Astronomy and Biomedical Science Research Complex, University of St Andrews, St Andrews, UK

The secondary structure of nucleic acids is emerging as an important determinant for a fine tuning of gene expression. For instance, DNA/DNA and mRNA/DNA duplex stability and mRNA structure have been proposed to control the efficiency of transcription and translation respectively by making helices more difficult to unwind. The intermediate species in the unwinding process are however difficult to characterize. Here, by analyzing thousands of base-pair opening and closing events from molecular simulations, we report a conserved asymmetry in the base (un)pairing process whereby the pyrimidine displacement is energetically favored with respect to the complementary purine one. We relate the recorded nucleobase dynamics to the behavior of small helicases and we predict the universal validity of a purine/pyrimidine bias during the unwinding of substrates with equal thermodynamic stability. Specifically, helicases would prefer those substrates that are rich in pyrimidine nucleotides in the displaced strand, whether RNA or DNA. As a proof of concept, we experimentally tested the unwinding efficiency of several helicase-duplex systems by biochemical and biophysical assays. The experiments showed that substrates with displaced strands rich in pyrimidines were—in all the cases—more efficiently unwound, thus corroborating the computational model. The unwinding pathways populated by RNA and DNA duplexes offer an intuitive understanding of the mechanisms underlying the workings of helicases, and our results suggest a fine and unexplored regulation of gene expression encoded in the direction-dependent unwindability of the double helix.

**501 Unbiased discovery of structured RNA recognition by cryptic RNA-binding regions***Qin Zhou, Vandita Bhat, Faruck Morcos, Zachary Campbell***University of Texas-Dallas, Richardson, TX, USA**

Eukaryotic genomes are resplendent with RNA-binding proteins. Understanding the specificity of these encounters is a fundamental step in the determination of their biological functions. Intriguingly, many RNA-binding proteins appear to lack canonical RNA-binding domains (1-3). While their mode of RNA interaction is cryptic, a commonality among these proteins is a preponderance of lysine and arginine rich regions. We hypothesized that because these regions are reminiscent of viral RNA-binding regions (e.g. HIV TAT and REV) they were also likely to recognize complex secondary structural elements. To this end, we have developed a novel methodology for unbiased discovery of structured RNA recognition that combines in vitro selection and high-throughput sequencing with direct coupling statistical analysis. The resulting compendium of specificities reveals pervasive recognition of structured elements by cryptic RNA-binding regions of viral, phage, and human origin. Key insights that have emerged from our models that include: (a) Mapping of endogenous sites of RNA recognition with base-pair resolution. (b) Identification of extended target structures up to 18 nucleotides in length. (c) Recruitment mechanisms for human miRNA processing. (d) Prediction and validation of permissive versus deleterious mutations in target structures. (e) Confirmation of functional interactions suggested by solution state structural ensembles. Taken together, our data suggest that cryptic RNA-binding regions facilitate a diverse array of biological functions via recognition of structured elements.

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**502 Hypoxic stress induces binding switch of HuR and AUF1 to controls FGF9 expression***Tsung-Ming Chen<sup>1,3</sup>, Yu-Chuan Wu<sup>1</sup>, Joseph T. Tseng<sup>2</sup>, Ying-Fan Chen<sup>1,3</sup>, Ming-Chang Hong<sup>3</sup>, Kuo-Hsun Chiu<sup>3</sup>, H. Sunny Sun<sup>1,2</sup>***<sup>1</sup>Institute of Molecular Medicine, National Cheng Kung University Medical College, Tainan, Taiwan;****<sup>2</sup>Institute of Bioinformatics and Biosignal Transduction, National Cheng Kung University, Tainan, Taiwan;****<sup>3</sup>Department and Graduate Institute of Aquaculture, National Kaohsiung Marine University, Kaohsiung, Taiwan**

Fibroblast growth factor 9 (FGF9) is an autocrine/paracrine growth factor which involves in many important physiological processes and human diseases. The expression of FGF9 is known to be under tightly controlled in transcriptional and posttranscriptional level. We have shown that AU rich element (ARE)-mediated decay is central to FGF9 expression through AUF1 binding and mRNA destabilization. HuR is another ARE- binding protein that functions as a stabilizing factor to regulate mRNA turnover and translational efficiency in stress conditions. In this study, we demonstrate that HuR regulates FGF9 expression under hypoxia by mediating FGF9 mRNA half-life. As HuR is a shuttle protein to carry HuR-bound RNA transporting between nuclear and cytoplasm, we examined cellular distributions of HuR in normoxia and hypoxia. Our data showed that HuR was significantly increased in cytoplasm in hypoxic cells. Using RNA interference to reciprocally knockdown these two genes, we demonstrated that HuR and AUF1 are collaboratively control FGF9 expression under normoxia. However, hypoxia altered the interplay between HuR and AUF1 and these two proteins turns to compete for FGF9 ARE binding. Consequently, HuR carries FGF9 mRNA to the cytoplasm for translation and thus increase FGF9 protein expression. This study provides more insights on post-transcriptional regulation of FGF9 mRNA to fine tune FGF9 homeostasis under hypoxia.



**503 switchSENSE: a new technology to study protein-RNA interactions***Antoine Cléry<sup>1</sup>, Thibault Sohier<sup>1</sup>, Thomas Welte<sup>2</sup>, Andreas Langer<sup>2</sup>, Frédéric H.T. Allain<sup>1</sup>*<sup>1</sup>ETH Zurich, 8093 Zurich, Switzerland; <sup>2</sup>Dynamic Biosensors GmbH, 82152 Martinsried, Germany

Characterization of RNA-binding protein interactions with RNA became inevitable to properly understand the cellular mechanisms involved in gene expression regulation. Structural investigations bring information at the atomic level on these interactions and complementary methods such as Isothermal Titration Calorimetry (ITC) and Surface Plasmon Resonance (SPR) are commonly used to quantify the affinity of these RNA-protein complexes and evaluate the effect of mutations affecting these interactions. The switchSENSE technology has recently been developed and already successfully used to investigate protein interactions with different types of binding partners (DNA, protein/peptide or even small molecules). In this study, we show that this method is also well suited to study RNA binding proteins (RBPs). We could successfully investigate the binding to RNA of three different RBPs (Fox-1, SRSF1 and Tra2- $\beta$ 1). Using small quantities of unlabelled biomolecules, we obtained  $K_D$  values very close to the ones determined previously by SPR or ITC for these complexes. These results show that the switchSENSE technology can be used as an alternative method to study protein-RNA interactions with  $K_D$  values in the low *micromolar* ( $10^{-6}$ ) to *nanomolar* ( $10^{-7}$  to  $10^{-9}$ ) and probably *picomolar* ( $10^{-10}$  to  $10^{-12}$ ) range.

Cléry A, Sohier TJ, Welte T, Langer A, Allain FH: **Switchsense: A new technology to study protein-RNA interactions.** *Methods* (2017)

**504 Molecular basis of human LARP4 interaction with RNA and protein partners***Isabel Cruz-Gallardo<sup>1</sup>, Luigi Martino<sup>2</sup>, Sandy Mattijssen<sup>3</sup>, Sergei Gaidamakov<sup>3</sup>, Andrew Atkinson<sup>1</sup>, Geoff Kelly<sup>2</sup>, Richard J. Maraia<sup>3</sup>, Maria R. Conte<sup>1</sup>*<sup>1</sup>Randall Division of Cell and Molecular Biophysics, King's College London, London, UK; <sup>2</sup>The Francis Crick Institute, London, UK; <sup>3</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

Human LARP4 belongs to a superfamily of eukaryotic RNA Binding Proteins (RBPs) called La-related proteins (LARPs) with important roles in different aspects of RNA metabolism, including transcriptional regulation, mRNA stabilisation and translation [1]. LARP4 promotes mRNA translation [2] and is been found to regulate cancer cell migration and invasion [3]. Its depletion increases cell elongation, which is correlated with an increment in the migration speed, while the overexpression decreases it and increases cell circularity [3]. LARP4 interacts with polyA RNA sequences, polyA binding protein (PABP) and RACK1 (Receptor for Activated C-Kinase) [2]. In this work, we analyse the molecular and structural basis of LARP4 binding to RNA and other proteins. We used a combination of EMSA, NMR and ITC to unveil the contribution and importance of different regions of the protein in its binding properties. Although LARP4 interaction site with RNA includes the "La-module", the RNA binding unit conserved in the LARPs superfamily, some differences arise in the interaction compared with other members of the family.

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## 505 Biochemical characterization of DDX3: the DEAD-box mechanism challenged

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DDX3X is the mammalian orthologue of Ded1, the yeast archetype of the DEAD-box helicase family. In the past years, DDX3 has been involved in many of the pathways of RNA metabolism from promoter regulation to translation initiation. Particularly, it has been shown to promote HIV-1 Gag translation initiation. DDX3 is an ATP-dependent RNA helicase whose biochemical activities as been poorly studied compared to Ded1. In this study, we produced DDX3 fused to the MBP and performed kinetic experiments to analyze its biochemical properties. Contrary to what has been previously described, DDX3 displays a strictly RNA-dependent ATPase activity with kinetic constants similar to those displayed by its yeast counterpart. We next evaluated DDX3 helicase activity towards RNA duplexes or RNA/DNA hybrids, with different lengths and single strand overhangs. Not only our results allow us to make new hypotheses about the DEAD-box helicases mechanism, but they also provide a framework that will allow further investigations on the molecular mechanism by which DDX3 promotes Gag translation initiation.

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## 506 Discovery of RNA-protein complexes by Grad-seq

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RNA-binding proteins (RBPs) are important factors in the regulation of gene expression. In bacteria, only two major families of regulatory RBPs are known: Hfq and CsrA, both of which act by regulating gene expression on mRNA level. While Hfq facilitates binding of small RNAs (sRNAs) to their target mRNAs and thereby leads to regulation, CsrA binds directly to mRNAs, altering their translation and stability. sRNAs are also a key factor in CsrA regulation, as they are able to sequester CsrA away from its mRNA targets. Furthermore, RBPs can be essential factors for bacterial virulence as it was shown for Hfq in the major human pathogen *Salmonella* Typhimurium. However, many bacterial species lack one or both of these two well-studied RBPs even though they express regulatory sRNAs, suggesting the existence of currently unknown RBPs.

Using gradient profiling by sequencing (Grad-seq), we are investigating the existence of overlooked RBPs. For this, whole bacterial cell lysates are run on a linear glycerol gradient, leading to partitioning of all soluble content. Since Grad-seq is performed under native conditions, RNA-protein complexes stay intact and sediment as a whole according to their biochemical properties. These interactions can then be investigated by fractionation of the gradient followed by RNA-seq and mass spectrometry of each of those fractions. The combined analysis of the resulting data sets allows us to draw conclusions which RNAs might interact with which proteins.

## 507 Identification of a lead compound affecting RNA-protein binding of mutant HTT RNA to counteract Huntington's disease

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The identification of small molecules able to recognize specific RNA secondary and tertiary structures is currently a key research topic for developing tools to modulate gene expression and therapeutic drugs. Expanded CAG trinucleotide repeats in Huntington's disease (HD) are known to be causative for neurotoxicity. These mutant CAG repeat RNAs do not only encode neurotoxic polyglutamine proteins, but also lead to a toxic-gain of function by aberrantly recruiting RNA-binding proteins. One of these RNA binding proteins that is captured by mutant HTT RNAs is the MID1 protein, which induces aberrant Huntingtin protein translation upon binding. Here we describe the selection and binding analysis of a set of commercially available small molecules that allowed identifying Furamidine as an effective CAG trinucleotide repeat binder. Our results show that Furamidine competes for the binding of MID1 to HTT mRNA *in vitro* and reduces the protein level of HTT in an HD cell line model. From our study, Furamidine emerges as a very promising lead compound for the future design of small molecules with high affinity for mRNA transcripts of pathogenic length contributing to HD and other CAG disorders.

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## 508 Molecular Basis of mRNA regulation by the TRIM-NHL protein LIN-41

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The TRIM-NHL family of proteins is conserved among metazoans and several members have well established roles in development and disease. A common feature of some members is a positively charged NHL domain, which has been shown to mediate the binding to RNA but the mechanisms, underlying the binding specificity of different members, remain unknown. LIN-41, a founding member of the TRIM-NHL family, functions as a post-transcriptional regulator in the *C.elegans* heterochronic pathway. Recent findings show that LIN-41 silences somatic mRNA by distinct position dependent mechanisms<sup>1</sup>. Additionally, we identified LIN-41 as a regulator of pluripotency in the germline<sup>2</sup>. Current experiments show that LIN-41 mRNA targets are different in the *C. elegans* germline and soma with distinct mechanisms of binding. In order to understand the molecular basis of mRNA regulation by LIN-41, we have solved the crystal structure of the NHL domain and are working towards identifying the RNA motif recognized by LIN-41. So far, among the TRIM-NHL proteins, RNA binding and motif recognition has been systematically studied only for the NHL domain of *Drosophila* Brat<sup>3</sup>. The amino-acid residues that make contact with RNA in the Brat NHL domain are not conserved in LIN-41 or human TRIM71. Consistently, we found that the RNA motif recognized by LIN-41 NHL domain is different from that of Brat. Hence, this study provides an interesting paradigm to study tissue specific mechanisms of mRNA regulation by LIN-41 and how minor changes in the RNA binding surface of a domain can lead to recognition of different RNA motifs.

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## 509 MS2-hairpin based affinity purification of lncRNP assembled in vivo

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RNA-protein interactions are vital for RNA stability and function. With recent identification of large quantity of lncRNAs, it is fascinating to know protein components in different lncRNPs and understand how these proteins are involved in the processing of lncRNA and its function. Thus purification of lncRNP is the first step to understand the detailed mechanism, here we describe an MS2-hairpin based affinity purification of lncRNP assembled in vivo.

For lncRNA expression, pcDNA5/FRT/TO vector was modified by inserting three copies of MS2 hairpins downstream of the MCS together with or without inserting optimized beta-globin intron 1 upstream of the MCS. Dox inducible stable cell line was established using Flp-In T-REx system by cotransfection of the modified pcDNA5/FRT/TO vector encoding the target lncRNA and pOG44 vector encoding the recombinase into HeLa cells with FRT site, puromycin screen was then performed for single colony. After verification of the lncRNA expression, cells were expanded into six 15cm plates and induced by Dox. Cells were then harvested after UV crosslink for nuclear extract (NE) or cytoplasmic extract (CE) preparation.

Depending on the localization of lncRNA, NE or CE was used for incubation with amylose beads pre-incubated with purified MS2-MBP fusion protein and lncRNP was eluted. Using this technique, lncRNP assembled on sense lncRNA was compared to that of the antisense lncRNA after separation on protein gel followed by silver staining. lncRNP of the same lncRNA assembled in nucleus was also compared to that assembled in cytoplasm. Protein components in the lncRNP were identified by mass spec analysis and verified by western blot. Further analysis will be needed to reveal proteins important for lncRNA function.

The technique provides the possibility for deep insights into how lncRNA functions via its binding proteins as well as the metabolism of different lncRNAs.

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## 510 Biochemical interrogation of lariat debranching enzyme with backbone-branched RNAs and inhibitors

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During splicing, introns are removed from the transcript as lariats. These lariat introns have their 5'-terminus linked to an internal branch-point adenosine ribosyl 2'-O through a 2'-5'-phosphodiester bond. Before the lariat intron can be processed further, the 2'-5'-phosphodiester bond is specifically cleaved by lariat debranching enzyme (Dbr1p). Following debranching, the RNA is degraded into constituent nucleotides for recycling, or more significantly, enters into miRNA or snoRNA biogenesis pathways or other important cellular regulatory processes. Additionally, Dbr1p has been implicated in the progression of amyotrophic lateral sclerosis (ALS) and HIV-1 infection. However, its role in these diseases remains unclear and the mechanism by which Dbr1p recognizes and specifically cleaves the backbone-branching 2'-5'-phosphodiester linkage in lariats is not well understood. To investigate how Dbr1p binds and cleaves its substrate, we have synthesized backbone-branched RNAs (bbRNAs) that contains the 2'-5' phosphodiester linkage. Solid-phase synthesis methods readily permit functional group modifications, unnatural linkages and labelling of the bbRNAs with fluorophores that are required for biochemical interrogation of the Dbr1p cleavage reaction. We have developed a FRET based assay to monitor Dbr1p cleavage in real time and determined kinetic parameters of the cleavage reaction of Dbr1p from *E. histolytica* and *S. cerevisiae*. Additionally, our FRET based assay has allowed us to evaluate inhibitors of the Dbr1p cleavage reaction. 'Click'-branched RNAs (cbRNAs) are similar to bbRNA, but contain a non-cleavable 2'-5'-triazole linkage. We find that these are competitive inhibitors of Dbr1p and aid in our examination of the binding modes of Dbr1p to branched RNA. The cbRNAs are easily tunable in size and we have created an assortment of Dbr1p inhibitors with varying inhibition constants.

**511 CLIP-seq guides the molecular characterization of CspC and CspE in Salmonella**

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Cold shock proteins (Csps) are defined by the presence of two RNA-binding domains and constitute one of the most conserved protein families in all three kingdoms of life. Bacterial species encode multiple Csps, several of which are constitutively expressed and whose biological function remains poorly understood. For example, *Salmonella* CspC and CspE are highly expressed during growth at 37°C and in infection-relevant conditions. To understand their physiological roles, we have used in vivo RNA-protein crosslinking followed by deep sequencing (CLIP-seq) [1] which provided a high-resolution map of their binding sites. Furthermore, in silico alignment of the common CLIP peaks highlighted a consensus motif, CUG, as a common feature in the CspC and CspE binding regions.

To obtain a detailed molecular mechanistic view of CspC and CspE function, we used *ecnB* mRNA as a model transcript. It contains two CLIP-predicted common binding sites and it is negatively regulated in the double *cspCE* deletion strain. We found that the expression of the *ecnB* mRNA depends on the presence of CspC or CspE, which bind and stabilize the transcript post-transcriptionally. To dissect the molecular determinants of Csp-mediated regulation of the *ecnB* mRNA, we used in vitro gel shift assays with different mutants of the predicted binding sites. Only one of the two CLIP peaks of *ecnB* is essential for binding of CspC and CspE. Moreover, a single point mutation in the CUG motif inside this peak decreased significantly the affinity to the proteins, validating the in silico prediction. Computation of *ecnB* mRNA folding revealed that the second peak is involved in the formation of a stem-loop, suggesting that both sequence and structural elements could indeed be required for binding to CspC and CspE.

**512 Ubiquitination controls miRNA-binding of HuR and augments extracellular export of miRNA**

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miRNAs, the 22 nucleotide long non-coding RNAs, form miRNP complexes with Argonaute proteins and regulate gene expression by imperfect base pairing to the 3'UTR of target messages. Human ELAV protein HuR is a RNA-binding protein which has strong affinity for AU rich elements (ARE) in the 3'UTR of target mRNAs. It binds with target mRNAs by replacing the miRNPs, stabilizes the mRNAs and facilitates their translation. Therefore, HuR is a negative regulator of miRNA function as it relieves the mRNAs from miRNA-mediated repression.

Exosomes are Extracellular Vesicles (EVs) of 30-90 nm and are secreted by a wide range of cells and contain proteins, RNAs and miRNAs. They help in cell-to-cell communication, by transporting various proteins, mRNAs and miRNAs. We have observed a reduction in cellular miR-122 content in amino acid starved human hepatic cells, due to their accelerated extracellular export. HuR accelerates this Extracellular Vesicles (EVs) mediated export of miRNAs. In stressed cells, HuR replaces miRNPs from target messages and is both necessary and sufficient for the export of corresponding miRNAs. HuR reversibly binds miRNAs and replaces them from Ago2 on endoplasmic reticulum (ER) and subsequently, itself gets freed from respective miRNAs upon ubiquitination on multivesicular bodies (MVBs). HuR-unloaded miRNAs get exported out via EVs thereby delimiting cellular miR-122 level during amino acid starvation. Therefore, by modulating extracellular export of miR-122, HuR controls stress response and autophagy in amino acid starved human hepatic cells. I will discuss the mechanism of HuR driven export of miRNAs in human cells.

1. Ref: Mukherjee, K., Ghoshal, B., Ghosh, S., Chakrabarti, Y., Swetha, S., Das, S. and **Bhattacharyya, S.N.**, (2016). Reversible HuR-microRNA binding controls extracellular export of miR-122 & augments stress response. *EMBO Rep.* e201541930.



### 513 A post transcriptional knights' festival in cancer - The oncogenic RNA binding protein IGF2BP1 shields mRNAs from miRNA-mediated silencing

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The RNA binding protein IGF2BP1 is a member of the insulin-like growth factor-2 mRNA-binding protein family (IGF2BPs) comprising three paralogues. All IGF2BPs share high homology but have distinct expression patterns. IGF2BP2 is ubiquitously expressed in adult organisms. In contrast, IGF2BP1 and IGF2BP3 show an oncofetal expression pattern with high abundance during embryogenesis and severe upregulation or even *de novo* synthesis in various cancers including ovarian carcinoma. In the latter, IGF2BP1 expression is associated with an overall poor prognosis. In ovarian carcinoma-derived tumor cells, IGF2BP1 promotes an oncogenic triangle consisting of the let-7 miRNA targets HMGA2, LIN28B and IGF2BP1 itself. IGF2BP1 enhances the expression of all triangle factors by recruiting the respective mRNAs in safe guard mRNPs lacking RISC components like AGO proteins and let-7 miRNAs.

The major proposed role of IGF2BP1 is the stabilization of target mRNAs and CLIP analyses indicate a pleiotropic target spectrum. In tumor cells the protein serves various oncogenic roles including the enhancement of tumor cell proliferation, migration, invasion and self-renewal. Consistently, the depletion of IGF2BP1 results in a significant downregulation of mRNAs encoding factors with roles in proliferation, adhesion, migration and extracellular matrix organization in ovarian carcinoma derived ES-2 cells. Intriguingly, downregulated mRNAs comprise long 3'UTRs that provide a bona fide landscape for a post-transcriptional "knights festival" including multiple IGF2BP1 binding sites identified by CLIP analyses and multiple miRNA targeting sites. By depleting either the shielding factor or the attacking miRNAs we identified and validated various novel target mRNAs like SIRT1 or MAPK6 in cancer-derived cells. These mRNAs are shielded by the safe guard RNA-binding protein IGF2BP1 from miRNA-mediated silencing.

### 514 To catch a thief: Finding the RNA binding proteins hijacked by HIV-1

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Due to their limited coding capacity of viral genomes, most viruses strongly rely on the host cellular machinery to accomplish their biological cycle. In particular, the human immunodeficiency virus type 1 (HIV-1) requires several host RNA-binding proteins (RBPs), including the RNA polymerase II, to transcribe, translate and encapsidate their RNA genome. In addition, RBPs play important roles as sensors of intermediaries of viral replication in the antiviral response. However, the complete repertoire of cellular RBPs utilised by HIV-1 remains largely unknown.

To identify RBPs playing a role in HIV-1 biological cycle, we applied here RNA interactome capture to a CD4<sup>+</sup> T-cell line infected with HIV-1. First, protein-RNA interactions are covalently linked by *in vivo* UV crosslinking. Protein-RNA complexes are purified using oligo(dT), applying very stringent washes. Finally, the repertoire of RBPs differentially regulated by HIV-1 is determined by quantitative proteomics of uninfected versus infected cells.

Because ~20% of the mRNA in HIV-1 infected cells is viral RNA, we expect that many of the upregulated RBPs will be interacting with this abundant transcript. Indeed, the HIV-1 RNA binding protein Rev is enriched in the dataset, indicating the successful purification of RBPs with the viral RNA.

On the other hand, HIV-1 is expected to shut off cellular functions that may impair viral replication. Our study uncovers dozens of host RBPs with differential RNA-binding activity in infected cells. Candidate based functional validation shows that these proteins may play important roles in HIV-1 infection.

In summary, we provide a comprehensive set of host proteins with differential RNA binding activity in mock and HIV-1 infected cells. These proteins may represent new targets for host-based antiviral therapy.

## 515 Matrin3 directly binds to intronic pyrimidine-rich sequences and controls alternative splicing

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Matrin3 is one of RNA binding proteins that are localized in nuclear matrix. Although various roles in RNA metabolism have been reported for Matrin3, in vivo target RNAs, which Matrin3 directly binds to, have not investigated comprehensively so far. Here, we show that Matrin3 predominantly binds to intronic regions of pre-mRNAs. Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP) analysis revealed that Matrin3 recognizes pyrimidine-rich sequences as binding motifs, including polypyrimidine tract, a splicing-regulatory element. Splicing-sensitive microarray analysis demonstrated that depletion of Matrin3 preferentially increases the inclusion of cassette exons that are adjacent to introns that contain Matrin3-binding sites. We further found that although most of the genes targeted by polypyrimidine tract binding protein 1 (PTBP1) were shared by Matrin3, Matrin3 can control alternative splicing in PTBP1-independent manner, at least in part. These findings suggest that Matrin3 is a splicing regulator that targets intronic pyrimidine-rich sequences.

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## 516 PKR senses signals from the nucleus and mitochondria by interacting with endogenous double-stranded RNAs

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As a member of innate immune response proteins, PKR has been regarded as a sensor of viral double-stranded RNAs (dsRNAs). However, recent evidences suggested that PKR can be activated in uninfected cells by cellular dsRNAs such as inverted Alu repeats (IRAlus). Here, we apply formaldehyde mediated crosslinking-immunoprecipitation sequencing to identify PKR-interacting dsRNAs in human cells. Our analyses reveal that PKR binds to diverse types of non-coding RNAs (ncRNAs) such as transposable elements, repeat elements, and even satellite RNAs and that IRAlus only occupy a small fraction. Particularly, a major class of PKR-interacting RNAs is provided by mitochondrial ncRNAs, which form intermolecular dsRNAs with their sense counterparts. We further demonstrate that PKR is localized in mitochondria and disruption of mitochondrial transcription affects PKR phosphorylation and phosphorylation status of eIF2 $\alpha$ , a downstream substrate of PKR. Our study reveals mitochondrial ncRNAs as PKR activators and suggests the role of PKR as a coordinator of mitochondrial-nuclear communication.

## 517 Structural and Functional Study of human Argonaute3

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Argonaute proteins are loaded with microRNAs to trigger posttranscriptional gene regulation by interacting with target messenger RNAs. Human possess four Argonaute proteins; Argonaute1, Argonaute2, Argonaute3, and Argonaute4, and only Argonaute2 has been reported to retain RNA cleavage activity. Nevertheless, Argonaute2 and Argonaute3 share the same catalytic tetrad, Asp-Glu-Asp-His. This fact raises questions as to why slicer deficient Argonaute3 has retained the catalytic tetrad through the molecular evolution and how Argonaute3 avoids target cleavage. Here we report that Argonaute3 can cleave RNAs though its activity is lower than that of Argonaute2. The recombinant protein as well as the immunoprecipitated one from HEK293T cells cleaved RNAs of 60-nucleotides containing a fully complementary sequence. However, they did not cleave short RNAs of 23-nucleotides. We also determined the crystal structure of human Argonaute3 in complex with guide RNA. The current structure shows that the Argonaute3-specific insertion in the N domain remains as a flexible loop, which seems to be involved in target recognition in the nucleic acid binding channel. Based on these structural and functional studies, we discuss differences in the target recognition and cleavage activity between Argonaute2 and Argonaute3.

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## 518 The RNA binding protein SERBP1 is a novel oncogenic driver in glioblastoma

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Glioblastoma (GBM) is the most common and most aggressive type of brain tumor. GBM patients' life expectancy is about 15 month after diagnosis, and less than 5% of patients survive more than five years. Recently, genomic studies conducted under the TCGA consortium identified important driver mutations, chromosomal alterations and changes in gene expression profile that improved tumor classification and our understanding of treatment response and survival. However, novel treatment strategies based on these findings did not meet expectations and researchers are still looking for effective ways to target GBM. Despite their central role in gene expression and large range of target genes, RNA binding proteins have been overlooked in this context. By combining genomics and biological methods, our lab has been searching for oncogenic RBPs and recently identified the poorly characterized SERBP1 as a major player in gliomagenesis. SERBP1 has all characteristics of an oncofetal RBP; on one hand, it is relevant during development but on the other, it promotes oncogenic activation. Using data from two independent sources (TCGA and Shanghai Military Hospital), we determined that SERBP1 is highly expressed in GBMs when compared to normal brain and similarly, highly expressed in high grade gliomas (GBMs) vs. low grade gliomas. High expression of SERBP1 correlates with poor prognosis and we showed that it can influence both chemo- and radio-resistance. SERBP1 knock-out CRISPR GBM lines do not survive, showing that tumor cells require SERBP1 expression. SERBP1 knockdown in GBM lines produced dramatic impact on cancer relevant phenotypes; it decreased proliferation, viability, colony formation, migration and invasion while promoting apoptosis. Importantly, Glioma Stem Cells which are considered the source of tumor initiation and relapse, are very sensitive to SERBP1 silencing. SERBP1 has exclusive cytoplasmic localization and regulates translation and mRNA decay by binding to GC rich motifs. Genomic analyses revealed that SERBP1 impacts glioblastoma development via multiple routes that includes blockage of neuronal differentiation and apoptosis, activation of angiogenesis and AKT pathways. In conclusion, due its broad impact on cancer relevant processes and pathways, we believe SERBP1 can emerge as an important target in GBM.

## 519 Exploring Macromolecular Interactions of DDX5 Helicase and Long Non-Coding RNA Rmrp Involved in Autoimmune Diseases

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The adaptive immune system is a powerful means by which higher organisms protect themselves from a variety of pathogens. T-helper 17 (TH17) cells are an important component of the adaptive immune system involved in the production of inflammatory cytokines responsible for this protection. ROR $\gamma$ t is a key transcriptional regulator of these cytokines, dysregulation of which causes tissue destruction and chronic inflammation in autoimmune disorders. Recent studies have revealed a non-coding RNA (ncRNA) Rmrp and an RNA helicase DDX5 as regulators of ROR $\gamma$ t. Rmrp is best known as the RNA component of RNase MRP and mutations in this RNA cause the severe autosomal disease cartilage hair hypoplasia (CHH). CHH is a pleiotropic disease characterized by a short stature, skeletal dysplasia, and in some cases immunodeficiency, consistent with a role for Rmrp in immune responses.

To understand how Rmrp and DDX5 regulate cytokine transcription through ROR $\gamma$ t, it is important to biochemically and biophysically characterize the molecular interactions between these key regulatory players. These studies are, however, challenging due to the low stability of the full-length human proteins expressed in bacterial systems. We were able to purify both DDX5 and ROR $\gamma$ t and characterize their interactions with Rmrp RNA by binding assays and nuclease footprinting *in vitro*. Our footprinting experiments revealed RNA modulations throughout most of the RNA sequence, with several strong sites of protections in peripheral regions. These sites of modulation may be involved in the conformational rearrangements of RNA and DDX5 for interactions with ROR $\gamma$ t and subsequent activation of transcription. Binding experiments showed strong affinity between DDX5 and Rmrp which appears to be non-sequence specific. Further binding experiments demonstrated that the intrinsically disordered and unstable termini of DDX5 are responsible for high affinity RNA binding while the helicase core alone contributes much lower affinity to Rmrp binding. Our study provides the framework for future analyses focused on understanding how disease related mutants of Rmrp affect RNA structure and protein binding. Ultimately, this work aims to clarify the role of Rmrp in inflammation and autoimmune diseases.

## 520 Impact of RNA structure on ZFP36L2 interaction with Luteinizing Hormone Receptor mRNA

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ZFP36L2 protein destabilizes AU-rich element-containing transcripts and has been implicated in female fertility. We have shown that only one of three putative AREs within the 3'UTR of murine LHR mRNA, *i.e.*, ARE 2197, is capable of interacting with ZFP36L2. To dissect the structural elements of ARE 2197 that mediate protein-RNA binding, we performed whole-transcript SHAPE-MaP (Selective 2' Hydroxyl Acylation by Primer Extension-Mutational Profiling). ARE 2301 contains only a pentameric sequence (AUUUA) which could explain its inability to bind to ZFP36L2; however our SHAPE-MaP data provides further explanation, because these nucleotides are poorly reactive, thus are not prone to interaction. The two other AREs are exposed in hairpin loops, but the non-binding ARE 2444 contains only a pentameric sequence (AUUUA) similar to ARE 2301 and is insufficient to mediate protein binding. Only the functional ARE 2197 contains a heptameric sequence of UAUUUUAU, which also has high SHAPE reactivity and is located in a hairpin loop. To further extend the molecular details of these AREs, we examined human LHR mRNA. Similar to mouse, only one ARE in hLHR mRNA was capable of binding to ZFP36L2, and also is located a hairpin structure. To further investigate the role of the secondary structure stability we mutated specific nucleotides located in the stem of this hairpin structure and found it to be paramount to explain the binding affinity. Collectively, our results suggest that a combination of minimal ARE sequence, likely a heptamer, and the structural location of the ARE in a hairpin loop as well as the secondary structure of the hairpin are important molecular components of ZFP36L2 binding.



## 521 Extending RNA sequence recognition of PUF proteins toward RNA sequence-specific regulation

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Sequence-specific RNA binding proteins are helpful to RNA targeting for controlling splicing, translation, RNA stability, and so on. However, they are not yet fully developed. Pumilio and fem-3-binding factor (PUF) proteins, a family of eukaryotic RNA binding proteins, provide an attractive scaffold to target RNA sequence-specifically since PUF can target arbitrary RNA sequences with each repeated unit recognizing one base. However, PUFs recognize only eight bases, which seems to be insufficient for targeting a specific mRNA. Taking into account of mRNA sequence diversity, it is required to develop proteins recognizing longer RNA sequences. In this research, we successfully extended RNA sequence recognition of PUF proteins toward RNA sequence-specific regulation.

Two PUFs were combined by inserting 8 RNA binding units of one PUF between units of another. To evaluate the RNA binding ability depending on the inserted position, we conducted luciferase reporter assay considering suppressed expression of luciferase as binding of PUFs to RNA. Repression activities of 16 units PUFs varied by their inserted position. 16 units PUFs inserted in specific positions showed stronger repression activities in common with different combinations of PUFs. Using electrophoretic mobility shift assays, these 16 units PUFs did not bind to the partially matched RNA including the binding site of the original successive 8 units, which suggests that 16 units PUFs recognize longer RNA sequences.

In order to control an endogenous mRNA metabolism, 16-repeats PUFs were designed to target the 3'UTR of the endogenous vascular endothelial growth factor A (VEGFA) mRNA. Artificial PUFs fused with tristetraprolin, which promotes mRNA degradation or translation repression, successfully repressed the production of VEGFA, suggesting that specific binding of 16 units PUFs to the endogenous mRNA.

This result provides an insight for designing tools regulating a specific endogenous gene at the post-transcriptional level.

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## 522 The subcellular localisation of EhRRP6 and its involvement in pre-ribosomal RNA processing in growth-stressed *Entamoeba histolytica*

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The eukaryotic exosome complex plays a pivotal role in RNA biogenesis, maturation, surveillance and differential expression of various RNAs in response to varying environmental signals. The exosome is composed of evolutionary conserved nine core subunits and the associated exonucleases Rrp6 and Rrp44. Rrp6p is crucial for the processing of rRNAs, other non-coding RNAs, regulation of polyA tail length and termination of transcription. Rrp6p, a 3'-5' exonuclease is required for degradation of 5'-external transcribed spacer (ETS) released from the rRNA precursors during the early steps of pre-rRNA processing. In the parasitic protist *Entamoeba histolytica* in response to growth stress, there occurs the accumulation of unprocessed pre-rRNA and 5' ETS sub fragment. To understand the processes leading to this accumulation, we looked for Rrp6 and the exosome subunits in *E. histolytica*, by insilico approaches. Of the nine core exosomal subunits, seven had high percentage of sequence similarity with the yeast and human. The *EhRrp6* homolog contained exoribonuclease and HRDC domains like yeast but its N- terminus lacked the PMC2NT domain. *EhRrp6* complemented the temperature sensitive phenotype of yeast *rrp6Δ* cells suggesting conservation of biological activity. We showed 3'-5' exoribonuclease activity of *EhRrp6p* with in vitro-synthesized appropriate RNAs substrates. Like the yeast enzyme, *EhRrp6p* degraded unstructured RNA, but could degrade the stem-loops slowly. Furthermore, immunolocalization revealed that *EhRrp6* was nuclearlocalized in normal cells but was diminished from nucleus during serum starvation, which could explain the accumulation of 5'ETS during stress. Our study shows functional conservation of *EhRrp6p* in *E. histolytica*, an early-branching eukaryote, and will help to understand the evolution of exosomal components and their regulatory function.

Keywords-*Entamoeba histolytica*, Exosome complex, rRNA processing, *EhRrp6*.



**523 RNA dependent localization of the RBP Bfr1p to the endoplasmic reticulum in *S.cerevisiae***Manchal Srinivas, Ralf-Peter Jansen**Interfaculty Institute of Biochemistry, University of Tuebingen, University of Tuebingen, Germany**

Brefeldin A resistance protein (Bfr1p) is a non-essential yeast protein conserved in *ascomycetes*. It was originally identified as a high-copy suppressor of brefeldin A-induced lethality in *Saccharomyces cerevisiae*. Deletion of BFR1 in yeast results in multiple defects, including chromosomal mis-segregation, altered cell shape and altered cell size. These defects suggest that the protein functions in the secretory pathway as well as in ploidy maintenance. Although Bfr1p does not contain canonical-RNA binding domains, UV-crosslinking and high resolution mass-spectrometry revealed mRNAs 6 binding sites in Bfr1p<sup>1</sup>. Analysis of mRNAs bound to Bfr1p revealed an enrichment of mRNAs translating at the endoplasmic reticulum (ER)<sup>2</sup>.

To understand the molecular mechanism and role of Bfr1p, we created mutations in the RNA binding sites and characterized their impact on its known functions. Our data shows that the N-terminus of Bfr1p (Bfr1<sub>(1-397)</sub>), which contains all RNA binding residues is sufficient to maintain correct chromosomal ploidy and to localize Bfr1p to the ER. Surprisingly, no mutation of the known RNA binding residues does affect ploidy. However, a single mutation (F239A) at the highly conserved RNA-crosslinking phenylalanine abolishes ER localization of Bfr1p. We speculate that RNA binding and ploidy maintenance are independent functions of Bfr1p and that the protein's role in secretion is mediated via its target mRNAs.

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**524 Nucleic acid-binding properties of two Hfq paralogs from *Aquifex aeolicus***Kimberly Stanek, Jennifer Patterson-West, Peter Randolph, Cameron Mura**University of Virginia, Charlottesville, VA, USA**

The bacterial host factor Hfq is an RNA-binding protein that facilitates the interaction of mRNAs with small regulatory RNAs (sRNAs) and acts as a hub in transcriptional regulatory networks. Hfq has been linked to numerous physiological pathways including stress response, quorum sensing, and biofilm formation. Bioinformatic evidence suggests that several bacterial species encode a second Hfq paralog, though the function and structure of these putative paralogs are unknown. We have identified two potential Hfq paralogs, denoted Hfq1 and Hfq2, in the genome of the deep-branching thermophile *Aquifex aeolicus* (*Aae*). To compare the structures and functions of Hfq1 and Hfq2, as well as elucidate any potential interactions between them, the proteins have been over-expressed, purified, crystallized, and biochemically characterized in terms of their nucleic acid-binding properties. The structures of *Aae* Hfq1 and Hfq2 have been determined to 1.5 and 2.0 Å resolution, respectively. Both proteins have also been co-crystallized with short RNA strands or single nucleotides. The overall folds and toroidal assemblies of both proteins are similar, but the RNA-binding profiles of the two homologs are quite different; notably, *Aae* Hfq2 exhibits higher-affinity binding at low pH and co-purifies with RNA as well as DNA. Hfq1 and Hfq2 do not appear to associate with one another, based on various lines of experimental data, and we suspect that each paralog serves a distinct functional role *in vivo*. Therefore, this may represent an example of neofunctionalization within the Hfq branch of the RNA-associated Sm superfamily.

## 525 The RNA-binding landscape of HCV NS3 and host DDX3 helicases

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Chronic hepatitis C virus (HCV) infection is an epidemic viral disease that threatens ~3% of the world population and may result in hepatic cirrhosis, liver failure, and hepatocellular carcinoma. During its replication in host cell, the plus-strand HCV genomic RNA must be first copied into a negative-strand RNA, which then serves as template for viral genome replication. In the process, an extended RNA duplex may form, thus predicting the involvement of RNA helicase(s) to unwind it. The HCV-encoded NS3, an SFII helicase, is conventionally thought to play such an unwinding role, yet persuasive biochemical evidence remains lacking. DDX3, a host DEAD-box protein, was also implicated in HCV life cycle. We seek to identify RNA targets of NS3 and DDX3, respectively, aiming to define their mechanistic roles in HCV replication cycle. We adopted a newly developed method, TRIBE (Targets of RNA binding protein identifying by editing), to identify their RNA targets under the conditions with or without HCV infection. Our preliminary data suggest that (1) neither NS3 nor DDX3 seems to appreciably interact with species of HCV RNAs; (2) the viral NS3 surprisingly makes physical contacts with a subset of cellular RNAs; (3) the landscape of cellular targets of DDX3 appears to be altered upon HCV infection. This last observation raises a possibility that HCV infection alters DDX3's binding partners to enforce a more favorable cellular environment for HCV replication. Further analysis is ongoing to explore this hypothesis.

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## 526 Regulation of AU-rich element RNA-binding proteins Zfp3611 and Zfp3612 in lipopolysaccharide-stimulated macrophages

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The tristetraprolin (TTP) family of mRNA-binding proteins contains three major members, Ttp, Zfp3611, and Zfp3612. All TTP family members have a highly conserved tandem zinc finger (TZF) domain, which is responsible for high-affinity binding to AU-rich elements (AREs) of mRNAs. Their N-terminal and C-terminal mRNA decay activation domains are <40% conserved. Ttp down-regulates the stability of ARE-containing inflammatory cytokine mRNAs and functions as an anti-inflammation regulator. The gene regulation and function of Zfp3611 and Zfp3612 in inflammation are unclear. Ttp mRNA and protein were highly induced by lipopolysaccharide (LPS) in mouse RAW264.7 macrophages, whereas Zfp3611 and Zfp3612 mRNAs were down-regulated and their proteins were phosphorylated during early LPS stimulation. Biochemical and functional analyses exhibited that the decrease of Zfp3612 mRNA was cross-regulated by Ttp, and the decrease of Zfp3611 mRNA was regulated by miR-27. Knockdown of Zfp3611 or Zfp3612 increased the basal level of Mkp-1 mRNAs by prolonging its half-life, which inhibited the activation of p38 MAPK under LPS stimulation and down-regulated Tnf $\alpha$  and Ttp mRNA. However, the Cox-2 mRNA level was increased only in Zfp3612 knockdown cells but not in Zfp3611 knockdown cells. The Site-directed mutagenesis combined with kinase analysis showed p38-MK2 phosphorylates Zfp3611(at Ser92 and Ser334 ) and Zfp3612 (at Ser127and Ser480) to modulate their interaction with 14-3-3 and Ccr4-Not deadenylase complex. Our findings imply that the expression and phosphorylation of Zfp3611 and Zfp3612 was tightly regulated to control their target mRNA stability during LPS stimulation. Zfp3611 and Zfp3612 play roles in innate immune response.

**527 Reforming the TRIBE: A more efficient approach to identify RNA-binding protein targets***Weijin Xu, Reazur Rahman, Michael Rosbash***Brandeis University, Waltham, MA 02454, USA**

RNA-binding proteins (RBPs) are important for many post-transcriptional regulatory events that act on pre-mRNA and mRNA. They include splicing, capping, polyadenylation, mRNA export from the nucleus to the cytoplasm and translation. Despite the fact that mutants in RBP genes are the primary cause of numerous human diseases including diseases of the nervous system, current methods to identify cell-specific RBP targets in small numbers of cells in vivo, especially small numbers of discrete neurons, are imperfect. This is because most of them require making and analyzing an extract from this small amount of material, with causes signal:noise problems. A previous publication from our lab reported the development of a genetic technique for this purpose, which we named TRIBE (Targets of RNA-binding proteins Identified By Editing). A TRIBE construct is made by fusing an RBP of interest to the catalytic domain of ADAR (ADARcd), which performs Adenosine-to-Inosine editing on RNA targets. The construct is expressed in specific cells, and the targets then identified by computational analysis of RNA-seq data from those cells, a much easier and cleaner approach than immunoprecipitation. However, the published TRIBE technique suffers the potential difficulty of a high false negative rate due to a strong substrate bias of the ADAR enzyme.

Here, we introduced a hyperactive mutation (E488Q) from a conserved key region of the ADARcd into the TRIBE construct, which creates a Hyper-TRIBE construct. Hyper-TRIBE identifies significantly more editing sites and target genes compared to the original TRIBE method. In addition, 70 percent of the Hyper-TRIBE identified genes now overlap much more successfully with CLIP results. This suggests that Hyper-TRIBE is not only much more efficient but also faithfully reflects the known binding specificity of the RBP. Comparisons of the editing percentages and the neighboring sequence preferences that surround the editing sites indicate that the increased number of editing sites are due to a higher editing rate as well as a reduced sequence bias of Hyper-ADARcd. Hyper-TRIBE therefore successfully addresses the high false negative rate of regular TRIBE and makes CRISPR knock-ins of Hyper-TRIBE attractive way to circumvent the potential negative impact of Hyper-TRIBE overexpression.

**528 Granule formation by the RNA binding protein heterogeneous nuclear ribonucleoprotein G (hnRNPG)***Katherine Zhou, Tao Pan***University of Chicago, Chicago, IL, USA**

Heterogeneous nuclear ribonucleoprotein G (hnRNPG) is a nuclear RNA binding protein that regulates diverse processes including transcription, alternative splicing, DNA repair, and sister chromatid cohesion. The domain structure of hnRNPG consists of an N-terminal RNA recognition motif and ~300 amino acids of low-complexity region rich in serine, arginine, glycine, and proline. We have previously shown that hnRNPG preferentially binds to RNAs containing *N*<sup>6</sup>-methyladenosine (m<sup>6</sup>A) using its low-complexity region, and that hnRNPG regulates the transcription and alternative splicing of hundreds of hnRNPG-bound transcripts containing m<sup>6</sup>A (Liu and Zhou et al., *N*<sup>6</sup>-methyladenosine alters RNA structure to regulate binding of a low-complexity protein, *Nucleic Acids Res.*, in press). We further investigated the formation of large complexes by hnRNPG *in vitro* and *in vivo*. *In vitro*, full-length hnRNPG protein forms large round particles with a diameter of ~200 nm, as well as 200-to-800-nm-long chains and clusters of smaller aggregates. RNA binding influences the size and conformation of hnRNPG complexes. In addition, a 58-residue segment of the low-complexity region of hnRNPG is sufficient to form complexes of similar shape but smaller size. In HEK293T cells, endogenous hnRNPG localizes to small nuclear granules. The cellular localization of hnRNPG depends on active transcription, and our results suggest that hnRNPG interacts with RNA polymerase II. The influence of RNA binding on the size and conformation of large hnRNPG complexes could have implications for the function of hnRNPG in transcriptional regulation.

## 529 RNA tools to dissect the underlying pathology of amyotrophic lateral sclerosis (ALS)

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a progressive degeneration of motor neurons in the brain and spinal cord, which leads to paralysis and death. ALS is characterized by the formation of cytoplasmic inclusion bodies containing aggregated proteins, which additionally sequester multiple RNA binding proteins (RBPs) causing their depletion from the nucleus where they normally function in mRNA processing.

It is currently unclear whether the functional depletion of nuclear processing factors or the granules per se are toxic. We aim to develop tools to separate the two potential pathological aspects of ALS by mimicking cytoplasmic mis-localization of disease-relevant RBPs without granule formation and monitor the consequences for motoneuron survival.

We will employ the inducible transcription of RNA-aptamers to reversibly sequester and concentrate selected nuclear RBPs in the cytoplasm and to functionally deplete them from the nucleus. As a proof of principle, we will set up the system with the splicing factor SRSF3. SRSF3 was chosen because it shuttles continuously from the nucleus to the cytoplasm and is present in stress granules. We have devised two different strategies:

In strategy one, we have designed a fusion RNA aptamer, which consists of two independent domains: a GFP-aptamer, which binds with high selectivity and affinity to GFP (Tome, 2014) and an extended single-stranded loop region, whose sequence is strongly bound by SRSF3 in vivo (Müller-McNicoll, 2016). Upon induction the fusion aptamer should trap SRSF3 onto GFP-tagged cytoplasmic residence proteins and prevent its nuclear re-import. Using confocal microscopy we could show that the fusion aptamer is expressed in vivo and co-localizes with cytoplasmic GFP. Moreover, SRSF3 co-immunopurifies with GFP in an RNA-dependent manner, only when the fusion aptamer is expressed. We are currently optimizing in vivo expression of the aptamers using different expression system and cell lines.

In strategy two, we are selecting specific RNA aptamers that bind the RS domain of SR proteins. This domain is crucial for nuclear re-import and aptamer binding blocks either its phosphorylation or binding to nuclear importers. Both strategies should lead to a specific and reversible nuclear depletion of SRSF3 and other disease-relevant RBPs.

## 530 Control of IL-2 homeostasis and CD4<sup>+</sup> T cell differentiation by HuR in allergic airway inflammation

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Naïve CD4<sup>+</sup> T cells differentiate into unique subsets (Th1, Th2, Th17, Tregs plus others) with characteristic signature transcription factors and cytokines. Posttranscriptional factors controlling these processes are poorly understood. We previously demonstrated that the RBP, HuR (*elavl1*), regulates prototypical Th2 factors (IL-4, IL-13 and GATA-3), which play important roles in asthma, by binding to their AU-rich (ARE) regions in their 3' UTRs to increase stability and translation. We made a gain of function transgenic HuR mouse model and demonstrated increased stability of Th2 factors, resulting in increased Th2 mediated inflammation. We hypothesized HuR plays a pivotal role in T cell activation and differentiation in allergic airway inflammation (asthma). We conditionally ablated HuR in T cells, using a novel distal *lck-cre* ROSA HuR<sup>fl/fl</sup> mouse. We demonstrated that HuR KO T cells cannot negatively regulate IL-2 expression (30-fold increase in mRNA, 7-fold in protein) and have defects in Th2, Th17 and Treg differentiation and cytokine production but not Th1. Since IL-2 and its signaling plays a critical role in T cell activation, we also investigated its' function. Using RIP and biotin pull-down techniques, we showed that HuR binds to CD25 (IL-2RA) 3' UTR and controls its translational efficiency but not transcript stability. Since Th2 cytokines are important in asthma, we asked whether HuR KO mice are able to develop allergic airway inflammation. We used the ovalbumin challenge model of asthma to demonstrate that HuR KO mice have decreases in eosinophils and IL-13 in bronchoalveolar lavage fluid from lungs; this completely ameliorated lung inflammation. Furthermore, when HuR KO mice are immunized with specific antigens, HuR KO T cells are unable to recognize and proliferate in response to antigen. Using RIP-SEQ and RNA-SEQ, we identified novel HuR-associated targets which we hypothesize play important roles in T cell biology. To our knowledge, this is the first time these techniques have been applied to primary murine T cells. We conclude that HuR plays critical roles in both multiple CD4<sup>+</sup> Th lineage differentiation, as well as T cell activation. Furthermore, HuR may also regulate genes involved in early TCR mediated activation events.



**531 Aberrant Splicing in B-Cell Acute Lymphoblastic Leukemia**

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One year ago, we began investigating the possibility of wide-spread mis-splicing in B-cell acute lymphoblastic leukemia (B-ALL), as had been reported for other types of chronic and acute leukemias. We began by comparing normal CD19-positive bone marrow samples with 12 B-ALL samples for which high-quality RNA-Seq datasets have been generated as part of the TARGET Initiative. We identified 808-1564 high confidence local splicing variation (LSV) events per leukemia sample (20% change with a 95% probability). Of particular importance was the consistency: 528 LSVs in 338 genes were observed in at least 20 out of 24 (>80%) possible pairwise (tumor-normal) comparisons. Following the release of updated TARGET data in July 2016, we extended this analysis to several hundred B-ALL samples. More than 25% of these alternative splicing events are exon skipping varieties, events known to be controlled, to a large extent, by the SR family of splicing factors (SF). We hypothesized that in the absence of genetic mutations, splice factors might be deregulated post-transcriptionally. Indeed, at least 5 of SRSF genes showed widespread variations in their own splicing patterns. For example, we observed in B-ALL samples an increase from ~20% to ~75% in the inclusion of the SRSF3 poison exon 4, which contains a premature termination codon and whose inclusion is known to decrease protein levels. Also deregulated at the exon usage level was one of its known targets TP53, which encodes the key tumor suppressor gene. Specifically, in several leukemia samples over 60% of TP53 mRNA included the poison exon 9 $\beta$ , which results in the expression of the hypomorphic p53 $\beta$  isoform. Of note, similar results were obtained for 13 out of 20 most commonly mutated B-ALL driver genes, TSG and oncogenes alike, fully consistent with the idea of aberrant exon usage as an oncogenic driver in B-cell acute lymphoblastic leukemia.

**532 Implication of Sam68 in migration of metastatic cells**

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**Context:** The formation of secondary tumors remains the leading cause of cancer-related deaths and most of the time defines the prognosis. Dissemination of cancer cells is a complex process which allows them to leave the primary tumor to form metastases in distant tissues. This process requires several interdependent biological mechanisms such as proliferation, adhesion and cell migration. Our laboratory focuses on RNA-binding proteins, including Sam68, which are known to regulate some of these mechanisms in the cell including adhesion and migration. Studies have revealed that overexpression of Sam68 is associated with a poor prognosis in some advanced stage cancers. Our hypothesis is that Sam68 would have an important role in tumor progression through the migration and adhesion processes of metastatic cells. Our first objective is to determine the effect of Sam68 on translation during adhesion and its effect on adhesion capacity. Our second objective is to determine the implication of the two functional domains of Sam68 in migration.

**Results:** For the first objective, we confirmed the importance of translation on the adhesion capacity. Indeed, we have shown that there is a strong translation of mRNA localized in early adhesion (SIC) structures favoring the formation of focal points of adhesion and that this would be regulated by RNA binding proteins such as Sam68.

For my second objective, we observed that a complete deletion of Sam68 negatively affects the migratory potential. We also used point mutations distributed in the RNA-binding domain and in the signaling domain of Sam68 in order to evaluate their effect on migration, invasion and cellular localization of the protein. In addition, we characterized the implication in migration of a Sam68 target mRNA and we are in the process of identifying new RNA targets.

**Conclusion:** Characterizing the role of Sam68 in the migration and adhesion of cancer cells would help to understand and potentially block metastatic progression and possibly improve prognosis.



### 533 The long non-coding RNA MALAT1 is upregulated in multiple sclerosis and involved in disease-associated alternative splicing events

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Multiple sclerosis (MS) is an autoimmune neurodegenerative disease, characterized by chronic inflammation, demyelination, and axonal damage. Alterations in the alternative splicing (AS) process are increasingly appreciated as pathogenic mechanisms underlying autoimmune diseases, including MS, in which an unbalance of AS isoforms has already been demonstrated to contribute to the disease etiology.

A comparative analysis of publicly available microarray and RNA-sequencing datasets highlighted a consistent dysregulation, among the non-coding genes, of the long non-coding RNA (lncRNA) MALAT1, which resulted upregulated in MS patients' blood compared to healthy controls. MALAT1 is an abundant lncRNA reported to influence AS through its interactions with pre-mRNA splicing factors within nuclear speckles. We first confirmed MALAT1 upregulation in peripheral blood mononuclear cells of MS patients compared to healthy controls by real-time RT-PCR assays (1.4- fold increase in MS patients;  $P < 0.05$ ). We then performed MALAT1 overexpression and downregulation experiments in HEK293, HeLa, SH-SY5Y, and Jurkat cells with the aim of analyzing the effects both on the expression of splicing factors and on the regulation of specific exons known to undergo AS. RNA-sequencing experiments and real-time RT-PCR assays revealed a differential expression of several splicing factors, including HNRNPF, HNRNPH, and CELF1. Moreover, MALAT1 modulation affects different AS events associated with MS, as demonstrated by in-vitro splicing assays performed both on endogenous transcripts and on minigene constructs. In particular, an analysis of the splicing pattern of IL7R (one of the top loci associated with MS by GWAS) showed that a particular AS event, consisting in the inclusion of exon 6, decreased upon MALAT1 overexpression, mimicking the functional consequence of the disease-associated polymorphism.

In conclusion, we provide evidence of a functional link between the lncRNA MALAT1, AS regulation, and MS-associated AS events, suggesting a potential role of MALAT1 in MS pathogenesis.

### 534 The RNA-binding protein LARP1 regulates the response to nutrient stress in cancer cells

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RNA-binding proteins play a crucial role in regulating gene expression, and consequently their aberrant expression and activity has been linked with driving many types of cancer. The RNA-binding protein LARP1 is overexpressed in a range of cancers, including ovarian and hepatocellular carcinoma, and has been shown to activate cancer progression. LARP1 is known to associate with over 3000 mRNAs and can regulate their expression by affecting their stability, translation and localisation. Accordingly, LARP1 may drive network-level shifts in gene expression to aid the survival and proliferation of cancer cells. Cancer cells typically exist under conditions of nutrient or hypoxic stress and therefore must reprogram gene expression to adapt to these conditions. This includes bypassing the integrated stress response, in which cells shut down protein synthesis and eventually undergo apoptosis.

Here, we show that LARP1 plays a central role in this process, allowing continued proliferation of cancer cells under conditions of nutrient stress. Knockdown of LARP1 diminishes the ability of cancer cells to adapt to stress conditions and sustain high-level proliferation. Furthermore, we demonstrate that LARP1 exists in a macromolecular complex that is substantially altered during nutrient stress, indicating that changes in protein-protein and protein-RNA interactions with LARP1 drive changes in post-transcriptional regulation of gene expression.

These findings illustrate the suitability of LARP1 as a target for rational drug design in those cancers in which it is overexpressed. Further, it reveals the potential of targeting other RNA-binding proteins, given their major role in regulating gene expression.

### 535 Intellectual disability-causing mutations in human TRMT1 enzyme abolish its function in tRNA modification, translation and oxidative stress survival

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Mutations in the tRNA methyltransferase 1 (TRMT1) gene have been identified as the cause of certain forms of autosomal recessive intellectual disability (ID). However, the molecular pathology underlying ID-associated TRMT1 mutations is unknown since the cellular functions and targets of the encoded TRMT1 protein remain to be determined. Here, we show that TRMT1 is responsible for catalyzing the dimethylguanosine (m<sub>2</sub>,2G) base modification in both nuclear and mitochondrial-encoded tRNAs. TRMT1-deficient cells lack the m<sub>2</sub>,2G modification in tRNA, exhibit decreased global protein synthesis and display perturbations in redox homeostasis, including increased basal ROS levels and hypersensitivity to oxidizing agents. Notably, ID-causing TRMT1 variants are unable to catalyze the formation of m<sub>2</sub>,2G due to defects in RNA binding and cannot rescue oxidative stress sensitivity. Our results uncover a critical biological role for TRMT1-catalyzed tRNA modification in redox metabolism and indicate that individuals with TRMT1-associated ID are likely to have major perturbations in cellular homeostasis due to the lack of m<sub>2</sub>,2G modifications.

### 536 Epistasis between genetic variants controlling alternative splicing of IL7R increases the risk of Multiple Sclerosis

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Multiple Sclerosis (MS) is an autoimmune disorder characterized by self-reactive T cell-mediated damage to neuronal myelin sheaths in the central nervous system leading to demyelination, neuronal death and progressive neurological dysfunction. A driver of increased MS risk is the soluble form of the interleukin-7 receptor alpha chain (sIL7R), produced by alternative splicing of IL7R exon 6. We have previously shown that the genetic variant rs6897932 in exon 6 of IL7R is strongly associated with MS risk and increases skipping of the exon leading to elevated sIL7R expression. Analysis of *trans*-acting splicing factors uncovered the RNA helicase DDX39B as a potent activator of this exon and a repressor of sIL7R. Moreover, we found strong genetic association of DDX39B variants with MS risk, and identified rs2523506 as a functional variant that reduces DDX39B protein levels by diminishing translation of DDX39B mRNAs. Importantly, this DDX39B variant showed strong genetic and functional epistasis with rs6897932 in IL7R, wherein the risk alleles at both loci work in concert to enhance skipping of IL7R exon 6, leading to a dose-dependent increase in MS risk. We functionally validated this epistasis both in cell lines and in primary CD4<sup>+</sup> T cells, where DDX39B depletion led to higher exon 6 skipping in the presence of the risk allele at rs6897932 compared to the protective allele. This study established the occurrence of biological epistasis in humans and provided mechanistic insight into the regulation of IL7R exon 6 splicing and its impact on MS risk.

To better understand how the exon is regulated, and to explore the potential therapeutic value of IL7R splicing modulation, we generated a set of tools that will facilitate screening of IL7R splicing-modulating compounds in cultured cells and *in vivo*. First, we generated a set of IL7R splicing fluorescent reporters that will enable high-throughput screening in cultured cells. Second, given that IL7R exon 6 is not regulated by alternative splicing in the mouse, we are currently generating mouse models of human IL7R exon 6 splicing carrying the alternative alleles of rs6897932, which will enable study of IL7R splicing and the efficacy of IL7R splicing-modulating compounds *in vivo*.

**537 Dynamics of RNA-bound proteome reveals master regulators of virus infection**

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RNA is a central molecule in virus biology, since it can function both as messenger RNA and as genome. While RNA metabolism is extremely complex, requiring more than 1,500 RNA-binding proteins (RBPs) [1], most viral genomes only encode a dozen of proteins of which only few bind RNA. Thus, viruses have developed sophisticated mechanisms to hijack the host resources for their own use. On the other hand, the host cell has also developed strategies to counteract viral replication through antiviral sensors that are often RBPs.

Adaptation of the "RNA interactome capture" [1-3] to cells infected with a cytoplasmic, positive stranded RNA virus model, named Sindbis virus (SINV), reveals a global alteration in the RNA-binding activity of hundreds of host RBPs in response to infection. Particularly, SINV inhibits nuclear functions such as splicing, 3'-end processing and export, and activates protein synthesis, 5' to 3' RNA degradation, stress RNA pathways and antiviral defences in the cytoplasm.

Using high-resolution microscopy and RNA-protein complex immunoprecipitation, we prove that most of the host RBPs with enhanced RNA binding activity in infected cells are re-localised to the viral replication factories, where they bind directly to SINV RNAs. Moreover, overexpression of some of these upregulated RBPs significantly delays viral replication, thus providing new insights into the host antiviral responses against SINV. Inhibition of upregulated RBPs with available drugs also negatively impact SINV replication, suggesting that these factors may represent novel targets for host-based antiviral strategies. Finally, we analysed the role of post-translational modifications and protein-protein interactions in the regulation of the RNA-binding activity of host RBPs. Strikingly, the altered association of host RBPs with RNA may be induced by the formation of different protein complexes upon infection. In summary, we show that RNA interactome capture offers an unprecedented opportunity to identify, in a system-wide manner, master regulators of virus infection.

[1] Castello et al., 2012 Cell

[2] Castello et al., 2013 Nature Protocols

[3] Castello et al., 2016 Mol Cell

**538 Variable penetrance of PARN (poly(A)-specific ribonuclease) loss-of-function (LOF) variants in a three generation pedigree**

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Dyskeratosis congenita (DC) and Hoyeraal-Hreidarsson (HH) syndrome are disorders of short telomeres that can be caused by biallelic pathogenic *PARN* variants. Telomere maintenance requires telomerase protein and RNA (hTR). Adenylation targets hTR for degradation. PARN deadenylates and stabilizes hTR. PARN loss-of-function (LOF) reduces hTR level and telomerase activity. Monoallelic LOF *PARN* variants cause pulmonary fibrosis, however, penetrance is incomplete.

To further investigate PARN LOF effect on health and telomere length, we studied a kinship with three children in the 3<sup>rd</sup> generation, two with HH and one unaffected. The proband had bone marrow failure, while the younger affected child did not. A rare *PARN* missense variant (PARN-Y91C) was transmitted to all children. A novel LOF variant (PARN-ins) was transmitted to the two affected children.

PARN-Y91C deadenylation activity *in vitro* was reduced 30-fold compared to wild type. PARN-ins transcript underwent nonsense-mediated decay. Therefore, hypofunctional PARN-Y91C was presumably the predominant protein in the affected siblings. In lymphoblastoid cell lines (LCLs) and compared to controls, PARN protein was reduced in proband and mother but similar in the affected sibling. PARN mRNA level in all family members was similar to controls. Together, these results indicate that additional factors modify PARN protein level in LCLs and differ between the proband and affected sibling. Also consistent with modifier presence, levels of multiple oligoadenylated H/ACA-box small nucleolar RNAs were increased in the proband, but not affected sibling, compared to controls.

Telomere flow FISH showed the mean B lymphocyte telomere length (B-TL) of the affected siblings was <1<sup>st</sup> percentile; father and unaffected sibling (carried PARN-Y91C) were ~10<sup>th</sup> percentile. The B-TL of the mother and her mother (carried PARN-ins) were ~50<sup>th</sup> percentile, maintaining telomere length despite reduced PARN level. This tolerance may reflect previously observed incomplete penetrance.

### 539 Pus10, a Pseudouridine Synthase and Apoptosis

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Pseudouridine ( $\Psi$ ) is the most common modified residue in RNAs. Members of six different families of  $\Psi$  synthases post-transcriptionally produce  $\Psi$  in various RNAs. Pus10 is the only known member of its family of  $\Psi$  synthases. Pus10 produces  $\Psi$ 54 and  $\Psi$ 55 in tRNAs of Archaea. *Pus10* homologs are present in Archaea and most Eukarya, but not in Bacteria and yeast. This coincides with the presence of  $\Psi$ 54 in the tRNAs of Archaea and higher Eukarya and its absence in Bacteria and yeast. The crystal structure of human Pus10 shows that the protein has two domains: an N-terminal THUMP-containing domain and a C-terminal  $\Psi$  synthase domain. The C-terminal domain contains the full set of conserved  $\Psi$  synthase active site residues, suggesting that human Pus10 may function as a  $\Psi$  synthase. Human *pus10* has also been suggested as a gene needed during TRAIL-induced apoptosis. As yet there is no evidence indicating any relationship between the roles of HuP10 in  $\Psi$  synthesis and in apoptosis. We analyzed the role of Pus10 in apoptosis after TRAIL treatment of TRAIL-sensitive PC3, MDA-MB-231 and RH30 cells, and cycloheximide-sensitized HeLa, LNCaP and MCF7 cells, by immunofluorescence, immunoblotting and several indicators of apoptosis. Pus10 is mainly present in the nucleus. It translocates mostly to mitochondria via CRM1-mediated nuclear export during TRAIL-induced apoptosis with concurrent release of cytochrome c. Caspase-3 is required for Pus10 translocation, which reciprocally amplifies caspase-3 activity, suggesting a role for nuclear components in major apoptosis pathways. The caspase-3-mediated movement of Pus10 and the release of mitochondrial contents that enhance caspase-3 activity may create a feedback amplification loop for all caspase-3-dependent apoptosis pathways. Furthermore, any defect in the interactions or translocation of Pus10 would reduce the sensitivity of tumor cells to TRAIL and other apoptosis inducing drugs. These findings would be useful to develop drugs for cancer therapy.

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### 540 The Anaplastic Thyroid Carcinoma and IGF2BP1 - from clinical finding to putative treatments

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The anaplastic thyroid carcinoma (ATC) is the least common but most aggressive type of all thyroid cancers with a median survival of approximately 4 months. The ATC is clinically presented by rapid tumor mass gain, accompanied by systemic metastasis to distant organs. By transcriptome profiling of primary ATCs we confirm a highly aggressive phenotype characterized by a severe trans-differentiation including the loss of typical thyroid marker expression and epithelial cell characteristics. This is associated with a severe upregulation of mesenchymal and stemness-promoting factors. The most severely upregulated mRNA in ATCs encodes the oncofetal RNA-binding protein IGF2BP1 (Insulin like growth factor II mRNA-binding protein 1). IGF2BP1 belongs to a family of conserved oncofetal RNA-binding proteins modulating the cytoplasmic fate of various mRNAs. In cancer, IGF2BP1 and IGF2BP3 were shown to promote the oncogenic potential of tumor-derived cells by various means. In agreement, we observe that IGF2BP1 depletion significantly impairs the viability, invasiveness and self-renewal potential of ATC-derived cells *in vitro* and the growth of *Xenografts* in nude mice. Aiming to reveal the up- as well as downstream effectors of IGF2BP1 in ATCs, we identify an epigenetic switch responsible for the *de novo* synthesis of IGF2BP1 in ATCs as well as several novel targeted mRNAs. The latter encode various oncogenic factors including MYC, FOXM1 and LIMK1. By interfering with the degradation of these target transcripts, IGF2BP1 promotes the proliferative, self-renewal and invasive potential of ATC-derived tumor cells.

In conclusion our findings indicate that IGF2BP1 is a potent molecular marker unambiguously distinguishing ATCs from other thyroid cancers and that targeting IGF2BP1-dependent oncogenic networks by epigenetic drugs may provide new hubs for ATC-treatment.



## 541 An engineered RNA binding protein with improved splicing regulation

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The muscleblind-like (MBNL) family of proteins are key developmental regulators of alternative splicing. Sequestration of MBNL1 proteins by expanded CUG/CCUG repeat RNA transcripts is a major pathogenic mechanism in the neurodegenerative disorder myotonic dystrophy (DM). The sequestration of the MBNL proteins by these toxic RNAs within nuclear foci leads to mis-splicing events responsible for disease symptoms. From a structural perspective, MBNL1 contains four zinc finger (ZF) motifs that form two tandem RNA binding domains (ZF1-2 and ZF3-4) that each bind YGCY RNA motifs. To better understand the difference between these two domains and to develop improved proteins for therapeutics, we designed and characterized synthetic MBNL1 proteins with duplicate ZF1-2 or ZF3-4 domains, referred to as MBNL1(1-2,1-2) and MBNL1(3-4,3-4), respectively. The biochemical characterization of these engineered proteins revealed significant differences in the activities of each ZF domain. Analysis of splicing regulation by the two synthetic proteins compared to wildtype MBNL1 revealed that MBNL1(1-2,1-2) had 5-fold increased splicing activity while MBNL1(3-4,3-4) had 4-fold decreased activity. Global RNA binding analysis indicated that the differences in splicing activity were due to differences in RNA binding specificities between the two ZF domains and was not dictated by binding affinity. ZF1-2 binds YGCY motifs with high specificity while ZF3-4 acts as a more general RNA binding domain. Our findings indicate that ZF1-2 drives alternative splicing via recognition of canonical YGCY RNA motifs and ZF3-4 allows for MBNL1 to bind a wider array of RNA substrates via its reduced requirements for specific sequence recognition. The characterization of these synthetic MBNL1 proteins has provided insights into the individual function of each ZF domain and their role in MBNL1-mediated alternative splicing. Our studies also serve as a proof of principle that MBNL1 can tolerate modifications and retain function. Further rational design strategies to modify MBNL1 can be utilized to continue to create a more stable and active synthetic MBNL1 for use as protein therapeutics for DM and other microsatellite diseases.

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## 542 The function and mechanisms of the low complexity domain of TDP-43

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Most patients suffering from motor neuron disease (MND) display TDP-43 protein aggregates, which gives TDP-43 a central role in the development of this disease. Aggregation of TDP-43 is promoted by its low-complexity (LC) domain, which is also the region containing most of the disease-causing mutations. While most studies suggest that the LC domain mainly mediates protein-protein interactions, its function is poorly understood. We therefore examined the role of the TDP-43 LC domain in protein-protein and protein-RNA interactions, and the functions of these interactions in regulating pre-mRNA processing. For this purpose, we created cell lines where the endogenous RBP is replaced by mutants lacking specific portions of the LC domain and assessed them with the use of individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP), RNA-Seq, PolyA-Seq and Mass Spectrometry.

We found that a partial deletion of the LC domain in TDP-43 leads to a loss of binding to several RBPs, many of which contain long LC domains. This indicates that LC domain serves as docking-platforms for protein-protein interactions, thereby potentially forming RNP granules. Finally, we show that deletions in the LC domain affect the function of TDP-43 in splicing and 3' end processing. In conclusion, our research uncovers a crucial role of the LC domain in protein-protein interaction, which affects TDP-43's regulatory functions.



### 543 Two microRNA clusters may determine the biological functions of microRNA-regulated pathways in underactive bladder

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**Introduction and Objectives:** MicroRNAs regulate diverse biological processes. Previously we identified miRNA-regulated pathways in bladder outlet obstruction (BOO)-induced bladder dysfunction. MiRNAs in a cluster reside in genomic proximity (<10 kb), and miRNA families have seed sequence homology. Expression of miRNA cluster might be mediated by common transcription factors, and clustered miRNAs often regulate same biological processes. Here we probed functional associations of BOO phenotype-specific miRNAs and identified several co-expressed miRNA sub-networks.

**Materials and Methods:** MiRNA sequences and genomic coordinates were extracted from miRBase version 21. Large scale chromosomal mapping of human miRNA structural clusters was done using MIREStruC-1.0 package. Next-generation sequencing datasets of patients' biopsies with urodynamically established BOO with and without detrusor overactivity (DO and BO groups, respectively) or with detrusor underactivity (UA group) were used to perform miRNA-mRNA integrated analysis and target pairing. We included the miRNA families extracted from miRBase version 21. Sequences were aligned with MAFFT version 7 and Clustal X 2.1 and manually refined with RALEE - RNA version 0.8.

**Results:** In DO group hsa-miR-376c-3p/hsa-miR-409-3p cluster was identified on chromosome 14. In BO group hsa-miR-889-3p/hsa-miR-410-3p/hsa-miR-409-3p cluster was detected on chromosome 14. Three miRNA clusters were detected in UA group: hsa-miR-25-3p/hsa-miR-106b-3p cluster on chromosome 7, and 2 clusters on chromosome 1: hsa-miR-199a-3p/hsa-miR-3120-3p cluster and hsa-miR-429/hsa-miR-200b-3p cluster belonging to miR-200bc/429/548a family. All clustered miRNAs were intergenic. Integrated miRNA-mRNA expression profiling in all miRNA clusters revealed no significant target overlap, 90% of targets of up-regulated miRNA clusters were also up-regulated. In DO and BO groups no major contribution of the miRNA clusters to the biological functions of miRNA-regulated pathways was detected. In contrast, in UA group 2 down-regulated miRNA clusters were necessary and sufficient to determine the functions of all miRNA-regulated pathways. The appropriately regulated targets of hsa-miR-199a-3p/hsa-miR-3120-3p and hsa-miR-429/hsa-miR-200b-3p clusters constituted the majority of miRNA-regulated pathway elements in the UA state.

**Conclusions:** Multiple co-expressed miRNAs may cooperatively influence biological processes and the acontractile urodynamic phenotype in the underactive bladder. Elucidating the down-regulation mechanisms of these miRNA clusters may help determine the "point of no return" for the loss of bladder function during BOO.

### 544 The cancer-associated U2AF35 Q157R mutation creates an alternative 5' splice site leading to a protein that contributes to the distinct splicing signature of Q157R patients

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Recent work has highlighted frequent mutations in spliceosome components to cause or contribute to malignant transformation. A prominent example is U2AF35, for which cancer-associated missense mutations in two zinc-finger (ZnF) domains have been identified. While numerous studies have analyzed splicing defects caused by U2AF35 point mutations, little is known about the functionality of the ZnF domains in general. Here we have analyzed key functionalities of U2AF35 and show that the second ZnF is required for protein stability and stable interaction with its heterodimerization partner U2AF65. In addition, we show that both ZnFs are indispensable for U2AF35 function in alternative splicing which is consistent with the ZnF domains being essential for RNA-binding. Further analyses focusing on two disease-associated mutations within the second ZnF revealed that the c.470A>G mutation not only leads to the Q157R substitution, but also creates an alternative 5' splice site (ss). Usage of this alternative 5'ss results in the deletion of four amino acids within the second ZnF, leading to reduced binding to U2AF65 and a changed specificity for U2AF35 target exons. We confirmed the presence of this deletion mutant in c.470A>G patients where it may contribute to disease development. Additionally, we show varying responsiveness of individual splicing targets to the ZnF2 mutations Q157R and Q157P in a knock down complementation assay with quantitative read-out. Furthermore, consistent with our cell culture data, patients with Q157R and Q157P mutations display distinct alternative splicing profiles, with unique target exons showing differential splice site sequence and strength. This supports the notion that disease formation is caused by mutation-specific gain of function and missplicing of various targets. Our data emphasizes the importance to explore mutations beyond altered protein sequence and deepens the molecular understanding of cancer-associated U2AF35 mutations.

## 545 miR-146a Directly Regulates Expression of 5-lipoxygenase Activating Protein (FLAP) in Lung Cancer Cells

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One commonly dysregulated inflammatory pathway in cancer is the metabolism of arachidonic acid (AA) by cyclooxygenase-2 (COX-2) or 5-lipoxygenase (5-LO) and its activating protein (FLAP) into prostaglandins (PGs) and leukotrienes (LTs), respectively. PGs and LTs act in an autocrine and paracrine fashion to promote inflammation as well as invasion, growth, and proliferation of cancer cells. Although COX-2, 5-LO, and FLAP inhibitors have shown promise for treating cancer, high toxicity and adverse side effects have limited their effective use in the clinic. Therefore, new methods of targeting these proteins are necessary. Our lab and others have shown that these molecules are regulated post-transcriptionally by microRNAs (miRNAs). miRNAs are small, non-coding RNAs that negatively regulate target gene expression through mRNA degradation or translational repression. They generally target multiple genes of similar biological function, making them attractive candidates for therapeutic use.

Here, we show that FLAP protein is overexpressed in lung cancer cells compared to normal lung cells. One miRNA in particular, miR-146a, is predicted to target FLAP via its 3' untranslated region (UTR). This computationally predicted binding site is evolutionarily conserved across numerous species. Previous work from our lab demonstrated that miR-146a directly regulates COX-2 expression through its 3' UTR. In this study, we have experimentally defined the specific regulation of FLAP by miR-146a. Transfection of synthetic miR-146a in lung cancer cells repressed endogenous FLAP mRNA and protein expression, and reporter assays indicated this regulation occurs through a direct interaction between miR-146a and the FLAP 3' UTR. This modulation of FLAP gene expression also resulted in decreased cancer cell LTB<sub>4</sub> production. This work suggests miR-146a can regulate both PG and LT production in lung cancer cells by directly targeting the 3' UTRs of COX-2 and FLAP, leading to dual inhibition of inflammatory eicosanoid production. Regulation of both arms of this metabolic pathway is significant in that it prevents shunting of AA, where blocking one pathway results in upregulation of the other due to more available substrate. Overall, our data suggest an elegant mechanism of miRNA-mediated regulation of AA metabolism that further supports a tumor suppressive role for miR-146a in lung cells.

## 546 Small Molecule Approaches to Reduce Toxic RNA Repeats in Myotonic Dystrophy Models

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RNA-mediated toxicity is an emerging mechanism of pathogenesis in dominantly inherited neurological and neuromuscular diseases. Many of these diseases are caused by unstable microsatellite expansions in non-coding regions of the genome, which give rise to RNAs that have a toxic gain of function. Typically, the toxic RNA aggregates into nuclear foci that sequester and alter the activities of various RNA-binding proteins required for normal RNA processing. Also, bidirectional transcription and repeat-associated non-ATG translation have emerged as common themes in expansion diseases. One possible treatment approach is to reduce or eliminate transcription of these microsatellite expansions. Our previous work has demonstrated that Actinomycin D (ActD), a potent transcription inhibitor and FDA-approved chemotherapeutic that binds GC-rich DNA with high affinity, decreases CUG transcript levels in a dose-dependent manner in myotonic dystrophy type 1 (DM1) models. In a DM1 mouse model, ActD significantly reverses DM1-associated splicing defects and RNA-seq analyses shows that low concentrations of ActD does not globally inhibit transcription. We have also demonstrated that pentamidine, a diamidine which is currently FDA-approved as an anti-parasitic agent, was able to partially reverse mis-splicing in multiple DM1 models, albeit at toxic concentrations. Here we demonstrate that furamidine and DB1242, pentamidine analogs, effectively reversed mis-splicing with equal efficacy and reduced toxicity compared to pentamidine in DM1 cell and mouse models. These results, paired with preliminary data in Myotonic Dystrophy type 2, indicate that transcription inhibition could be a viable treatment approach across many GC-rich microsatellite expansion diseases.

**547 Sensitive and specific detection of miRNAs using SplintR DNA ligase***Jingmin Jin<sup>1</sup>, Wen Cai Zhang<sup>2</sup>, Alexander Zhelkovsky<sup>1</sup>, Frank Slack<sup>2</sup>, Larry McReynolds<sup>1</sup>*<sup>1</sup>New England Biolabs, Ipswich, MA, USA; <sup>2</sup>Institute for RNA Medicine, Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

We had described a simple, sensitive and specific microRNA (miRNA) detection method that utilized Chlorella virus DNA ligase, SplintR<sup>®</sup> Ligase (1). This two-step method involves ligation of two adjacent DNA oligonucleotides hybridized to a target miRNA and real-time quantitative PCR with a FAM labeled double quenched DNA probe. SplintR Ligase is 100X faster than either T4 DNA Ligase or T4 RNA Ligase 2 for RNA splinted DNA ligation. Only a 4-6 bp overlap between one of the two DNA probes and miRNA splint was required for efficient ligation by SplintR Ligase. This property allows the specific detection of miRNA isoforms; for example, members of the let-7 and miR-34 families. Here we update the application of this SplintR qPCR method in miRNA detection from lung cancer cell lines. The individual tumor miRNAs, including miR-21 were measured by SplintR qPCR in human lung cancer cell lines. The qPCR SplintR ligation assay is sensitive; it can detect a few thousand molecules of miR-122. It is at least 40X more sensitive than the TaqMan assay. When coupled with NextGen sequencing, it allows multiplex detection of miRNAs from total RNAs of brain, kidney, testis and liver. The rapid kinetics and ability to ligate DNA probes hybridized to miRNA targets with short complementary sequences makes SplintR Ligase a useful enzyme for miRNA and miRNA isoform detections.

(1) Jin, J., Vaud, S., Zhelkovsky, A., Posfai, J. and McReynolds, L. A. (2016) Sensitive and specific miRNA detection method using SplintR Ligase. *Nucleic Acids Research*, doi: 10.1093/nar/gkw399

**548 The miR-24-3p/p130Cas: a novel axis regulating the migration and invasion of cancer cells***Eunbyul Ji<sup>1</sup>, Hoin Kang<sup>1</sup>, Sojin Ahn<sup>1</sup>, Chongtae Kim<sup>1</sup>, Eun Kyung Lee<sup>1,2</sup>*<sup>1</sup>Department of Biochemistry, College of Medicine, Catholic University of Korea, Seoul, Republic of Korea;<sup>2</sup>Cancer Evolution Research Center, Catholic University of Korea, Seoul, Republic of Korea

microRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression by suppressing translation or facilitating mRNA decay. Differential expression of miRNAs is involved in the pathogenesis of several diseases including cancer. Here, we investigated the role of miR-24-3p as a downregulated miRNA in metastatic cancer. miR-24-3p was decreased in metastatic cancer and lower expression of miR-24-3p was related to poor survival of cancer patients. Consistently, ectopic expression of miR-24-3p suppressed the cell migration, invasion, and proliferation of MCF7, Hep3B, B16F10, SK-Hep1, and PC-3 cells by directly targeting p130Cas. Stable expression of p130Cas restored miR-24-3p-mediated inhibition of cell migration and invasion. These results suggest that miR-24-3p functions as a tumor suppressor and the miR-24-3p/p130Cas axis is a novel factor of cancer progression by regulating cell migration and invasion.

## 549 Crystal structures of CAG and CUG repeats with PNA oligomers: toward antisense therapy against TREDs

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RNA-mediated diseases are genetic disorders in which a mutation results in toxic mRNA. The mutated gene contains an abnormally expanded microsatellite repeat sequence which are transcribed and form hairpin structure showing abnormal affinity for several cellular proteins. Diseases are progressive with no effective treatment. One of proposed therapeutic approaches relies on antisense oligonucleotides which can serve as blockers binding directly to complementary target.

Here we present crystal structures of two duplexes of RNA, containing CUG and CAG repeats, with their antisense PNA oligomers; and one PNA-PNA duplex with T-T mismatches. To our knowledge these are the first crystallographic RNA-PNA structures and PNA-PNA model containing non-Watson-Crick pairs. The two RNA/PNA duplexes are isomorphic, having identical helical parameters despite different sequences. Both are fully complementary with Watson-Crick base pairs. They have form of A-RNA with an unusually low twist and low rise. The PNA oligomers form antiparallel right and left-handed helices. The helical twist is low while the rise is very high. The T-T pairs can be classified as WC-WC pairs or as an asymmetric homo-pyrimidine XVI. The results shows that RNA and PNA have a similar ease of adapting to one another and the resulting duplex is halfway between PNA/PNA and RNA/RNA structures. The relative instability of heteroduplexes containing mismatches can be explained by the additional need of the two strands to overcome their different conformational preferences.

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## 550 Regulation of Neuroblastoma Cell Proliferation and Differentiation by ELF4

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miRNA-transcription factor networks are implicated in various developmental and physiological processes. Neurogenesis is driven by several miRNAs and previously we found that proneurogenic miR-124 regulates a highly connected group of transcription factors during neurogenesis. Neuroblastoma, the most frequent extracranial solid tumor of childhood, originates from neural crest cells that fail to differentiate. miR-124 is downregulated in neuroblastomas and overexpression of miR-124 with miRNA mimics induces differentiation similarly to the clinically used all-trans retinoic acid, but the mechanism is poorly understood. To understand how miR-124 induces differentiation, we selected 28 transcription factors that we identified as targets of miR-124 and knocked down their expression in neuroblastoma cells and examined the subsequent effects on proliferation, neuronal differentiation, and apoptosis. Knockdown of E74 like ETS transcription factor 4 (ELF4), produced the most significant phenotypic changes: inhibition of cell proliferation, induction of neuronal differentiation, and cellular apoptosis within 48hrs. Next, we characterized the impact of ELF4 regulation in neuroblastoma via RNA-seq. Down-regulated genes in siELF4-treated cells were enriched in cell cycle and DNA replication pathways, which further highlights the importance of ELF4 in proliferation. We compared the ELF4 down-regulated genes with miR-124 targets we previously identified and discovered significant overlap, indicating antagonistic roles for miR-124 and ELF4 in regulating proliferation and differentiation. Finally, we analyzed ELF4 expression in tumors collected from neuroblastoma patients and found that the five-year survival in patients with high ELF4 expression was significantly reduced compared to patients with low ELF4 expression. While futures studies will help determine mechanisms by which ELF4 and other transcription factors can contribute to neuroblastoma development, our work has established a critical role for miR-124-ELF4 in neuroblastoma proliferation and maintenance of an undifferentiated phenotype in these cells. Furthermore our work highlights the important interaction between miRNA-transcription factors in cancer development and differentiation.



## 551 Phosphorylation of an RNA-binding protein RBM20 on an RSRSP stretch, whose missense mutations cause familial dilated cardiomyopathy, is required for proper nuclear localization and splicing regulation

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A giant sarcomeric protein titin spans from Z-disk to M-band and functions as a molecular spring to determine myocardial passive stiffness. In the adult cardiac muscles, titin is produced as two major isoforms from the *TTN* gene by alternative pre-mRNA splicing: a larger and highly compliant isoform N2BA and a shorter and stiff isoform N2B. Recently, deficiency or missense mutations in a gene encoding an RRM-type RNA-binding protein RBM20 have been reported to greatly increase N2BA/N2B ratio of the *TTN* transcript in a rat strain with spontaneous heart failure and in a patient with dilated cardiomyopathy (DCM). Although tens of other direct target transcripts for RBM20 have been identified by RNA-seq and CLIP-seq experiments, it is not yet clear why the missense mutations identified in DCM patients are enriched in a highly conserved RSRSP stretch located outside of the single RRM domain.

Here we constructed a bichromatic fluorescence *Ttn* splicing report minigene that expresses red and green fluorescent proteins upon N2B-type and non-N2B-type splicing, respectively. Co-expression of wild-type RBM20 increased the ratio of the N2B-type isoform, mimicking the splicing regulation of the *Ttn* gene *in vivo*. Missense mutations in the RSRSP stretch significantly affected the splicing regulation. Immunofluorescence staining revealed that the mutant RBM20 proteins were excluded from the nucleus in HeLa cells and forced nuclear localization of the mutant RBM20 restored splicing regulation of the *Ttn* reporter. Polyacrylamide gel electrophoresis with phostag-containing gel revealed that RBM20 is phosphorylated on multiple sites in the steady state and Western blotting with a phosphorylation-specific antibody confirmed phosphorylation within the RSRSP stretch. These results indicate the phosphorylation of the RSRSP stretch is critical for nuclear localization and splicing regulation for RBM20.

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## 552 microRNA-200a-3p increases 5-fluorouracil resistance by regulating dual specificity phosphatase 6 expression

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Acquisition of resistance to anti-cancer drugs is significant obstacle to effective cancer treatment. Although several efforts have been made to overcome drug resistance in cancer cells, the detailed mechanisms are not fully elucidated. Here, we investigated whether microRNAs (miRNAs) function as pivotal regulators in the acquisition of anti-cancer drug resistance to 5-fluorouracil (5-FU). A survey using a lenti-virus library containing 572 precursor miRNAs revealed that five miRNAs promoted cell survival after 5-FU treatment in human hepatocellular carcinoma Hep3B cells. Among five different clones, the clone expressing miR-200a-3p (Hep3B-miR-200a-3p) was further characterized as 5-FU resistant cells. The cell viability and growth rate of Hep3B-miR-200a-3p cells were higher than those of control cells after 5-FU treatment. Ectopic expression of miR-200a-3p mimic increased, while inhibition of miR-200a-3p downregulated viability in response to 5-FU, doxorubicin, and CDDP. We showed that dual specificity phosphatase 6 (DUSP6) is a novel target of miR-200a-3p and regulates resistance to 5-FU. Ectopic expression of DUSP6 mitigated the pro-survival effects of miR-200a-3p. Taken together, we propose that miR-200a-3p enhances anti-cancer drug resistance by decreasing DUSP6 expression.



### 553 MiR-221/222 activate Wnt/ $\beta$ -catenin signaling to promote triple negative breast cancer progression

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Triple-negative breast cancer (TNBC), characterized by the lack of expression of the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2), is an aggressive form of the disease which convey unpredictable and poor prognosis due to limited treatment options and lack of proven effective targeted therapies. Wnt/ $\beta$ -catenin signaling is hyperactivated in TNBC, which promotes the progression of TNBC. However, the molecular mechanism of Wnt/ $\beta$ -catenin activation in TNBC still remains unknown. Here we report that miR-221/222 directly target and suppress multiple negative regulators of the Wnt/ $\beta$ -catenin signaling cascade, including WIF1, SFRP2, DKK2, and AXIN2. In TNBC cell line MDA-MB-231, miR-221/222 inhibitors inhibited proliferation, migration and promoted apoptosis. Notably, miR-221/222 were markedly upregulated in TNBC primary tumor samples from patients and were associated with overall survival. These results demonstrate that miR-221/222 maintain constitutively activated Wnt/ $\beta$ -catenin signaling and may represent a therapeutic target for TNBC.

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### 554 The contrasting roles of RNA binding protein RBM10: Although traditionally a tumor-suppressor, RBM10 promotes transformatory processes in small cell lung cancer and is directly regulated by RBM5

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Lung cancer is the leading cause of cancer-related deaths, with small cell lung cancer (SCLC) being the most aggressive form. SCLC treatment options are usually quite limited, as diagnosis typically occurs when the disease has reached an advanced and often metastatic state. An astounding 95% of SCLC patients succumb to the disease within five years, highlighting the need for more effective screening and treatment options. Interestingly, the earliest and most frequent genetic alteration in lung cancer results in decreased levels of the RNA binding protein RBM5. We have recently shown that this reduction in RBM5 expression may be a key step in SCLC development and may thus be useful indicator of SCLC risk. Interestingly, RBM5 is structurally and functionally similar to another RNA binding protein, RBM10. We set out to determine if RBM10 shared RBM5's tumor-suppressor properties in SCLC. We hypothesized that RBM5 and RBM10 would indeed have similar roles in this system and accordingly, that RBM10 expression levels may also be a prognostic tool for assessing SCLC risk. Surprisingly, RNA-Seq and functional studies showed opposing roles for RBM5 and RBM10; RBM10 actually promoted a number of hypoxia-related transformation-associated processes, including angiogenesis, epithelial to mesenchymal transition and glycolysis, in an RBM5-null SCLC cell line. In coordination with these findings, we demonstrated that although RBM5 and RBM10 shared many similar targets in SCLC, gene expression was affected in contrasting ways by both proteins. We also determined that RBM5 post-transcriptionally regulates RBM10 expression. This work is the first to show that RBM10's function is significantly altered by RBM5. As RBM5 is downregulated in many cancers, not only SCLC, these findings could be of great clinical importance. It also highlights the scope of the influence RNA binding proteins, and their isoforms, can have on the cell, and how this role can be so dramatically modified by other interacting factors.

## 555 The role of HAX1 and MCP1P proteins in regulation of transcripts involved in pro-inflammatory response of cancer cells

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Recent epidemiological and clinical data clarified the close association between inflammation and carcinogenesis process. During carcinogenesis, a microenvironment influences cancer and cancer influences a microenvironment. The cells surrounding tumor, like activating lymphocytes, macrophages or stromal fibroblasts produce pro-inflammatory factors to support cancer growth. An inflammation is tightly associated with the development of cancer and growing cancer promotes the expression of pro-inflammatory factors.

HAX1 (HCLS1-associated protein X 1) protein is involved in apoptosis, migration, regulation of calcium ions homeostasis and probably plays a role in innate immune response (Kostmann disease). HAX1 is a protein engaged in development of cancer but still its role in cancerogenesis stays unresolved.

RNase MCP1P1 (Monocyte chemoattractant protein-induced protein 1) has a well-established role in inflammation and indirectly, in carcinogenesis. MCP1P1 controls an immune response by destabilization of mRNAs encoding immune related proteins including IL-6 and IL-12p40 via their 3' untranslated regions (UTR). MCP1P1 binds to a conserved stem-loop element (~30 nt) within the 3' UTR of *IL-6*. MCP1P1 auto-regulates its own mRNA level through interaction with similar stem-loop in the transcript. So far, the molecular basis of MCP1P1 interaction with RNA remains unclear, since no sequence specificity has been found.

HAX1 was identified also as an RNA binding protein with known interaction with vimentin (2003) and DNA polymerase beta (2007) transcripts. Those interactions suggest its post-transcriptional regulatory function.

To verify the hypothesis that HAX-1 can interact with MCP1P1 protein we performed the co-immunoprecipitation experiment and found out that both proteins co-precipitate. Additionally, in Western-blot and IHC experiments, we have observed that HAX-1 protein level as well as MCP1P1 level decrease in clear cell renal carcinoma clinical samples.

The intended studies should clarify whether HAX-1 and MCP1P1 can regulate transcripts involved in inflammation process during carcinogenesis. Results of this project are expected to bring the new knowledge about mechanisms how and where HAX-1 and MCP1P1 proteins cooperate in the cells to maintain the internal homeostasis between cells and inflammation process.

### Acknowledgments

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## 556 Manipulation of Recurrent Alternative Splicing Events across Human Cancers by Antisense Oligonucleotides as a Novel Treatment Strategy

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A growing body of evidence has revealed that the process of alternative splicing is misregulated in many human diseases, including cancer. These alternative splicing changes can contribute to cancer development and progression. In order to identify these cancer-driving events, we analyzed RNA-seq data from The Cancer Genome Atlas (TCGA; <https://cancergenome.nih.gov>). We examined the association between significantly altered exon inclusion events in tumor vs. normal samples, and patient survival. Splicing alterations significantly associated with survival (p-value<0.05), were further examined and divided into two categories: skipping events that potentially produce a dysfunctional protein and inclusion events that present in numerous cancer types. In order to identify the predicted functional role of either included or skipped exon in the protein, we examined overlap of the encoded exon with a known protein domain (PFAM domain). We found a set of events that could produce an opposing functional biological activity and chose two events in which the inclusion of the exon generates a functional protein while the skipping does not: exon 25 in *LTBP1* and exon 1 in *FN1*. For the second category, we selected events that were altered significantly in at least five cancer types: exon 17 in *EHBPI* and exon 5 in *FN1*.

In order to manipulate the levels of the isoforms in cancer cells, we designed antisense RNA oligonucleotides (ASO) to modulate the alternative splicing of these four genes, favoring skipping of the specified exons, which is expected to inhibit cancer progression and survival. These ASOs mask the 3' splice-site between the designated exon and the upstream intron, and prevent the inclusion of that exon. All four ASOs that caused a switch toward increased exon skipping, inhibited the anchorage-independent growth and colony formation of lung, glioblastoma, metastatic breast and cervical cancer cell lines. These results suggest that manipulation of the alternative splicing of these four genes by ASOs is a potential treatment for several types of cancer.

## 557 Suppression of pro-oncogenic eicosanoid signaling by miR-708-5p in lung cancer cells

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Our objective is to define dysregulated post-transcriptional mechanisms in the arachidonic acid pathway relevant to cancer progression and the tumor microenvironment (TME). Many cancers maintain an inflammatory microenvironment to promote their growth. Besides being its own hallmark of cancer, inflammation influences other characteristics, such as proliferation, invasion, angiogenesis, and immune evasion. Researchers are beginning to better appreciate how communication between cancer cells and the tumor stroma promotes tumor growth. These signals are also commonly found in wound healing and the inflammatory response. Hence, resolving how inflammation is dysregulated in cancer may provide fresh opportunities for therapeutic development to more comprehensively treat tumors.

One inflammatory pathway commonly dysregulated in cancer is the metabolism of arachidonic acid by Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO) into potent lipid-signaling molecules called eicosanoids. Eicosanoids promote proliferation, migration, angiogenesis, and immune evasion through autocrine and paracrine signaling to cancer cells and the tumor stroma. While the arachidonic acid pathway is commonly upregulated in cancer, the mechanisms governing this deregulation are not well understood. Major regulators of expression are microRNAs (miRNAs). miRNAs are commonly misexpressed in cancer and can perform oncogenic or tumor suppressive functions. One miRNA in particular, miR-708-5p, has been shown to be downregulated in cancer and represses many oncogenic pathways. Interestingly, miR-708-5p is predicted to target the COX-2 and 5-LO 3' UTRs, possibly contributing to arachidonic acid pathway dysregulation in cancer.

We show miR-708-5p does indeed directly target both COX-2 and 5-LO 3' UTRs in lung cancer cells, decreasing their mRNA and protein levels. This direct targeting of COX-2 and 5-LO decreased oncogenic eicosanoid production, resulting in decreased proliferation of lung cancer cells *in vitro*. We are continuing to extend our research to phenotypic effects of miR-708-5p on lung cancer cells and other non-cancerous cell types commonly found within the TME. The ultimate goal of our research to better understand the role of arachidonic acid signaling in cancer, as well as miR-708-5p's role in carcinogenesis.

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## 558 Modeling RNA exosome-linked disease in *Drosophila*

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The RNA exosome is an evolutionary-conserved 3'-5' ribonuclease complex critically important for both precise processing and complete degradation of a variety of cellular RNAs. One of the most critical cellular functions of the RNA exosome is the production of mature, properly trimmed rRNAs required within the ribosome. Given, the crucial role of the RNA exosome in post-transcriptional regulation of RNA, it is not surprising that the complex is essential in systems examined thus far. The recent discovery that mutations in genes encoding structural exosome subunits cause tissue-specific diseases, makes defining the role of the exosome within specific tissues critically important to understand the basis of these diseases. The *Drosophila* system provides ideal tools to examine tissue-specific gene function. Mutations in RNA exosome subunit 3 (*EXOSC3*) cause Pontocerebellar Hypoplasia type 1b (PCH1b), which is an autosomal recessive neurodegenerative disease, while mutations in RNA exosome subunit 2 (*EXOSC2*) cause a variety of distinct tissue-specific phenotypes including mild intellectual disability. The disease-causing mutations identified are not null mutations but rather amino acid changes in evolutionarily-conserved residues. The tissue-specific defects these changes cause are challenging to understand based on current models of RNA exosome function with only limited analysis of the complex in any multicellular model *in vivo*. The goal of this study is to provide insight into how mutations in *EXOSC2* and *EXOSC3* cause disease. *EXOSC2* and *EXOSC3* are evolutionarily conserved subunits, termed Rrp4 and Rrp40, respectively, in *Drosophila*. To assess tissue-specific requirements for the *Drosophila* RNA exosome subunit Rrp40 (*EXOSC3*), we utilized tissue-specific RNAi drivers and assessed viability. My preliminary data show that we can assess RNA exosome function in a tissue-specific manner. Furthermore, we have identified at least one tissue, the mushroom bodies (MB), the seat of learning and memory of the fly, where exosome function is critical but not absolutely required for viability. Our initial studies reveal severe morphological defects in the MBs upon depletion of Rrp40. These data provide evidence for the first multicellular model in which to study the tissue-specific role of specific RNA exosome subunits providing insight both into RNA exosome function and disease mechanism.

## 559 Spliceosomal snRNA Mutations in Human Diseases

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Higher eukaryotic genomes contain two orthogonal types of spliceosomal introns that are removed by structurally and functionally distinct spliceosomes termed U2-dependent and U12-dependent. The U12-dependent snRNAs, with the exception of the shared U5 snRNA, are encoded by single copy genes in mammals. Analysis of SNP databases shows that the U11, U12, U4atac and U6atac genes are unusually polymorphic in human populations in spite of being highly evolutionarily conserved. A consequence is that defective mutations occur often and produce autosomal recessive diseases when combined in homozygous or compound heterozygous configurations. Two somewhat distinct clinical syndromes called Microcephalic Osteodysplastic Primordial Dwarfism Type 1 (MOPD1) and Roifman Syndrome (RS) have been linked to such mutations in the U4atac snRNA gene (*RNU4ATAC*). In both diseases, the primary gene expression defect appears to be inefficient splicing of U12-dependent introns. Functional studies of mutant U4atac snRNAs reveals that MOPD1 patients generally carry more severe combined mutations than RS patients. Biochemical studies of individual U4atac mutant RNAs show that different mutants have stability defects and/or protein binding defects that lead to disruption in the formation or function of the U4atac/U6atac di-snRNP or the U4atac/U6atac.U5 tri-snRNP complexes. Mouse models of two fairly severe MOPD1 mutations show characteristics similar to human MOPD1 with regard to growth retardation and skeletal defects as well as displaying an unexpected type of diabetes due to progressive loss of insulin producing pancreatic beta cells and disruption of islet structure without inflammation. Patient derived iPS cells have been produced and are being phenotyped for their developmental potential.

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## 560 The RNA-binding protein Musashi1 is a potent post-transcriptional enhancer of tumor cell stemness in glioblastoma

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The RNA-binding protein (RBP) Musashi1 (MSI1) controls the fate of various target mRNAs. It is highly abundant during development and observed in neural and other stem cells where it was proposed to modulate cellular plasticity and differentiation. In some cancers, the upregulation of MSI1 is associated with an aggressive stemness-like tumor cell phenotype. In agreement, recent studies confirmed MSI1 to modulate the fate of glioblastoma multiforme (GBM) derived tumor cells and iCLIP studies indicated various novel candidate target mRNAs. Aiming to reveal MSI1's role in GBM-derived stem-like tumor cells, we analyzed differential gene expression in response to MSI1 depletion. This revealed a substantial number of deregulated mRNAs suggesting that MSI1 next to its reported role in mRNA translation also controls mRNA turnover. Among candidate target mRNAs downregulated upon MSI1 depletion and associated with MSI1 according to iCLIP analyses were several transcripts encoding stemness factors including CD44. Studies in primary GBM-derived stem-like tumor cells confirmed MSI1-dependent regulation of CD44 expression and stem-like cell properties like self-renewal and anoikis resistance. Taken together our findings provide strong evidence that MSI1 is a potent RBP promoting stem-like tumor cell properties in GBM by interfering with the degradation of mRNAs encoding stem cell factors.



## 561 *H.sapiens* tRNase Z<sup>L</sup> (ELAC2) Mutations Associated with Hypertrophic Cardiomyopathy Reduce Mitochondrial Pre-tRNA 3'-End Processing and Translation

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tRNase Z is a widely conserved enzyme responsible for endonucleolytic 3' end processing of pre-tRNAs. The eukaryotic long form of tRNase Z (tRNase Z<sup>L</sup>), encoded by *ELAC2* and dually targeted to the *H. sapiens* nucleus and mitochondria, cleaves 3' ends of both nuclear encoded pre-tRNAs and mitochondrial (mt) tRNAs from the primary, polycistronic transcripts. Recently, we showed that compound heterozygous or homozygous mutations within the nuclear *ELAC2* gene associated with hypertrophic cardiomyopathy reduce mt tRNA 3' end processing (Haack et al., *AJHG* **93**:211, 2013). Five individuals identified from three independent families harbor a combination of the substitutions F154L, L423F and T520I. Establishing pathogenicity of novel variants remains challenging and functional studies should be central to the evaluation, complementing the increased utility of genetic testing and targeted exome sequencing. These three mutant tRNase Z<sup>L</sup> enzymes reduced the efficiency of mt tRNA processing relative to wild-type. Two more tRNase Z<sup>L</sup>s were analysed based on another recent report (patient 27 from Taylor et al., *JAMA*, 312(1), 68, 2014). Mutations from 10 new patients were added to the analysis in collaboration with an international consortium coordinated by one of us (MM). Substitution mutations are amenable to enzymatic analysis and frameshifts that occur close to the carboxy end can be characterized, while splice junction mutations cannot be biochemically evaluated. Combined with results obtained using patient cell lines (detection of precursor in Northern blots and the deficiency of mitochondrial translation), the impairment observed using mt tRNA substrates suggests a molecular role of tRNase Z<sup>L</sup> mutation in mitochondrial disease.

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## 562 A mutation in the 3'UTR of IL-10RB leads to VEO-IBD

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The 5' and 3' untranslated region (UTR) of an mRNA are non-coding and harbor important regulatory elements consisting of binding sites for proteins and microRNAs. These elements regulate mRNA processing, localization, stability or directly affect translation. Mutations in these regions can disturb the complex regulatory mechanism of eukaryotic gene expression and lead to severe diseases. Here, we investigate a 3'UTR mutation in the Interleukin-10 receptor beta (IL-10RB) gene that was found in a patient with very early onset inflammatory bowel disease (VEO-IBD). IL-10RB is part of the IL-10 receptor complex that recognizes the anti-inflammatory cytokine IL-10. Binding of the ligand leads to the expression of different immunosuppressive genes. Thus a receptor defect leads to a permanent activation of the immune system and a severe inflammation of the gastro intestinal tract. We generated IL-10RB minigene constructs harboring the wild type or mutant 3'UTR. Interestingly, the mutation neither leads to a reduction of IL-10RB mRNA amount nor to an mRNA export defect. In addition, the IL-10RB protein quantity is identical for the wild type and the mutant construct. Furthermore, the IL-10RB protein encoded by the mutant mRNA shows the same glycosylation pattern as the wild type protein. Therefore, we hypothesize that the protein localization to the plasma membrane might be disturbed in the patient's cells due to the loss of a binding motive in the mutant 3'UTR. To verify this hypothesis we are currently testing membrane anchored GFP reporter constructs containing the wild type or the mutant IL-10RB 3'UTR for their subcellular localization.

**563 The Role of PIWIL1 in Gastric Cancer**Shuo Shi<sup>1</sup>, Na Liu<sup>2</sup>, Zifeng Wang<sup>1</sup>, Zhaoran Zhang<sup>1</sup>, Sanhong Liu<sup>1</sup>, Haifan Lin<sup>1,2</sup><sup>1</sup>Shanghai Institute of Advanced Immunochemical Studies, Shanghai, China; <sup>2</sup>The Yale Stem Cell Center and Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut, USA

PIWI proteins, a clade of the Argonaute family proteins, are expressed predominantly in the germline and bind to PIWI-interacting RNAs (piRNAs). There are four PIWI proteins in humans, PIWIL-1, -2, -3, and 4. They are normally expressed only in the germline but become strongly expressed in many types of cancers in somatic tissues. Despite this, little is known about how PIWI proteins are involved in cancers. Therefore, we have initiated a large-scale, systematic study to address this question. Here we found that one of the four PIWI proteins in humans, PIWIL1, is highly expressed in both gastric cancer tissues and gastric cancer cell lines. Knock out PIWIL1 in gastric cancer cell line drastically impairs the cell proliferation and cell migration ability, and significantly decrease the *in vivo* tumorigenesis ability. Our transcriptome and proteome analyses reveal that these functions are partially achieved via glycolysis and oxidative phosphorylation pathways. These findings indicate an important role of PIWIL1 in tumorigenesis and reveal PIWIL1 as a potential therapeutic target for gastric cancer.

**564 Muscle-specific RNA biogenesis and satellite cell defects in a novel mouse model of OPMD**Katherine Vest<sup>1</sup>, Ayan Banerjee<sup>2</sup>, Luciano Apponi<sup>1</sup>, Hyojung Choo<sup>1</sup>, Bin Tian<sup>3</sup>, Grace Pavlath<sup>1</sup>, Anita Corbett<sup>2</sup><sup>1</sup>Emory University Department of Pharmacology, Atlanta, GA, USA; <sup>2</sup>Emory University Department of Biology, Atlanta, GA, USA; <sup>3</sup>Rutgers University, New Brunswick, NJ, USA

Oculopharyngeal muscular dystrophy (OPMD) is a late onset disease that affects eyelid, pharyngeal, and proximal limb muscles. The vast majority of OPMD patients harbor a dominant polyalanine expansion mutation in the gene encoding the nuclear polyadenosine [poly(A)] binding protein (PABPN1). The PABPN1 protein is ubiquitously expressed and is involved in multiple steps of RNA processing but is most well-characterized for its roles in poly(A) tail addition and poly(A) signal (PAS) selection. Given the ubiquitous expression and general role in RNA processing, the mechanisms by which mutant PABPN1 causes pathology in a specific subset of muscles remains unknown. We have developed a novel mouse model of OPMD that expresses native levels of alanine-expanded PABPN1 in all tissues and is the closest available genocopy to OPMD patients (*Pabpn1*<sup>+/A17</sup>). Initial studies in rectus femoris muscle in *Pabpn1*<sup>+/A17</sup> mice revealed that native levels of alanine-expanded PABPN1 lead to a global shortening of poly(A) tails. In contrast to previous studies using *Pabpn1* knockdown or alanine-expanded PABPN1 overexpression, no global shift in PAS usage was detected, though modest changes in PAS usage in a subset of RNAs were detected. Mitochondrial abnormalities were also detected at the RNA and protein levels. Subsequent studies focused on satellite cells, a pool of stem cells closely associated with muscle tissue that are normally quiescent but can be activated to regenerate muscle tissue in the event of injury. Interestingly, studies in animal models have shown that many of the muscles most severely affected by OPMD including pharyngeal and extraocular muscles have unusually active satellite cell populations. In *Pabpn1*<sup>+/A17</sup> mice, the satellite cell population associated with pharyngeal muscle is impaired. This pharyngeal satellite cell defect may contribute to the muscle-specific pathology associated with OPMD.

**565 Sandwich-typed assays for microRNA quantification using pyrene-modified probes***Yu Warari<sup>1</sup>, Jumpei Ariyoshi<sup>1</sup>, Yuta Sugihara<sup>1</sup>, Asako Yamayoshi<sup>2,3</sup>, Akira Murakami<sup>4</sup>, Akio Kobori<sup>1</sup>*

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MicroRNAs (miRNA) are small non-coding RNAs (18-25 nt) that regulate gene expression. Recently, it has been reported that the expression levels of miRNAs in human blood are closely related to various cancers<sup>(1)</sup>. Thus, miRNAs are could be promising biomarkers for cancer diagnosis. However, it is difficult to quantify miRNAs due to low concentration. Therefore, quantification systems with accuracy and simpleness were required. We have studied sandwich-typed assays using pyrene-modified probes that specifically produce a strong fluorescent signal in the presence of complementary RNAs.

In this study, we chose four miRNAs, whose expression levels in blood or cancer cells of breast cancer patients are changed, as target miRNAs.

First, detection probe sets with biotinylated capture probes and the 2'-O-pyrene-modified oligonucleotides that were complementary to their target miRNAs were prepared for sandwich-typed assays for detection of miRNAs. The biotinylated capture probes contained locked nucleic acid (LNA) to improve thermal stability of the duplex between capture probes and targets. After mixing detection probe sets and various concentration of miRNAs, avidin-modified agarose beads were added and stirred for 1 h. Then, fluorescent signals on avidin-modified agarose beads were measured using fluorescent microscope without washing steps. As a result of fluorescent measurements, we accomplished quantitative detection of miRNAs (LOD = 3 fmol). Next, the sequence selectivity of detection probe sets was evaluated using non-complementary miRNAs and microRNA families of the target miRNA. Consequently, strong fluorescent signals were selectively observed on avidin-modified agarose beads only in the presence of the target miRNAs. Finally, quantitative capability of sandwich-typed assay was evaluated using miRNAs extracted from HeLa cells. From quantification of endogenous total RNA, ca 320 fmol of miR-21 was included in the lysate of  $1.8 \times 10^7$  HeLa cells.

(1) N. Kosaka, *et. al.*, *Cancer Sci.*, **2010**, 101, 2087-2092.

**566 Analysis of the RNA methyltransferase EMG1 and the molecular basis of Bowen-Conradi syndrome***Ahmed S. Warda<sup>1</sup>, Bernard Freytag<sup>2</sup>, Sara Haag<sup>1</sup>, Katherine E. Sloan<sup>1</sup>, Dirk Görlich<sup>2</sup>, Markus T. Bohnsack<sup>1</sup>*

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Bowen-Conradi syndrome (BCS) is a severe monogenetic disorder that is characterised by bone marrow failure, developmental abnormalities and early infant death. This disease is caused by a single mutation (D86G) in EMG1, an essential nucleolar RNA methyltransferase. EMG1 is required for the synthesis of the small ribosomal subunit and is involved in the hyper-modification in position 1191 of the 18S ribosomal (r)RNA during early steps in ribosome synthesis. The BCS mutation leads to a reduction in EMG1 levels and decreased nucleolar accumulation of the protein, however, the dynamics and recruitment of EMG1 and the molecular basis of the disease have remained poorly characterised.

To understand why the mutation of this ribosome biogenesis factor causes BCS, we have followed the EMG1 pathway from its translation in the cytoplasm to its site of action in the nucleolus. We have identified the nuclear transport receptors that mediate nuclear import of EMG1. Our data show that EMG1 forms a sub-complex with the ribosome biogenesis factors NOP14, NDC4L and UTP14A, and we demonstrate the roles of these proteins in the hierarchical recruitment of EMG1 into the nucleolus. The co-functionality of these proteins is supported by their requirement for the same steps in precursor rRNA processing.

Interestingly, immunofluorescence analysis of the BCS mutant has revealed its accumulation in nucleoplasmic aggregates, which is coupled to protein degradation. Furthermore, import assays using permeabilised cells and protein precipitation assays have revealed that the nuclear import receptors suppress the aggregation of the EMG1 BCS mutant, suggesting that the importins play an essential role in chaperoning EMG1 into the nucleoplasm. Together, these data give novel insights into the step-wise recruitment of EMG1 for its functions in ribosome production and we propose a model for how this pathway is perturbed by the D86G mutation leading to Bowen-Conradi syndrome.

## 567 The splicing factor SRSF1 associates with the 5S RNP and is important for both 5S RNP-mediated p53 signalling and the efficient incorporation of the 5S RNP into the ribosome

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The 5S RNP (RPL5, RPL11 and the 5S rRNA), an essential assembly intermediate of the large ribosomal subunit, is a key signalling factor important for the cellular reaction to stalled or reduced levels of ribosome production. Perturbations in ribosome production lead to the accumulation of the 5S RNP, which binds to, and inhibits MDM2, the main regulator of the tumour suppressor p53, resulting in p53 activation. The activation of p53, a transcription regulator, leads to anti-proliferative responses such as cell cycle arrest, apoptosis or senescence. Several additional proteins, such as the splicing factor SRSF1, have been linked to regulating p53. SRSF1 was shown to bind the ribosomal protein RPL5 and influence RPL5's ability to regulate MDM2 and it was suggested that RPL5 can act independently of the other 5S RNP components. However, we have shown that all three components of the 5S RNP are needed to regulate p53. We therefore set out to investigate SRSF1 function in regulating p53 and 5S RNP biology in more detail.

To our surprise we found that knocking down SRSF1 resulted in a two-fold decrease in 5S RNP incorporation into the ribosome. Consistent with this, knockdown of SRSF1 affected the subcellular localisation of both RPL11 and RPL5. Immunoprecipitation data demonstrated that SRSF1 is associated with the 5S RNP and we could show direct interactions between SRSF1 and both RPL5 and RPL11 using recombinant proteins. Knockdown of SRSF1 resulted in a reduction of p53 activation in response to defects in ribosome biogenesis. Conversely, overexpressing SRSF1 resulted in p53 activation. Overexpression of either of the 5S RNP proteins, RPL5 and RPL11, leads to p53 activation and this effect was blocked by the knockdown of SRSF1. Preliminary data indicate that treating cells with SRPIN 340, an inhibitor of SRSF1 phosphorylation, blocked p53 activation in response to defects in ribosome biogenesis. Taken together, our data indicate that SRSF1 does not work with RPL5 alone, but is required for optimal 5S RNP function in both p53 signalling and ribosome biogenesis.

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## 568 Structural basis for the translational repression of *hunchback* mRNA by the Brat-Pumilio-Nanos complex

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Embryonic development in eukaryotic organisms is tightly controlled by precise post-transcriptional RNA processing. In *Drosophila*, correct abdominal segmentation is governed by translational repression of maternally derived *hunchback* (hb) mRNA in the posterior pole of the embryo. This generates a gradient of hunchback protein in the cell, and is controlled through the anterior-posterior gradient of Nanos (Nos). The zinc finger domain of this protein has been shown to bind directly to two Nanos response elements (NREs) within the 3' untranslated region (UTR) of *hb*, in complex with two other proteins, Pumilio (Pum) and Brain Tumour (Brat). However, it remains unclear how this complex assembles and how its components interact to drive translational repression.

Although Nanos is absolutely required for translational repression, both Brat and Pum have been shown to bind RNA independently of each other and of Nanos (Loedige et al., 2014, Wang et al., 2002).

Here, we demonstrate that they are able to form a compact, ternary complex with a 23mer RNA based on NRE2 using NMR, SAXS, SANS and ITC, even in the absence of Nanos. However, there is no cooperativity in RNA binding and protein-protein interactions were not observed. Comparing our model to the recently solved structure of Pum and Nanos in complex with NRE2 (Weidmann et al., 2016), reveals that Nanos may form a bridge between Brat and Pum to stabilise the complex and to coordinate RNA interaction by Pum and Brat to their respective RNA binding sites. Indeed, we were able to confirm this using an integrated structural biology approach.

Loedige, I., et al. 2014. *Genes Dev.* 28(7) pp 749-64

Wang, X., et al. 2002. *Cell* 110(4) pp 501-12

Weidmann, C.A., et al. 2016. *Elife*.



## 569 A Network Approach Reveals Novel Roles of Low Complexity Domains in Spliceosomal Proteins

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The spliceosomal protein U1-70K aggregates in the brains of Alzheimer's disease patients, and contains two disordered low complexity domains, LC1 and LC2. Low complexity domains are characterized by their limited amino acid diversity and are hypothesized to have a role in nuclear granule formation and protein aggregation in neurodegenerative disease. The LC1 domain of U1-70K is necessary and sufficient for aggregation in AD brain homogenate, and contains highly repetitive tandem arrays of basic (R/K) and acidic (D/E) residues. This unusual pattern of amino acids has been previously described as a 'polar zipper' motif, without well-defined functions. Defining the function of the polar zipper motif in U1-70K and other related spliceosome proteins has been limited because they are difficult to crystallize or sequence. To overcome this limitation, we performed co-immunoprecipitation (co-IP) and mass spectrometry of rU1-70K, and mutants lacking one or both LC domains. A network approach was then used to map interacting proteins to distinct domains of U1-70K. Out of the total 716 interacting proteins, 190 mapped to one of two LC1 dependent interacting groups. This group of LC1 interacting proteins were determined to be significantly enriched in 'polar zipper' domains homologous to the LC1. By immunofluorescence, endogenous U1-70K was observed to colocalize with other 'polar zipper' proteins in nuclear granules consistent with their biochemical association. Co-IP and Western blot analysis confirmed that the 'polar zipper' motifs of recombinant LUC7L3 and RBM25 were necessary for reciprocal interactions with U1-70K. Furthermore, the LC1 domain was both necessary and sufficient for U1-70K self-association and nuclear granule localization in cells. Together our data suggest that 'polar zipper' motifs in RNA binding proteins facilitate nuclear localization and serve as a scaffold for granule formation via protein-protein interactions with other homologous 'polar zipper' proteins.

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## 570 Structure of the 90S pre-ribosome prior to A1 pre-rRNA cleavage

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The 40S small ribosomal subunit (SSU) is co-transcriptionally assembled in the nucleolus as part of a giant chaperoning complex called 90S pre-ribosome or small subunit processome. In addition to rRNA and ribosomal proteins, several small RNAs, most prominently the U3 snoRNA, and more than 60 non-ribosomal proteins contribute to formation and activity of this assembly machine. Here, we present the structure of the 90S pre-ribosome preceding pre-rRNA processing from the eukaryotic thermophile *Chaetomium thermophilum* at 3.8 Å resolution. Molecular models for 34 assembly factors, the 5' ETS RNA, the U3 snoRNP and the immature 40S subunit were placed in the architectural context of the 90S scaffold. The structure reveals in pseudo-atomic details the highly-intertwined network of protein and RNA interactions, which is centered around the 5' ETS with U3 snoRNP as a principal organizer. Thereby, a dynamic structural scaffold is provided for early 40S subunit assembly, which involves the sequential and independent folding of the individual pre-40S domains.

## 571 Unusual semi-extractability as the hallmark of nuclear body-associated architectural long noncoding RNAs

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Mammalian paraspeckle nuclear bodies are giant, spheroidal RNPs (diameter: 360 nm) that are constructed by scaffolding NEAT1\_2 long noncoding RNAs (lncRNAs). Physiologically, NEAT1 plays pivotal roles in pregnancy and mammary gland development. Like NEAT1\_2, several other lncRNAs play similar architectural roles in the formation of specific nuclear bodies; we proposed classifying these nuclear body-constructing RNAs as a distinct subclass of lncRNA called "architectural RNA (arcRNA)" (Chujo et al., 2016). Formation of arcRNA-dependent nuclear bodies occurs under various stresses and developmental stages, and is initiated by induced expression of the arcRNAs. Accordingly it is likely that as-yet-unknown arcRNA-dependent nuclear bodies remain to be identified in various cellular contexts.

Here, we report an unusual feature of cellular NEAT1\_2: it is inefficiently extracted by conventional RNA purification procedures, a property we term "semi-extractability." Extensive needle shearing of cell lysate improved NEAT1\_2 extraction by 20-fold, whereas using a conventional method NEAT1\_2 was trapped in the protein phase. Improved extraction enabled us to estimate that approximately 50 NEAT1\_2 molecules are present in a single paraspeckle. The semi-extractability of NEAT1\_2 correlated with its association with paraspeckle proteins, and required the prion-like domain of the RNA-binding protein FUS. This observation suggests that RNA-protein and protein-protein interactions, which are the driving forces of RNA-mediated nuclear body formation, are responsible for semi-extractability. Another architectural lncRNA, IGS16, exhibited similar features. A comparison of RNA-seq data from needle-sheared and control samples revealed the existence of multiple semi-extractable RNAs, many of which were localized in subnuclear granule-like structures. Our findings provide a foundation for the discovery and study of the arcRNAs that constitute nuclear bodies.

## 572 The effect of CHH-mutations on RNase MRP biochemistry

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Human RNase MRP is a ribonucleoprotein composed of at least 7 proteins and single RNA subunit. RNase MRP is an endoribonuclease, which mainly localizes to the nucleoli where it cleaves the precursor of ribosomal RNA. Besides rRNA cleavage, RNase MRP also cleaves the viperin and cyclinB2-mRNAs.

Mutations in the RNA component cause cartilage hair hypoplasia (CHH), a rare pleiotropic disease characterized by short-limbed dwarfism and hypoplastic hair. The effect of some CHH-mutations on cleavage activity, RNA stability or the binding of specific proteins has been investigated in several studies. However, there is no comprehensive overview of how each of these mutations affects all these aspects. In addition, these mutations might interfere with RNase MRP's proper subcellular localization.

We introduced the streptavidin-binding S1m-RNA aptamer to determine the stability and localization of the wild type and mutant MRP RNAs in HEK293 cells. Furthermore this method is used to isolate RNase MRP complexes in order to characterize the protein composition and in vitro cleavage activity. In conclusion, this method allows us to study the effects of CHH-mutations on MRP RNA stability and localization and RNase MRP complex composition and cleavage activity.

### 573 The diversified role of RNA binding proteins in *Trypanosoma cruzi*

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Gene expression regulation comprises a series of events that involves transcriptional and post-transcriptional steps. RNA binding proteins (RBPs) are key players in all events after transcription; they are a diverse family of proteins with a variety of domains involved in splicing, nuclear-cytoplasmic transport, translation and mRNA decay. Trypanosomatids are an interesting model to study the post-transcriptional regulation due to the lack of transcriptional control, relying on RNA-binding proteins (RBPs) to control and define fate of the mRNA in the cell. This work aimed to characterize RBPs and the post-transcriptional network in *Trypanosoma cruzi*, the causal agent of Chagas disease. TcZC3H39 is a cytoplasmic RBP with a zinc finger domain (CCCH) that forms granules in the epimastigote form of the parasite. It was observed that during nutritional stress this protein binds a different subset of mRNAs mostly encoding ribosomal proteins and transcripts for the cytochrome c complex (COX), highly expressed mRNAs in the replicative form. We hypothesized that TcZC3H39 acts as a downregulator during stress conditions. TcNRBD1 is a cytoplasmic RBP that presents one RRM domain and is associated to 80S monosomes and polysomes. Ribonomics of TcNRBD1 allowed the identification of several transcripts that encode ribosomal proteins and rRNAs. These results indicate that TcNRBD1 is associated with different parts of the translation process, either by regulating mRNAs that encode ribosomal proteins or by acting in some step of ribosome assembly in *T. cruzi*. *TRRM2 is a nuclear RBP that is associated to mRNAs and found near the open chromatin borders, indicating a role in sites of active transcription. On the contrary, RBSR1 is also a nuclear RBP that presents a unique RRM domain in its amino terminal region, followed by a Ser/Arg-rich motif, defined as an SR-protein. Phylogenetic analysis shows that RBSR1 is an ortholog of the human SRSF7 protein, suggesting its possible involvement in pre-rRNA processing, confirmed by RNA-seq, where it was found associated to sn and snoRNAs. Altogether, our results show the diversity of the roles that the RBPs play in T. cruzi, like during stress, translation, transcription and rRNA processing.*

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### 574 RNA structural dynamics govern H/ACA ribonucleoprotein function

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In Eukaryotes and Archaea pseudouridine (Ψ) - the most abundant modification in RNAs - can be synthesized by H/ACA-ribonucleoprotein (RNP) particles. This enzyme class consists of the genetically conserved H/ACA guide RNA, which binds the substrate RNA via base pairing, and a set of four proteins (L7Ae, Nop10, Cbf5 and Gar1 in Archaea). To date, most knowledge of these pseudouridylases has been deriving from crystal structures of archaeal H/ACA-RNPs; hence the dynamic understanding of the isomerization process remains scarce. Using single molecule Förster resonance energy transfer (smFRET) spectroscopy we investigate and compare the assembly and catalytic process of an archaeal (*Pyrococcus furiosus*) and a eukaryotic (*Saccharomyces cerevisiae*) H/ACA-RNP. Starting with the archaeal ribonucleoprotein we established labeling sites and developed a new approach to immobilize the entire complex. Using these techniques and a variety of modified substrate RNAs we obtained new insights into assembly and dynamics of RNA guided pseudouridylation. Combining this approach with biochemical analysis, we could show which proteins are essential for substrate binding, how lack of some of these proteins influence the overall RNA structure and that the complex seems to be able to discriminate against mismatched substrates. In addition to that we will also present our newest data of a RNA guided pseudouridylase derived from yeast, and compare structure and assembly of the archaeal to the eukaryotic H/ACA-RNPs.

**575 Telomerase attraction to DNA: What is behind processivity enhancement by human TPP1***Ctirad Hofr***CEITEC and Faculty of Science, Masaryk University, Brno, Czech Republic**

Human telomere maintenance is a highly coordinated process. Telomere length misregulation is connected closely to cancer and telomere-shortening syndromes. Human shelterin component TPP1 increases DNA affinity of telomerase. Recent studies have shown that the OB domain of TPP1, containing TEL-patch a cluster of amino acids on the surface, recruits telomerase to the telomere in human cells. However, there has been only basic biochemical analysis of the role of OB domain of TPP1 for telomerase DNA binding and processivity. Our initial studies suggested that TPP1 bind DNA nonspecifically, similarly to processivity promoting factors in DNA replication. In order to assess whether the OB domain is necessary for DNA binding and hence stable DNA extension by telomerase, we described the effect of OB fold of TPP1 on telomerase activity and processivity *in vitro*. Additionally, we quantified the interactions of TPP1 OB domain with telomerase domains that are essential for telomerase recruitment. Finally, our quantitative observations indicate how OB domain stabilizes the association between telomerase and telomeric DNA substrates, providing a molecular explanation for its contributions to telomerase recruitment, activity and processivity.

**576 U6-specific factor SART3 participate in biogenesis of Sm-class snRNPs***Klara Klimesova, David Stanek***Institute of Molecular Genetics ASCR, Prague, Czech Republic**

Spliceosomal small nuclear RNPs are complexes containing snRNA, Sm or LSm protein ring and set of proteins specific for each particle. U1, U2, U4 and U5 (so called Sm-class snRNPs) are synthesized in a multi-step biogenesis pathway that involves nuclear and cytoplasmic stage and their final assembly takes place in a sub-nuclear compartment, the Cajal body. However, molecular details of snRNP targeting to the Cajal body remain mostly unclear. Our previous results revealed that SART3 protein is important for accumulation of incomplete U4, U5 and U6 snRNPs in Cajal bodies, but the molecular mechanism of the SART3-dependent locking of incomplete snRNPs to the Cajal body is elusive. SART3 was identified as a U6 snRNP interaction partner and a U4/U6 di-snRNP assembly factor. Here, we provide evidence that SART3 interacts also with U4 mono-snRNP and with U2 snRNP, and that it binds specifically immature particles that are localized in Cajal bodies. Next, we show that SART3 associates with U2 snRNP via Sm proteins, which form a stable core of all the Sm-class snRNPs. Thus, we propose that the interaction between SART3 and Sm proteins represents a general SART3-snRNP binding mechanism. We further speculate that the SART3-Sm interaction is important part of a quality control mechanism that detects incomplete snRNPs and tethers them in Cajal bodies.



**577 A novel dsRNA-binding module from prolyl isomerase FKBP25**

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Prolyl isomerases are defined by a catalytic domain that facilitates the cis-trans interconversion of proline residues. In most cases, additional domains are also present in these enzymes that add important biological functions. While several isomerases have been located in the nucleus, very little is known about the range of native substrates and the cellular function of nuclear FK506-binding proteins (FKBPs). In order to gain insight into the biological role of the nuclear prolyl isomerase FKBP25, we have performed proteomic assays to identify partners of FKBP25. The proximity dependent biotin identification (BioID) approach provides an historical snapshot of neighboring protein interactions to FKBP25 fused to the E. coli biotin ligase BirA. Biotinylated proteins in the FKBP25-BirA sample belong to RNA-binding proteins and ribosome biogenesis factors, including 22 ribosomal proteins and several proteins involved in the early maturation events of ribosome production. These FKBP25-associated proteins were further validated by using a complementary co-immunopurification assay. Strikingly, the addition of RNase A in a follow-up assay strongly reduced the interacting partners of FKBP25, implicating a role for RNA in mediating the contacts. Furthermore, we find that RNA is necessary for the localization and recruitment of FKBP25 to nucleolar sites of ribosome biogenesis, with FKBP25 co-localizing with the rRNA transcription factor UBF in an RNA-dependent manner. We therefore predicted a direct interaction with RNA to explain these findings, and a series of in vitro experiments were used to elucidate the nature of the RNA association by FKBP25. We find that FKBP25 in fact harbours a module that binds specifically to double-stranded RNA (dsRNA) and is selective for dsRNA over dsDNA, ssRNA and ssDNA. A minimal dsRNA length of eight base-pairs is required for the interaction, and in the context of ribosome biogenesis this interaction also occurs with nucleolin-bound rRNA stemloop structures. The atomic details were used to design a minimal mutation to abrogate the interaction with RNA in vitro, and this mutant also prevents interaction with RNA in vivo. We hypothesize that the repertoire of substrates for FKBP25 is defined by cellular localization, granted in this case by selective binding to dsRNA.

**578 Components of the SMN complex associate with pre-box H/ACA RNPs during their assembly**

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Spinal muscular atrophy (SMA) is a neuromuscular disease characterized by the degeneration of motor neurons of the spinal cord leading to muscular weakness and atrophy. Reduced level of the essential SMN (Survival of Motor Neuron) protein is the underlying cause of SMA. SMN is part of a large multi-protein complex, which functions in multiple cellular pathways related to RNA metabolism. The best-known role of the SMN complex is in spliceosomal UsnRNP assembly.

The conserved box H/ACA RNPs consist of one box H/ACA RNA and 4 core proteins: Dyskerin, NHP2, NOP10 and GAR1. A subset of these RNPs catalyze the formation of pseudouridine residues on various RNAs. Others participate in the nucleolytic processing of pre-ribosomal RNA and in the synthesis of telomeric DNA. Moreover, during the past years, dozens of H/ACA RNAs of unknown function have been described. The assembly of the H/ACA RNPs is a stepwise process which requires several assembly factors: SHQ1, NAF1 and the R2TP complex. It implicates the co-transcriptional assembly of pre-particles containing nascent RNAs, Dyskerin, NOP10, NHP2 and NAF1. The latest keeps the H/ACA RNP inactive, and needs to be replaced by GAR1 to produce mature and functional H/ACA RNPs in the Cajal bodies.

It has previously been shown that the SMN protein interacts directly with GAR. By glycerol gradient sedimentation, immunoprecipitation and Duolink experiments, we observed that some NAF- and NHP2-containing complexes also include NUFIP and components of the SMN complex. The association between NAF1 and the SMN complex occurs in the nucleus, and may be mediated by direct interactions. These data, altogether with data obtained by RNA interference, are in agreement with a role of the SMN complex in box H/ACA RNP assembly, and allow us to propose the stepwise formation of several transitory complexes during this process.

## 579 Functions of Bcd1p in box C/D snoRNP biogenesis

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Ribosome biogenesis is notably dependent on the action of small RNA protein complexes called small nucleolar ribonucleoproteins (snoRNPs). These are divided in two main families: the so-called box C/D snoRNPs and box H/ACA snoRNPs. Each category performs specific enzymatic processes that induce chemical modifications on target rRNAs. The box C/D snoRNPs are formed by association of a box C/D snoRNA with a set of four invariant proteins, and catalyze rRNA 2'-O-methylation. Biogenesis of these RNPs relies on the action of several proteins complexes which constitute a specific assembly machinery. Rsa1p, Hit1p, and components of the R2TP complex are the best characterized protein actors of this machinery.

Additional protein factors probably participate in box C/D snoRNP biogenesis; Bcd1p (Box C/D snoRNA protein 1) is such a candidate as it is essential for the *in vivo* stability of box C/D snoRNAs in yeast. Moreover, Bcd1p was shown to associate with snoRNP core proteins and assembly factors both in yeast and human cell lines. Despite these observations, the role of Bcd1p in the biogenesis of box C/D snoRNP is poorly understood.

Our preliminary data suggest that Bcd1p downregulation has no effect on snoRNA gene transcription, reinforcing the idea that this protein act downstream at the snoRNP biogenesis level. Interestingly, chromatin immunoprecipitation (ChIP) experiments suggested an interaction of Bcd1p with snoRNA gene loci both in yeast and human cell lines. Moreover, experiments performed in yeast showed that this interaction is RNA-dependent, which is in favor of a co-transcriptional recruitment of Bcd1p on nascent snoRNA transcripts.

Reinforcing this idea, we performed a series of RNA immunoprecipitation (RIP), co-immunoprecipitation and gene disruption experiments indicating that most of the other assembly actors could not be recruited on C/D snoRNAs in the absence of Bcd1p, suggesting that the assembly machinery is recruited on nascent snoRNAs only in presence of Bcd1p. Therefore, our current analyses are dedicated to unveil these early steps of C/D snoRNPs biogenesis, where Bcd1p seems to play a decisive role.

## 580 Mechanism of PWI Domain Binding to Nucleic Acids

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PWI domains are found in several proteins predicted or known to be involved in the regulation of pre-messenger RNA processing, such as constitutive and alternative splicing and the 3'-end modification of transcripts. The spliceosome also contains several proteins that have PWI domains, including pre-messenger RNA processing proteins PRP2, PRP3 and PRP22. The domain is named for a nearly invariant Proline-Tryptophan-Isoleucine tripeptide sequence, and is composed of a structured, four-helix bundle PWI motif and a potentially dynamic region rich in positively charged (basic) amino acid residues. Sequence homology, the location of the domain within the protein and the relative position of the basic region define three distinct sub-families of canonical PWI domains. In RBM25 and SRm160 sub-families, the basic region is N-terminal to the PWI motif, while the basic region is C-terminal of the motif in the PRP3 sub-family. In each instance, the PWI motif and the basic region are essential for optimal nucleic acid binding activity, but their individual roles in the cooperative binding mechanism are still not known. Several biophysical techniques, including electrophoretic mobility shift assays, gel filtration and fluorescence and NMR spectroscopies, are being employed to investigate the mechanism and stoichiometry of PWI domain binding to nucleic acid targets. We will present our recent results on the structural and functional features of the unique C-terminal basic region in the spliceosome-associated PRP3 PWI domain.

## 581 Genome-wide identification of RNA sensors in prokaryotes and eukaryotes

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RNAs are well-suited to act as cellular sensors that detect and respond to metabolite changes in the environment due to their ability to fold into complex structures. Here, we introduce a genome-wide strategy named PARCEL that directly and experimentally identifies RNA sensors in a high-throughput manner. By applying PARCEL to a collection of prokaryotic and eukaryotic organisms, we have revealed 58 new RNA sensors to three key metabolites, greatly expanding the list of known RNA sensors. Concordant with their functional roles, the newly identified RNA sensors exhibit significant sequence conservation and are highly structured, but show an unexpected prevalence outside of non-coding regions and possess new structural motifs. We identified a prokaryotic precursor tmRNA that acts as a vitamin B2 (FMN) sensor to facilitate its maturation. We also discovered new eukaryotic RNA sensors that respond to FMN, suggesting that RNA-based sensing and gene regulation is more widespread than previously appreciated.

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## 582 Transcript specific mRNP capture of locally translated mRNAs in *Drosophila* oogenesis

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A common and important feature of polarized cells is the active and directed localization of mRNAs combined with spatially restricted protein synthesis. Transported mRNAs are associated with proteins in ribonucleoprotein (mRNP) complexes that precisely control and mediate different aspects of directed movement along cytoskeletal structures, ensure translational silencing of the mRNA in transport and translational activation when needed. The maternally deposited mRNAs of *Drosophila* encoding embryonic axis determinants such as *oskar*, *nanos*, *bicoid* and *gurken* are well established model systems to study long-range mRNA transport and the translational regulation of local protein synthesis. However our knowledge of the molecular mechanisms underlying the spatiotemporal translational regulation of these mRNAs, as well as the identity and function of their mRNP components is incomplete. Recently, hundreds of proteins binding to polyadenylated mRNAs have been identified, yet the mRNP composition of a distinct mRNA species is largely unknown.

In order to identify the functional building blocks that impart the spatial and temporal specificity of mRNA translation, we established a protocol that allows isolation of endogenous mRNPs in a transcript specific manner and unbiased identification of associated proteins by mass spectrometry. We show that our method is capable of identifying many of the known regulators of the important maternally deposited mRNA *oskar*, as well as novel trans-acting factors. Furthermore, we show that the transcript specific mRNP composition is dynamic in response to environmental or genetic perturbations. Together with high-resolution single molecule fluorescence *in situ* hybridization (smFISH) co-localization analysis, this study allows a comprehensive view on the spatiotemporal dynamics of mRNP compositions and the regulation of localized translation.

**583 Atomic structure of the activated spliceosome***Chuangye Yan, Ruixue Wan, Rui Bai, Gaoxingyu Huang, Yigong Shi***Tsinghua University, Beijing, China**

Pre-messenger RNA (pre-mRNA) splicing is carried out by the spliceosome, which undergoes an intricate assembly and activation process. Here, we report an atomic structure of an activated spliceosome (known as the B<sup>act</sup> complex) from *Saccharomyces cerevisiae*, determined by cryo-electron microscopy at an average resolution of 3.52 angstroms. The final refined model contains U2 and U5 small nuclear ribonucleoprotein particles (snRNPs), U6 small nuclear RNA (snRNA), nineteen complex (NTC), NTC-related (NTR) protein, and a 71-nucleotide pre-mRNA molecule, which amount to 13,505 amino acids from 38 proteins and a combined molecular mass of about 1.6 megadaltons. The 5' exon is anchored by loop I of U5 snRNA, whereas the 5' splice site (5'SS) and the branch-point sequence (BPS) of the intron are specifically recognized by U6 and U2 snRNA, respectively. Except for coordination of the catalytic metal ions, the RNA elements at the catalytic cavity of Prp8 are mostly primed for catalysis. The catalytic latency is maintained by the SF3b complex, which encircles the BPS, and the splicing factors Cwc24 and Prp11, which shield the 5' exon-5'SS junction. This structure, together with those determined earlier, outlines a molecular framework for the pre-mRNA splicing reaction.

**584 Insights into the Functions of SF3b1 and Prp5 during Branchsite Recognition and in Spliceosome Fidelity***Tucker Carrocci, Joshua Paulson, David Beier, Aaron Hoskins***University of Wisconsin-Madison, Madison, WI, USA**

RNA and protein components of the U2 snRNP play critical roles in recognizing the branchsite sequence (BS) during pre-mRNA splicing. While the molecular architecture of the U2 snRNP in the context of the activated spliceosome has been recently revealed, the importance of specific RNA:protein contacts and conformational changes remains largely uncharacterized. We have previously studied how mutations in the U2 protein SF3b1 linked to myelodysplastic syndromes (MDS) change BS fidelity in yeast (Carrocci, *et al.*, *Nucleic Acids Research*, 2017). Our results showed that MDS alleles alter BS usage at specific positions flanking the branchpoint. Here, we investigated the RNA binding domains of SF3b1 that directly contact the U2/BS duplex and the hypothesis that the activity of Prp5 is regulated by large-scale structural rearrangements. Disruption of contacts between SF3b1 and the U2/BS duplex result in splicing defects when BS substitutions are found at -2, -1 and +1 positions relative to the branchpoint adenosine. The affected positions are identical to what we have previously observed for mutations linked to MDS and other cancers. This suggests a mechanism by which MDS-linked mutations alter splicing by changing the stability of the SF3b/BS complex, an effect that can be recapitulated by direct mutation of the RNA site binding itself. These same positions within the BS are also altered by mutations in the Prp5 protein. We have used single molecule FRET to characterize Prp5 conformational dynamics in the presence of ATP, RNA, and other splicing factors. In the absence of substrates, we do not detect structural transitions within Prp5, and the FRET values are consistent with the Prp5 crystal structure in which the RecA domains are separated. In the presence of ATP and RNA, Prp5 begins to transition to a short-lived state consistent with closure of the RecA domains. The addition of ADPNP stabilizes this state, suggesting a link between ATP hydrolysis and the lifetime of the closed conformation. We are now using these methods to investigate the impact of Prp5 mutations that alter BS fidelity on conformational switching. Together our results from SF3b1 and Prp5 reveal the importance conformational dynamics likely play in splicing fidelity.



## 585 The Hydrolytic Splicing of Group II Intron Ribozymes: A Novel Two-Metal-Aided Mechanism Revealed by QM/MM MD Simulations

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Group II introns are mobile  $Mg^{2+}$ -dependent ribozymes found in prokaryotes and eukaryotic organelles capable of self-splicing and retrotransposition.<sup>1</sup> They have been found to share common evolutionary origins with the eukaryotic spliceosome, thus representing an ideal model system to understand the mechanism of conversion of premature messenger RNA (mRNA) into mature mRNA.<sup>2</sup> These RNA macromolecules can self-catalyze their excision from an RNA strand via two transesterification events following a two-metal-aided catalysis.<sup>3</sup>

Here, we have investigated the mechanism of the first step of the splicing reaction catalyzed by this class of ribozymes by means of classical and QM(CP)/MM molecular dynamics simulations in combination with thermodynamic integration to explore the free energy surface of the process.

Our computational studies reveal a concerted one-step dissociative mechanism in which the bulk water accepts the nucleophile's proton during its attack on the scissile phosphate. The two  $Mg^{2+}$  ions play different crucial catalytic roles in the reaction. While one  $Mg^{2+}$  facilitates the cleavage by coordinating the oxyanion leaving group, the other stabilizes the activated electrophile. Hence, our results elucidate a novel two- $Mg^{2+}$ -ion mechanism which differs from the one observed in protein enzymes most likely due to the rather rigid architecture of the catalytic site which is exclusively formed by the RNA sugar-phosphate backbone.<sup>4</sup>

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## 586 Protein-RNA Interactions during Catalytic Steps of Pre-mRNA Splicing

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The spliceosome is assembled *via* sequential interactions of the pre-mRNA with five small nuclear RNAs and many proteins. Stepwise changes of these interactions play roles in positioning of splice sites and formation of the RNA catalytic core. To understand how protein-RNA interactions facilitate the splicing reaction, we probed interactions across the 5' splice site, branch site and 3' splice site at various steps of the spliceosome pathway by site-specific photo-crosslinking. We found that Prp8 interacts with the 5' splice site after activation of the spliceosome, and also interacts with the branch site after destabilization of SF3a/b, presumably to stabilize 5' splice site-branch site interactions for the first reaction. Step one factor Cwc25 contacts the branch site only downstream of the Prp8 binding site. Prp8 made a profound switch from interacting with the branch site to the 3' splice site after Prp16-mediated spliceosome remodeling, but only in the presence of step two factors Slu7/Prp18/Prp22. Slu7 and Prp22 directly bind to the intron and exon 2 sequences flanking Prp8, with Slu7 proximal to Prp8 on both sides, and the interactions retain after exon ligation. Our results suggest that Prp8 may play a key role in stabilizing the interaction between the 5' splice site and the branch site during the first reaction and between the 5' and 3' splice sites during the second reaction. The catalytic step factors may only play accessory roles in facilitating or stabilizing the interactions of Prp8 with the splice sites to promote catalytic reactions.

## 587 Structure and Function of U1 snRNP

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Splicing of pre-mRNA in eukaryotes is essential for gene expression and is catalyzed by the spliceosome, a large protein-RNA complex composed of U1, U2, U4, U5, and U6 small nuclear ribonucleoprotein complexes (snRNPs) and numerous non-snRNP related proteins. Despite the abundance of recent structural data, we have limited understanding of the initial intron recognition and early spliceosomal assembly. U1 snRNP is the most abundant snRNP and is critical for the recognition of the pre-mRNA 5' splice site (ss) through base pairing with the 5' end of U1 snRNA. Due to this role, U1 snRNP is often targeted by factors associated with alternative splicing to prevent or assist U1 snRNP binding to the 5' ss. In contrast to the human 250kDa U1 snRNP, the yeast U1 snRNP is nearly 800kDa and much more complex. The yeast U1 snRNP is composed of a U1 snRNA nearly 3.5-fold larger than the human U1 snRNA and has seven more stably associated proteins (Luc7, Nam8, Prp39, Prp40, Prp42, Snu56, and Snu71). These stably associated proteins have human homologs which are weakly associated with the human U1 snRNP and are implicated in alternative splicing. We have recently determined the cryo-EM structure of the yeast U1 snRNP at 3.7Å resolution. Based on this structure, we carried out biochemical and genetic experiments to decipher the role of the much larger yeast U1 snRNA and U1C in U1 snRNP assembly as well as the role of human accessory U1 snRNP proteins such as PrpF39 in alternative splicing. Our results will shed light on the structure and function of yeast U1 snRNP. Furthermore, they will provide valuable information on alternative splicing regulation in higher eukaryotes.

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## 588 Modulation of the spliceosomal catalytic triplex by U6 snRNA and *cwc2* alleles

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A highly conserved region in U6 is paired to U4 before spliceosome formation, subsequently forms an internal U6 stem-loop (ISL) structure during spliceosome activation and later forms a triplex with U2 thought to be the closed catalytic conformation. U6-C67a mutation within the ISL causes a severe cold-sensitivity (cs) phenotype, which is suppressed by a compensatory U4-G14u mutation (Shannon & Guthrie 1991; McManus et al. 2007). However, even in the presence of U4-G14u, the U6-C67a mutant still exhibits a splicing defect, as evidenced by analysis of ACT1-CUP1 reporters. We show that this defect stems from the destabilization of the catalytic conformation of the ISL, resulting from an altered U6-U80-U6-C61:U2-G21 base triple interaction within the catalytic triplex. In the competing, open U6-ISL structure, U6-C67 is juxtaposed against U6-U80 (Sashital et al. 2003), and this interaction is stabilized by the U6-C67a mutation. Mutations in U2 and U6 that hyperstabilize the triplex partially rescue the cs phenotype of U6-C67a and improve splicing of BS-c introns (limiting for both catalytic steps) but inhibit BS-g introns (limiting for the 2<sup>nd</sup> step).

We have previously identified a number of U6 alleles that suppress the cs defect of U6-C67a; their distribution resembles Cwc2-U6 physical contacts identified by Rasche et al. 2012. Compatible with this large Cwc2-U6 interface, we identified through a genetic screen a large number of *cwc2* mutants that suppress the U6-C67a cold-sensitivity. These *cwc2* alleles also suppress the cold-sensitivity of *prp16-302* (defective exit from the first step conformation), suggesting that destabilization of the Cwc2-U6 interaction allows for the reorganization of interactions within the catalytic spliceosome, facilitating first-to-second step conformational transitions at the catalytic center. Some of the identified *cwc2* alleles exacerbate cs defects of U6-C61a and -C61g mutants within the catalytic triplex, further supporting our model that they destabilize the catalytic triplex.

Recent cryo-EM spliceosome structures show that the catalytic triplex forms in the pre-catalytic complex Bact and is present in catalytic complexes C and C\*. Our results suggest that U6-ISL toggles between the open (pre- and post-catalytic) and closed (catalytic) conformations, and that these altering conformations affect transitions within the catalytic phase of splicing.

## 589 A novel type of intronic circRNA in nuclear genes of euglenids (Euglenida)

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Nonconventional introns found in nuclear genomes of euglenids (Euglenida), phytoflagellated Excavate protozoans, possess some unusual traits such as variable, noncanonical borders and an ability to form a stable RNA secondary structure, which brings together intron ends, placing adjacent exons in close proximity. Although they were first described in the early nineties, their mechanism of removal still remains undiscovered.

To gain insight into splicing pathways of nonconventional introns, we have investigated the physical form of excised introns in model organism, *Euglena gracilis*. Its *tubA* gene contains three conventional spliceosomal introns, which obey the GT-AG rule, two nonconventional ones and one intron combining features of both aforementioned types, i.e. an intermediate intron.

Using experimental approach based on cDNA synthesis and an inverse PCR, we observed, as expected, conventional lariats consistent with a two-step transesterification pathway for spliceosomal introns. This was not the case for the nonconventional introns and so-called intermediate one, which are released as circular RNA particles with full-length ends. We suggest that this new type of intronic circRNA might play a role in intron mobility due to the frequent insertions of nonconventional introns at a new positions within the genes.

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## 590 Impaired spermatogenesis in U12 intron splicing-defective *Zrsr1* mutant mice

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The U2AF35-like ZRSR1 protein has been implicated in 3' splice site recognition, but ZRSR1 knockout mice do not show abnormal phenotypes. To analyze the ZRSR1 function and its precise role in RNA splicing, we generated ZRSR1 mutant mice containing truncating mutations within its RNA-recognition motif. Homozygous mutant mice exhibit severe defects in erythropoiesis, muscle function and spermatogenesis, ultimately leading to azoospermia and male sterility. Histological analysis of mutant mice testis revealed seminiferous tubules arrested at the round spermatid stage, very few spermatozoa and germ cell sloughing with a high level of apoptosis. These results indicate an essential role for *Zrsr1* in spermatogenesis, when transcription of its principal paralog *Zrsr2* is repressed through X-chromosome inactivation. Testis RNA-Seq analyses revealed changes in the expression of 874 genes and increased intron retention of both U2- and U12-type introns, including U12-type intron events in genes with key functions in spermatogenesis. Interestingly, affected U2 introns were commonly found flanking U12 introns, and minigene analyses revealed functional cross-talk between the two spliceosomes for the definition of exons flanked by the two intron classes. The splicing and tissue defects observed in ZRSR1 mutant mice reveal a physiological role for this factor in U12 intron splicing.

**591 The Function of the Cassette Exon 7b of the Transcription Factor ERG***Samantha Jumbe, Bahareh Vahabi, Ian Wilson, Michael Lodomery***University of the West of England, Bristol, UK**

In cancer the transcription factor *ERG* is generally associated with aggressive disease and poor prognosis. It is now recognised as a key oncogene in prostate cancer where it is found in 50% of cases. Several splice isoforms of *ERG* with variable biological activity exist. The inclusion of the cassette exon 7b has been shown to be associated with progression of disease as well as aggressiveness in prostate cancer. This splicing event also changes the size of the alternative domain which contains binding sites for other transcriptional proteins.

Splice switching oligonucleotides (SSO) designed against the splice sites for exon 7b were designed to induce exon skipping. *ERG* splicing expression was detected at mRNA and protein level. Biological function was studied using assays to detect cell maturation, migration and cell viability.

Successful skipping of exon 7b after SSO treatment was confirmed in several cell lines including the osteosarcoma MG63 cell line, in which *ERG* has not previously been studied. Cell viability is not affected by SSO treatment. Increased alkaline phosphate activity in MG63 indicates cell maturation and this is attenuated with SSO induced splice skipping of exon 7b.

**592 Structural studies of diverse regulatory layers of U2AF in 3' splice site recognition***Hyun-Seo Kang<sup>1,2</sup>, Carolina Sanchez-Rico<sup>1,2</sup>, Reymond Sutandy<sup>3</sup>, Stefanie Ebersberger<sup>3</sup>, Sophie Bonnal<sup>4,5</sup>, Juan Valcarcel<sup>4,5</sup>, Julian König<sup>3</sup>, Michael Sattler<sup>1,2</sup>*

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Pre-mRNA splicing is an essential mechanism in eukaryotic mRNA processing and greatly contributes to proteome diversity by alternative splicing. A critical early step involves defining the exon/intron boundaries in the pre-mRNA transcripts, such as the branch-point sequence (BPS), polypyrimidine (Py) tract and 3'-AG dinucleotide in the 3'-splice site. Our previous structural studies have demonstrated, first, importance of the conformational dynamics of RNA-binding domains U2AF65-RRM1,2 for recognizing loosely defined Py-tracts, second, its modulation by the presence of U2AF35 for the regulation of 3'-splice site recognition.

Here, we present yet another regulatory layer for 3'-splice site selection by U2AF65 where the dynamic interaction of domain linker to RRM1,2 domains plays an autoinhibitory role for preventing non-specific RNA interactions to ultimately help selecting bona fide 3'-splice sites. We further tested the contributions of the domain linker on binding natural RNA sequences by using in vitro iCLIP experiments.

Additionally, we are investigating, another perspective of regulating 3'-splice site recognition by U2AF, where hnRNP A1 was reported to help discriminating U2AF from binding 3'-splice site in a sequence-dependent manner of the dinucleotide AG, using various biochemical methods.



**593 Human variant U2 snRNAs provide diverse platforms for intron recognition***Brian Kosmyna, Varun Gupta, Charles Query***Albert Einstein College of Medicine, Bronx NY, USA**

snRNAs are essential components of the spliceosome, which catalyzes the removal of introns from nascent pre-mRNA transcripts. The spliceosome comprises five small nuclear RNAs (U1, U2, U4, U5, and U6 snRNAs), each of which binds a specific set of core proteins to form stable small nuclear ribonucleoproteins (snRNPs). In metazoans, many pre-mRNA transcripts are alternatively spliced, increasing the diversity of mRNAs and number of functional splice sites. The proper identification of splice sites is cell-type specific and developmentally regulated. Mutations in core spliceosome components are associated with diseases such as myelodysplastic syndromes, retinitis pigmentosa, and spinal muscular atrophy.

Although base pairing between snRNAs and the pre-mRNA substrate is important for splice site identification, positioning the substrates in the active site, and coordinating catalytic magnesium ions, it is currently unclear how spliceosomes accommodate the substrate variability found in vertebrates. While snRNAs are known to be highly and ubiquitously expressed ( $10^5$ - $10^6$  molecules/nucleus), their expression and regulation are not well understood. Early characterization of snRNAs showed sequence heterogeneity of vertebrate snRNAs, and it was suggested that expression of snRNA variants could regulate alternative splicing. Since sequencing of the human genome, the repetitive nature of snRNA gene loci has become evident. However, almost all current RNA-Seq protocols actively select against the sequencing and alignment of snRNAs. Therefore, the diversity of variant snRNAs expressed has been inadequately addressed.

We have discovered previously undescribed U2 snRNA variants that are expressed and assembled into snRNPs. Spliceosomes assembled with variant U2 snRNAs are expected to have altered affinity for splice sites based on nucleotide variation in sequences that form Watson-Crick base pairs with the pre-mRNA substrate. These U2 snRNA variants are differentially expressed in hematopoietic cell lines suggesting variant U2 snRNAs contribute to cell-type specific alternative splicing. Additionally, these snRNAs may have altered snRNP assembly kinetics due to sub-optimal spliceosomal protein binding sequences, which would allow for snRNP assembly to be a major point of regulation for variant snRNAs. Expression and regulation of these variant spliceosomes may help explain changes in splicing that take place during development and disease that are currently not understood.

**594 Capture of mRNA fragments and genic *trans*-splicing mediated by a bacterial group II intron during circularization***Felix LaRoche-Johnston, Caroline Monat, Samy Coulombe, Benoit Cousineau***McGill University, Department of Microbiology and Immunology, Montreal, Quebec, Canada**

Group II introns are functional RNA elements present in a wide spectrum of organisms, ranging from bacteria, to archaea, to the organelles of certain eukaryotes. From an evolutionary standpoint, they are thought to have substantially shaped the origin and evolution of eukaryotic genomes. They are considered as the progenitors of hallmark genetic components such as nuclear introns and the snRNAs of the spliceosome. In bacteria they are highly autonomous, containing an intron-encoded protein (IEP) which assists the intron to self-splice from the pre-mRNA of invaded genes. Bacterial group II introns mainly splice through the conventional branching pathway, where they are released as RNA lariats. However, these retroelements can also splice through less well-characterized secondary pathways such as circularization, where they are released as circles.

Here we present a new splicing model wherein the group II intron L1.LtrB, from the gram-positive bacterium *Lactococcus lactis*, can incorporate cellular mRNA fragments at its circle splice junction and catalyse the genic *trans*-splicing of bacterial mRNAs.

We demonstrate that released L1.LtrB lariats can recognize and reverse splice into specific recognition motifs found abundantly in various bacterial mRNAs. After having catalysed its own insertion, the group II intron can base-pair with the same initial recognition site and excise itself through conventional forward splicing. However, the intron can also catalyse the ligation of an external nucleophile to the downstream interrupted mRNA, which generates a chimeric mRNA molecule and liberates the intron 3'-end. Following this *trans*-splicing reaction, L1.LtrB can either circularize conventionally, leading to the formation of head-to-tail intron circles; or alternatively circularize by base-pairing with another recognition motif upstream on the invaded mRNA, which leads to the incorporation of mRNA fragments at the intron circle splice junction.

Overall, this work details a new splicing model for group II introns in bacteria. We provide the first evidence for in vivo genic *trans*-splicing of bacterial mRNAs, which has wide-ranging implications for the overall genetic diversity of group II intron-containing bacteria.

## 595 Dissecting the Role of Dib1 in Spliceosome Assembly

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Assembly of the spliceosome is a highly ordered process that involves many protein-protein and protein-RNA rearrangements. In this study, we sought to characterize the role that the small, essential protein, Dib1, has in this assembly process. Recent cryo-EM structures of the U4/U6-U5 triple snRNP indicate that Dib1 is housed in a central location in the U4/U6-U5 triple snRNP in close proximity to the large U5 protein Prp8 and loop I of the U5 snRNA (1, 2). This region is thought to interact with the pre-mRNA upon its association with the U4/U5-U6 triple snRNP, suggesting Dib1 must depart from the spliceosome for the pre-mRNA to properly join. Indeed, previous mass spectrometry studies suggest that Dib1 leaves the splicing machinery prior to spliceosome activation (3). Therefore, the departure of Dib1 from the spliceosome may serve as a checkpoint in spliceosome assembly, although this potential biochemical function has not been previously studied.

To begin to understand how Dib1 interacts with the spliceosome and what may lead to its departure, we determined whether Dib1 could exchange readily on the spliceosome. Our findings show that recombinant Dib1 protein can reconstitute a *dib1* defective splicing extract to restore *in vitro* splicing, thus highlighting the dynamic nature of Dib1 on the spliceosome. In order to further understand the role of Dib1 in splicing, we sought to characterize residues that affect its function by identifying temperature sensitive (*ts*) mutations in *S. cerevisiae*. We will present two *ts* mutants of Dib1 that have different growth and splicing defects as assayed with *in vitro* splicing assays. These mutations are located in distinct areas of Dib1, a hydrophobic pocket and a loop region. We have further characterized the ability of these mutants to perturb spliceosome assembly using native gel analysis and observe stalled assembly. Together, these findings suggest that Dib1 plays a significant role in spliceosome assembly.

(1) Nguyen *et al.*, *Nature*. **530**, 298–302 (2016). (2) Wan *et al.*, *Science*. **351**, 1–17 (2016). (3) Fabrizio *et al.*, *Mol Cell*. **36**, 593–608 (2009).

## 596 Deep conservation of the neural-specific microexon alternative splicing program across bilaterians

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The vertebrate central nervous system (CNS) is a complex network of cells whose development and functioning depends on a finely orchestrated regulation of gene expression that includes the interplay of RNA-regulatory networks and the synthesis of a vast repertoire of protein variants. Alternative splicing (AS) is a major post-transcriptional process involved in regulating gene expression and generating transcript and proteome diversity. Notably, AS shows the highest prevalence and the lowest divergence evolutionary rates in vertebrate neural tissues (Barbosa-Morais N. *et al.* 2012, Irimia M. *et al.* 2014). Moreover, a specific-class of very small exons from 3 to 27nt, termed microexons, shows a sharp neural inclusion and a high evolutionary conservation among vertebrates (Irimia M. *et al.* 2014), indicating a role of these exons in the function and development of the vertebrate CNS. Despite these observations, little is known about the impact of AS on the evolution of the vertebrate CNS. In order to address this question; in the present work we explore the conservation of AS events using tissue-specific transcriptomes of 7 vertebrate (human, mouse, cow, chicken, western claw frog, zebrafish and elephant shark) and 5 non-vertebrate species (amphioxus, sea urchin, centipede, fly and octopus). Results on microexon conservation show that neural microexons are widely conserved among vertebrate species. Remarkably, a fraction of them are also conserved and show neural-specific inclusion in bilaterians. This suggests a general role of microexon AS in the evolution of the animal nervous system, where the appearance of the neural-specific microexon AS program precedes the separation of the non-vertebrate and vertebrate lineages followed by its expansion in the vertebrate one.

**597 Prp8 affects conformational changes in the spliceosome between the catalytic steps***Katarzyna Matylla-Kulinska, Lukasz Widlo, Magda Konarska***University of Warsaw, University of Warsaw, Poland**

Prp8 is the largest, centrally positioned protein component of the spliceosome, yet its influence on structural rearrangements between the catalytic steps is still poorly understood. We performed a genetic screen for *prp8* alleles that suppress cold-sensitivity (cs) of *prp16-302*, an allele that limits exit from the first- and entry to the second step of splicing. Previous analyses of *prp8* alleles that improve the second step and are located in the central and C-terminal domains of Prp8, failed to identify any suppressors of *prp16-302* defects; therefore, we have now focused our analysis on the N-terminal domain of Prp8. The identified alleles are clustered at the interface of Prp8 and Snu114 (spanning *prp8* region pos.330-380&400-445) and Cwc2 (pos.588-605), suggesting that suppression of *prp16-302* defects involves alterations of Prp8 contacts with these proteins. In addition, we identified a cluster of Prp8 mutations (pos.608-620) located in the proximity of U6-ISL and the intron branch-site in complex C. These alleles inhibit splicing of suboptimal substrates: e.g. 5'SS-G5a, -A3u, -A3c, BS-c and BS-g. They also exacerbate the cs phenotype of U6-ISL mutants (e.g. U6-C67a and -A59c) that hyperstabilize the non-catalytic structure of ISL (see abstract by K.Eysmont and M.Konarska). These results, together with location of these alleles in the spliceosome, suggest that this region of Prp8 is in a direct contact with both U6-ISL and the substrate positioned for catalysis.

Interestingly, relative positioning of Prp8 regions bearing the identified alleles and their putative contact molecules: U6, Snu114, or Cwc2 does not change significantly between complexes C and C star (Yan et al, 2017, Rauhut et al 2016), suggesting that first step interactions between these molecules may be only temporarily disrupted during the transition phase, returning to the previous state for the second step. Furthermore, the identified *prp8* alleles represent a new class of mutants, distinct from the previously characterized second-step *prp8* alleles (Liu et al, 2007). We suggest that whereas second-step alleles primarily stabilize the substrate repositioned for the second step, the newly isolated alleles destabilize the closed catalytic conformation of the spliceosome.

**598 Brr2 is a pre-mRNA splicing fidelity factor***Megan Mayerle, Christine Guthrie***University of California San Francisco, San Francisco, California, USA**

Pre-mRNA splicing must proceed with high fidelity in order to ensure accurate gene expression. A series of DExD/H box helicases use kinetic proofreading to help minimize errors during splicing. Mutations that decrease the ATPase activity of these helicases facilitate splicing of introns containing weaker, less consensus splice sites. Brr2 is a spliceosomal DExD/H box helicase required for spliceosome activation. Multiple *brr2* alleles associated with Retinitis Pigmentosa (RP), a form of hereditary blindness, have been shown to impact Brr2's ATPase activity and lead to defects in spliceosome activation (Maeder et al. 2009; Zhao et al. 2009; Santos et al. 2012). We show that yeast carrying *brr2*-RP alleles exhibit widespread intron retention. Furthermore, using the ACT-CUP reporter system, we show that *brr2*-RP yeast exhibit both error-prone and hyperaccurate phenotypes when challenged with splicing reporter constructs, particularly those bearing non-consensus pre-mRNA branch-site. Preliminary genetic data indicate that *brr2*-RP alleles interact genetically with *prp16* alleles known to impact branch-site fidelity. Together these data implicate Brr2 in the regulation of splicing fidelity, and suggest that Brr2 cooperates with Prp16 to proofread pre-mRNA prior to the 1<sup>st</sup> catalytic step.

**599 Interrogating the Importance of RNA Constituency in Splice Site Selection***Linus F. Reichenbach<sup>1</sup>, Andrew Jobbins<sup>2</sup>, Ian C. Eperon<sup>2</sup>, Glenn A. Burley<sup>1</sup>*<sup>1</sup>University of Strathclyde, Glasgow, UK; <sup>2</sup>University of Leicester, Leicester, UK

This project aims to probe the mechanism by which exonic splicing enhancers (ESEs) present within pre-mRNA exert their effect in alternative and constitutive RNA splicing. ESEs up to several hundred nucleotides away from a splice site are known to influence splicing to an alternative site.<sup>1</sup> Their ability to influence the selection of splice sites is a hallmark of alternative splicing and subsequently protein diversity in general.

One of the most accepted models by which ESEs exert their effect is the initial binding of an SR protein (e.g., SRSF1) to an ESE which subsequently interacts with a splice site by a three-dimensional (3D) diffusion or looping mechanism. This is supported by evidence that ESE activity depends on the distance to the splice site and cross linking experiments.<sup>2</sup>

The 3D diffusion mechanism implies that the authenticity of the RNA backbone should not greatly influence splice-site selection, as long as the RNA construct still behaves as a freely diffusing random coil. We have recently shown that this is not always the case. For example, the insertion of an ESE connected by an alkyl or hexaethylene glycol (HEG) linker on the 5' end of pre-mRNA using click chemistry, results in complete abolition of ESE function but it does not inhibit splicing.<sup>3</sup> This implies that RNA structure plays an influential role in ESE activity.

Here we describe current efforts towards understanding how the nature of linkers between an ESE and a pre-mRNA sequence influences splice-site selection by preparing synthetic ESEs containing non-natural linkers (HEG, abasic ribonucleoside) followed by splint ligation to a pre-mRNA construct.

In contrast to previous results both new linkers support splicing activity and ESE function, although the overall splicing efficiency was decreased. This shows that the constituency of the RNA construct is essential for ESE function. These results will further be supported by single molecule work and pull-down studies using biotinylated constructs.

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2. a) Graveley et.al. Mol. Cell. 1998, 1, 765-771. b) Shen et.al. Molecular Cell 2004, 13, 367-376.
3. a) Lewis et.al. Angewandte Chemie Int.Ed., 2012, 51, 9800.

**600 5' splice-sites selection by hnRNP L through multiple exon motifs in KLF6 and Bcl-x pre-mRNA alternative splicing***Tiing Jen Loh, Namjeong Choi, Jiyeon Ha, Jagyeong Oh, Xuexiu Zheng, Haihong Shen*

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In alternative splicing, selection of 5' splice-sites (5'SS) plays an important role in alternative exon skipping or inclusion. Although regulatory mechanisms of heterogeneous nuclear ribonucleoprotein L (hnRNP L), a well-known splicing regulatory protein, have been studied in a substantial level, its role in 5'SS selection is not thoroughly defined. By using a KLF6 pre-mRNA alternative splicing model, we demonstrate in this report that hnRNP L inhibits proximal 5'SS but promotes two consecutive distal 5'SS splicing, antagonizing SRSF1 roles in KLF6 pre-mRNA splicing. In addition, three consecutive CA-rich sequences in a CA cassette immediately upstream of the proximal 5'SS are all required for hnRNP L functions. Importantly, the CA-cassette locations on the proximal exon do not affect hnRNP L roles. We further show that the proximal 5'SS but not the two distal 5'SSs are essential for hnRNP L activities. Notably, in a Bcl-x pre-mRNA model that contains two alternative 5'SS but includes CA-rich elements at distal exon, we demonstrate that hnRNP L also suppresses nearby 5'SS activation. Taken together, we conclude that hnRNP L suppresses 5'SS selection through multiple exonic motifs.



## 601 Therapeutic splicing correction by U1 snRNP-mediated redefinition of a critical exon of spinal muscular atrophy gene

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Spinal muscular atrophy (SMA) is caused by deletions or mutations of *Survival Motor Neuron 1 (SMN1)* gene. *SMN2*, a nearly identical copy of *SMN1*, cannot compensate for the loss of *SMN1* due to predominant skipping of exon 7. However, correction of *SMN2* exon 7 splicing holds the promise for SMA therapy. Intronic splicing silencer N1 (ISS-N1) located immediately downstream of the 5' splice site (5'ss) of exon 7 constitutes one of the major regulatory elements responsible for *SMN2* exon 7 skipping and an antisense oligonucleotide (ASO) that targets ISS-N1 is currently the only approved drug for SMA. The stimulatory effect of an ISS-N1-targeting ASO is ascribed to the displacement of the inhibitory factor(s) hnRNP A1/A2 and the structural rearrangement favoring recruitment of the U1 snRNP to the 5'ss of exon 7. Similar stimulatory effects on *SMN2* exon 7 inclusion have been observed employing engineered U1 snRNPs (eU1 snRNPs): the suppressor U1 with improved complementarity to the 5'ss of exon 7 as well as the U1 snRNAs that target downstream intronic sequences. A recently reported individual patient with severe SMA showed complete skipping of *SMN1* exon 7 due to a splice site mutation (G to C substitution at the first position of intron 7, G1C) that destroyed the authentic 5'ss. Here we employed eU1 snRNPs that promoted exon 7 inclusion in the context of G1C mutation by activating a strong cryptic 5'ss (N1-5'ss) located within ISS-N1. While suppressed in the presence of the authentic 5'ss of exon 7, the N1-5'ss is preferentially activated by U1 snRNPs targeting ISS-N1 as well as other sequences upstream and downstream of ISS-N1 in *SMN1* carrying G1C as well as other splicing mutation associated with different types of SMA. Our findings open up a new therapeutic avenue for patients with pathogenic splicing mutations in a critical exon, skipping of which causes SMA, one of the leading genetic causes of infant mortality.

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## 602 The role of splicing factor gene mutation in craniofacial disorders

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The related craniofacial disorders Burn-McKeown Syndrome (BMKS) and MandibuloFacial Dysostosis Guion-Almeida type (MFDGA) are characterized by mandibular and malar hypoplasia, microcephaly, choanal atresia, external ear anomalies and other variable craniofacial and developmental defects. We have recently found that mutations that reduce expression of the U5 snRNP gene *TNXL4A (DIB1)* cause BMKS. Interestingly, reduced expression of the U5 snRNP gene *EFTUD2 (SNU114)* causes MFDGA providing a strong link between U5 snRNP function and these craniofacial disorders, as well as a possible functional link between the DIB1 and Snu114 proteins during splicing. The mechanisms by which reduced expression of essential splicing factor genes, required for splicing all pre-mRNAs, bring about these particular craniofacial disorders are unclear. Our hypothesis is that reduced expression of DIB1 and SNU114 partially disrupts spliceosome function, resulting in missplicing of a subset of pre-mRNAs required during craniofacial development.

Reduced expression of DIB1 and SNU114 in yeast does indeed cause missplicing of some pre-mRNAs but not others. In addition, we have found that reduced expression of DIB1 and SNU114 cause defects in snRNP assembly, including defects in tri-snRNP formation. We are currently investigating whether there is a functional link between DIB1 and Snu114 within the spliceosome. Growth of yeast models under a variety of conditions has identified conditions that induce ER stress as affecting growth of yeast models more than wild type cells. Related to ER stress sensitivity, we have identified the intron containing gene *CNB1*, as being particularly susceptible to reduced DIB1 and SNU114 expression. *CNB1* codes for Calcineurin B, the regulatory subunit of calcineurin, a Ca<sup>++</sup>/calmodulin-regulated type 2B protein phosphatase. Calcineurin is important in the signaling pathway that promotes cell survival under stress.

Reduced levels of Calcineurin cause ER stress, which in turn can induce apoptosis. Apoptosis of neural crest cells, at certain times and locations during development, is critically important for proper craniofacial development. We are now investigating whether patient cells are more sensitive to ER stress and setting up mouse models to determine exactly how reduced DIB1 and SNU114 expression leads to BMKS and MFDGA.

### 603 Birth of a bipartite exon by intragenic deletion

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We show a new type of pseudoexon activation in genetic disease. The pseudoexon was derived from two separate introns as a result of a COL4A5 deletion in a typical Alport syndrome. The DNA deletion encompassed exons 38 through 41 and activated a cryptic 3' and 5' splice site located in intron 37 and intron 41, respectively, normally separated by >10 kbp. The deletion breakpoint was in the middle of the pseudoexon, with the two exonized parts exhibiting substantial self-complementarity, potentially bringing the 3' and 5' splice site into spatial proximity. The pseudoexon introduced a stop codon in the shortened COL4A5 mRNA and was fully included in mature transcripts, which was recapitulated *ex vivo* following transfections of the deletion-containing plasmid into 293T cells. The pseudoexon 3' splice site, polypyrimidine tract and branch site were derived from an antisense, 5' truncated LINE-1 (L1) retroposon. This ancient L1 copy sustained several point mutations that created a conserved CAG consensus at the 3' splice site early in primate development. The deletion did not result in any gain/loss of known splicing enhancer/silencer 6-mers across the breakpoint. We are investigating structural and functional requirements for this exonization using a combination of *in vitro/in vivo* probing, RNA pull downs, transfection and biophysical studies. Together, these results expand the repertoire of mutational mechanisms that alter RNA processing, illustrate extraordinary versatility of transposons in shaping the new exon-intron structure and disease severity, and provide a valuable model for studying exon definition.

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### 604 Structural investigations of the spliceosome: insights into the transition between two steps during RNA splicing

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Each cycle of pre-mRNA splicing comprises two sequential transesterification reactions, first freeing the 5'-exon and generating an intron lariat-3'-exon, and then ligating the two exons and releasing the intron lariat. Both reactions are catalyzed by a stepwise-assembled molecular machine called spliceosome. The first reaction results in the catalytic step I complex (known as C complex), which needs remodeling for catalysis of the next transesterification. The second reaction is executed by the step II catalytically activated spliceosome (known as the C\* complex). Here we present the 3.4 Å structure of C complex and 4.0 Å structure of C\* complex from *Saccharomyces cerevisiae*, revealing the transition mechanism from the first transesterification to the second by the spliceosome. In our C complex structure, the 2'-OH of the invariant adenine nucleotide in the branch point sequence (BPS) is covalently joined to the guanine nucleotide at the 5' end of the 5' splice site (5'SS), forming an intron-lariat. The splicing active site is stabilized by protein components including Prp8, Snu114 and splicing factors. Compared to C complex, the lariat junction has been translocated by 15-20 Å to vacate space for the incoming 3'-exon sequences **in the C\* complex**. Two catalytic motifs from Prp8 (the 1585-loop and the **b**-finger of the RNaseH-like domain), along with the step II splicing factors Prp17 and Prp18 and other surrounding proteins, are poised to assist the second transesterification. **These two structures, together with the previously reported near-atomic structures of spliceosomal complexes (U4/U6.U5 tri-snRNP, B<sup>act</sup> complex and ILS complex), yield a near-complete mechanistic picture on the splicing cycle.**

## 605 Caught in Action: How DExD/H-box protein Prp28p Interacts with Spliceosome during Pre-mRNA Splicing

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Although DExD/H-box proteins are known to unwind RNA duplexes and/or modulate RNA structures *in vitro*, it seems plausible that, *in vivo*, some may function as RNAPases to dissociate proteins from RNA or to remodel RNA-protein complexes. Precisely how the latter can be achieved is largely unknown. We approach this issue by using the yeast Prp28p, an evolutionarily conserved DExD/H-box splicing factor, as a model system. Previous studies suggest that Prp28p counteracts the stabilizing effect by U1-snRNP proteins on the interaction between U1 snRNA and intron 5' splice site (5'ss). To probe how Prp28p contacts its targets in a splicing-dependent manner, we strategically placed a chemical cross-linker, benzoyl-phenylalanine (BPA), along the length of Prp28p *in vivo* using a nonsense-suppressor-mediated approach. Extracts prepared from these strains were then used for splicing at various ATP concentrations and for UV-activated cross-linking reactions. Prp28p appears to transiently interact with the spliceosome at low ATP concentration, which is known to accumulate A2-1 (or B; mammalian system) complex. Under such a condition, we observed that Prp28p cross-links with a small number of proteins, which is dependent on the presence of UV, ATP, RNA, and, importantly, functional 5'ss and branch site. Furthermore, the cross-linked products are resistant to RNase A treatment. We then showed that Prp28p-K136<sup>BPA</sup> and Prp28p-K82<sup>BPA</sup> cross-link to Prp8p, Brr2p, Snu114p, and U1C. Furthermore, Prp28p-K27<sup>BPA</sup> and Prp28p-K41<sup>BPA</sup> were cross-linked to Brr2p, Snu114p, and U1C. Notably, Prp28p-E326<sup>BPA</sup> cross-links to Npl3p, an SR-like protein, which appears to associate with spliceosome prior to Prp28p. The biological relevance of such interactions is supported by parallel genetic analysis. And ChIP analyses showed that elimination of the BPA site(s) on Prp28p delays the release of U1 snRNP from spliceosome. Finally, detailed biochemical probing revealed that Prp28p directly contacts pre-mRNA at positions very close to the 5'ss. Our data suggest that Prp28p approaches the complex spliceosomal milieu by making prior contact with Npl3p and U1C, then contacts with Prp8p, Snu114p and 5'ss of pre-mRNA to effect U1 snRNP dissociation and then with Brr2p, which may transmit the signal for coupling U1 dissociation with the U4/U6 unwinding, a key step in spliceosome activation.

## 606 Towards the understanding of the mechanisms responsible for alternative splicing modulation by mild heat shock

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The cellular response to various stresses largely depends on the nature and the intensity of the stressing conditions. While it is well established that severe stress conditions are often cytotoxic and associate with global repression of gene expression, growth arrest or cell death, less is known concerning cell adaptation to mild stress conditions. During these last years, transcriptome-wide analyses reported that mild heat stress affects global transcriptional and post-transcriptional regulations of gene expression notably by modulating the alternative splicing of pre-mRNAs. However, the mechanisms underlying heat-induced splicing alterations still remain poorly understood. Heat shock-induced changes of the phosphorylation status and/or the relocalization of splicing factors such as SR or hnRNP proteins have been proposed to participate to alternative splicing modulation. Indeed, the selective recruitment of splicing factors within nuclear or cytoplasmic stress granules is expected to change their relative abundance in the nucleus with predictable effects on alternative-splicing profiles. So far, however, this has only been verified for one splicing reporter: the adenoviral E1A gene.

In order to better understand the mechanisms underlying splicing alterations in response to mild heat-stress, we performed a genome-wide transcriptome analysis on mild heat shocked HeLa cells using Human Junction Array (HJAY) from Affymetrix. Our results highlight significant alternative splicing variations of about 450 pre-mRNAs. We then tested the contribution of the formation of nuclear or cytoplasmic stress granules to heat shock induced splicing alterations and established that these events do not contribute to the establishment of splicing alterations nor to the return to normal splicing pattern after a long recovery time. In agreement with this result, none of the SR or hnRNP protein depletions we performed modulate alternative splicing in the same way as mild heat shock did. We also demonstrated the HSF1-independence of heat-induced splicing modulations. Finally, in collaboration with Juan Valcarcel's lab, we observed a similarity between profiles of alternative splicing changes induced by mild heat shock and the knockdown of some spliceosome core components. These results were further confirmed, establishing for the first time an impact of mild heat shock on spliceosome biogenesis and assembly.

**607 In vivo dynamics of SR protein-RNA interactions**

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SR proteins are well known to play a key role as regulators of constitutive and alternative splicing. They show a modular structure characterized by one or two N-terminal copies of an RNA recognition motif (RRM) and a C-terminal serine/arginine (RS) dipeptide-rich domain. SR proteins act as splicing activators by binding ESE sequences via their RRM domain and then recruiting components of the splicing machinery. To select splice sites, SR proteins recognize short degenerated motifs present in multiple copies at ESEs. Similar cryptic motifs are also frequently present in pre-mRNAs and this low specificity of binding contrasts with the great fidelity splicing and exon definition. Our aim is to provide a detailed kinetic analysis of SR proteins-RNA interaction in living cells. To this aim, we used the CRISPR system to tag the endogenous SRSF1 gene with GFP and HaloTag. We then tracked single molecules of SRSF1 and measured their residency time on nascent RNAs in living HeLa cells. The data shows that SRSF1 remains bound to pre-mRNAs for only few seconds in average. This rapid binding kinetics was confirmed by a FRAP analysis at a transcription site containing an array of SRSF1 high-affinity binding sites. Similar analyses with other splicing factors also revealed rapid binding kinetics. These data suggest a model where splicing factors constantly come on and off pre-mRNAs, highlighting the plasticity of early splicing factors in vivo.

**608 A novel function for Exon Junction Complex as a repressor of recursive splicing of canonical exons**

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We have recently demonstrated that vertebrate introns can undergo splicing in a two-step process known as Recursive Splicing. This process requires the presence of a Recursive Splice Site (RS-site) within the intron, which consists of a 3' splice-site (ss) immediately followed by a 5' ss. In addition, the RS-site is followed by a cryptic RS-exon, which is spliced to the upstream exon during initial steps of recursive splicing. Here we find that there are many canonical RS-exons preceded by such RS-sites, which cause their skipping due to recursive splicing. Normally, deposition of the Exon Junction Complex (EJC) structurally blocks recursive splicing, which ensures high inclusion of these canonical exons. However, when EJC-dependent repression is less effective, recursive splicing can allow skipping of exons in spite of their strong definition. This mechanism is conserved in mice, where loss of EJC repressive function is associated with microcephaly, but not in *Drosophila*, where canonical RS-exons are less abundant and tend to be more alternative.



## 609 Unravelling the molecular determinants of splice site selection with chemical biological tools

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Alternative RNA splicing is a biological process that involves the excision (introns) and reshuffling of sequences (exons) of RNA directly after transcription to produce multiple isoforms of mature mRNA. Both the site of excision and formation of the new phosphodiester bond is catalysed by the spliceosome; a large biomacromolecular complex consisting of >150 proteins and RNA. Despite significant progress made in elucidating the key steps involved in the two transesterification steps of splicing, our understanding of the molecular determinants that define sites of phosphodiester cleavage (*i.e.*, splice site selection) is still poorly understood. This is particularly compelling when considering aberrant splicing patterns are involved a range of disease states ranging from neurodegenerative disease through to cancer.

Our research programme is aimed at addressing this gap in our knowledge of RNA splicing by developing an integrated molecular toolbox of methods to interrogate the key molecular events leading to the selection of splice sites and the development of small molecules that modulate the outcome of alternative splicing events. This presentation will highlight developments in both areas with particular emphasis on the targeting of RNA G quadruplexes present in pre-mRNA with small molecules to modulate the outcome of splicing in the Bcl-x oncogene.

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## 610 Spliceosome and Micro-processing interactions

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**Introduction:** Splicing is a process in which introns are removed and exons are catalytically joined via the Spliceosome, a mega-complex of hundreds of proteins and small nuclear ribonucleo proteins (snRNPs). MicroRNAs (miRNAs) are small non-coding RNAs of ~22 nucleotides that guide post-transcriptional repression of protein-coding genes by base-pairing with the 3' untranslated region (3' UTR) of a target mRNA. The Microprocessor consists of several proteins, including Drosha and DGCR8, responsible for cleaving pri-miRNA to produce pre-miRNA. The majority of human miRNA are located within intronic regions of either coding or noncoding genes and are transcribed by RNA polymerase II as part of their hosting transcription units.

**Scientific Question:** How do intronic miRNA processing and coding-gene splicing, interact? In other words, how do the machineries - the Microprocessor and the Spliceosome - coordinate processing of the same transcript?

**Aim:** The aim of this study is to examine the interactions between the splicing and micro-processing cellular mechanisms, using an unbiased and global approach.

**Method:** In order to determine which miRNAs activate/inhibit splicing, we inserted WT melastatin (TRPM1) intron 6 between two GFP exons. The construct we assembled allows us to use a natural intron containing a miRNA in order to study the micro-processing and splicing interactions.

**Results:** We observed an intricate crosstalk between splicing and micro-processing, in particular a competition between snRNAs and miRNAs in the vicinity of the splice sites. We found that miRNAs can promote splicing by weakening the physical interaction between snRNAs and specific splice sites. By using miRNA over-expression, we were able to correct the splicing defect in several cell lines and in a genetic disease known as Wagner syndrome.

**Conclusions:** We observed a previously unreported interaction between splicing and micro-processing indicating a possible novel route for controlling either process, and the development of innovative therapeutic strategies to treat various splicing-related diseases.

## 611 Regulation of circRNA formation by SR proteins and MALAT1 in hypoxia stress

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Hypoxia is associated with several diseases, including ischemia and cancer, and occurs when tissues are deprived of adequate oxygen supply. Transcriptional responses to hypoxia are mediated by the hypoxia-inducible factors (HIFs), which control the expression of hundreds of genes important in cellular stress response as well as long non-coding RNAs (lncRNAs) such as *MALAT1* and *NEAT1* (Choudhry, 2014).

CircRNAs are lncRNAs that are formed via back-splicing. We have recently identified circRNAs that are induced upon hypoxia in human endothelial cells (HUVECs) and demonstrated that *circZn292* promotes angiogenic sprouting (Boeckel, 2015). Yet, the factors that regulate circRNA formation particularly under hypoxia stress conditions remain unknown.

Performing **RNA-Seq** of HUVECs and HeLa cells we identified thousands of mRNAs, hundreds of lincRNAs and several highly expressed circRNAs that change significantly in abundance/splicing upon hypoxia. Although HUVECs and HeLa cells react differently to decreased oxygen levels, a substantial number of mRNAs and lincRNAs were similarly regulated, e.g. *NEAT1*, *MALAT1* and *circZn292* were up-regulated in both cell types.

*MALAT1* scaffolds nuclear speckles, which act as storage sites for SR proteins, a family of essential splicing factors. Their activities are regulated by phosphorylation and sequestration to nuclear speckles. iCLIP revealed that all SR proteins bind massively to *MALAT1* (Müller-McNicoll, 2016). Splicing inhibition by isogingketin decreased *MALAT1* level and caused SR proteins to accumulate in large, round, inactive storage speckles. In contrast, during hypoxia, *MALAT1* levels rose, nuclear speckles increased in number and SR proteins re-located completely from the nucleoplasm to these active speckles. The same was observed with 24h CoCL<sub>2</sub> treatment, which stabilizes HIF $\alpha$  and mimics the hypoxia response.

The boost in transcription of hypoxia-regulated genes and *MALAT1* may overwhelm the capacity of SR proteins to bind to target exons and promote linear splicing, which would increase exon skipping circRNA formation. In support of this hypothesis, SRSF6 binds close to back-splice junctions of circRNAs that are induced during hypoxia (iCLIP) and SRSF6 depletion causes exon skipping and increased circRNA formation. Moreover, SRSF6 phosphorylation is substantially decreased in the first 30-60 minutes of hypoxia, indicating an enhanced involvement in splicing.

## 612 A splicing-based thermometer controls circadian gene expression in mammals

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The core body temperature of all mammals oscillates with the time of the day. However, direct molecular consequences of small, physiological changes in body temperature remain largely elusive. Here we show that body temperature cycles drive rhythmic SR protein phosphorylation to control an alternative splicing program. A temperature change of 1°C is sufficient to induce a concerted splicing switch in a large group of functionally related genes, rendering this splicing-based thermometer faster and much more sensitive than previously described temperature-sensing mechanisms. Alternative splicing of two exons in the 5'-UTR of the *TATA-box binding protein* (*Tbp*) highlights the general impact of this mechanism, as it results in rhythmic TBP protein levels with an impact on global gene expression *in vivo*. Together our data establish body temperature driven alternative splicing as a novel, core clock-independent oscillator in mammalian peripheral clocks.

### 613 SPSB1-Mediated hnRNP A1 Ubiquitylation Regulates Alternative Splicing and Cell Migration in EGF Signaling

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Extracellular signals have been shown to impact on alternative pre-mRNA splicing; however, the molecular mechanisms and biological significance of signal-induced splicing regulation remain largely unknown. Here, we report that epidermal growth factor (EGF) induces splicing changes through ubiquitylation of a well-known splicing regulator, hnRNP A1. EGF signaling upregulates an E3 ubiquitin (Ub) ligase adaptor, SPSB1, which recruits Elongin B/C-Cullin complexes to conjugate lysine 29-linked polyUb chains onto hnRNP A1. Importantly, SPSB1 and ubiquitylation of hnRNP A1 play a critical role in EGF-driven cell migration. Mechanistically, EGF-induced ubiquitylation of hnRNP A1 together with the activation of SR protein kinases (SRPKs) results in the upregulation of a Rac1 splicing isoform, Rac1b, to promote cell motility. These findings unravel a novel crosstalk between protein ubiquitylation and alternative splicing in EGF/EGFR signaling, and identify a new EGF/SPSB1/hnRNP A1/Rac1 axis in modulating cell migration, which may have important implications for cancer treatment.

### 614 VastDB: an Atlas of Alternative Splicing Profiles and Functional Associations in Vertebrate Tissues and Cell Types

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Alternative splicing (AS) generates remarkable regulatory and proteomic complexity in metazoans. However, the functions of most AS events are not known and programs of regulated splicing remain to be identified. To address these challenges, we describe ‘Vertebrate Alternative Splicing and Transcription Database’ (VastDB), the largest resource of genome-wide, quantitative profiles of AS events assembled to date. VastDB provides readily accessible quantitative information on the inclusion levels and functional associations of AS events detected in RNA-Seq data from diverse vertebrate cell and tissue types, as well as developmental stages. The VastDB profiles reveal extensive new intergenic and intragenic regulatory relationships among different classes of AS, as well as previously unknown and conserved landscapes of tissue-regulated exons. Contrary to recent reports concluding that essentially all human genes express a single major isoform, VastDB provides evidence that more than 25% of multiexonic protein-coding genes express multiple splice variants that are highly regulated in a cell/tissue-specific manner, and that more than 11% of genes simultaneously express multiple, conserved major isoforms across diverse cell and tissue types. Isoforms encoded by the latter set of genes are generally co-expressed in the same cells and are engaged by translating ribosomes. Moreover, they are encoded by genes that are significantly enriched in functions associated with transcriptional control, thus implying they have an important and wide-ranging role in controlling cellular activities. VastDB thus provides an unprecedented resource for investigations of AS function and regulation. (Access to VastDB is via: <http://vastdb.crg.eu>).

## 615 A Repressor Candidate of Cancer Specific mRNA Re-splicing: A Key factor for splicing fidelity or mRNA quality control?

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Proper regulation in essential pre-mRNA splicing is crucial for the eventual biological/physiological functions. Therefore splicing system must be faithful and robust, and mis-regulation in this system immediately causes disorders in cell functions—often ends up in serious disease including cancer. We discovered mRNA re-splicing in cancer cells that occurs on mature spliced mRNA and generates aberrant transcripts/proteins [*Nucleic Acids Res.* **40**, 7896 (2012)]. The mRNA re-splicing in various cancer cells implies an important mechanism that prevents deleterious extra re-splicing in normal cells. The control of the re-splicing could be promoted by unknown activator upregulated and/or repressor downregulated in cancer cells.

Recent striking findings represent a breakthrough in the study of mRNA re-splicing. (1) The function of cancer-specific spliced product of *TSG101* gene was discovered. The human TSG101Δ190–1090 (TSG101Δ) mRNA was a major aberrantly spliced product detected in various cancer cells/tissues, and we first demonstrated that TSG101Δ mRNA was generated by re-splicing of normally spliced TSG101 mRNA [*Nucleic Acids Res.* **40**, 7896 (2012)]. It was demonstrated that TSG101Δ protein, generated *via* re-splicing of TSG101, specifically enhances TSG101-stimulated cell proliferation and tumor growth [*Oncotarget* **7**, 8240 (2016)]. (2) mRNA re-splicing is repressed by the expression of tumor suppressor p53 (*TP53*) often induced by cellular stress. This result indicates that the regulation of mRNA re-splicing is under the control of p53. (3) We have identified a repressor candidate of mRNA re-splicing by screening of siRNA library (including 156 kinds of RNA-binding proteins). Since the identified repressor is a known tumor suppressor, we postulate that global prevention of aberrant mRNA re-splicing, maintaining fidelity of splicing, is critical for the consequent tumor suppression. To prove this hypothesis is underway.

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## 616 Identification and Characterization of a Minimal Functional Splicing Regulatory Protein

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The process of alternative splicing is regulated in part by RNA binding proteins that bind to cis-acting elements and influence the assembly of a functional spliceosome at nearby splice sites. Aberrant splicing has been identified in cancer and neurodegenerative diseases, underscoring the importance of alternative splicing regulation. The Polypyrimidine Tract Binding Protein 1 (PTBP1) is a well characterized RNA binding protein with roles in alternative splicing regulation, mRNA localization, and IRES-mediated translation initiation. PTBP1 binds to pyrimidine rich motifs located either upstream or within exons to repress their inclusion, and downstream of an exon to promote its inclusion in the spliced mRNA. PTBP1 contains four RNA Recognition Motifs (RRMs) connected by three flexible linker regions. Solution structures of individual RRM bound to a RNA hexamer highlight amino acid residues involved in RNA recognition and binding. However, the arrangement of RRM domains on a target RNA during either splicing repression or activation is not understood. We created a series of PTBP1 mutants with deletions in both linker 1 and 2 regions. Mutants were tested for protein expression and splicing repression of neuronal exons *in vivo*. Our results indicate that we have identified a minimal PTBP1 mutant that can repress splicing of certain exons similar to full-length PTBP1.



## **617 Loss of p105 function induces aberrant mRNA splicing and DNA damage**

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p105 plays a crucial role in the regulation of diverse biological events including cell cycle, apoptosis, pre-mRNA splicing etc. Its overexpression is found in several human cancers, however its role as a pre-mRNA splicing factor in relation to cancer remains unclear. Here, we show that, p105 controls pre-mRNA splicing to prevent DNA damage in cancer cells. Depletion of p105 led to a severe inhibition in clonal survival of cancer cells, and induced the intron retention in pre-mRNAs for HDAC6, NDUFB1, ZFAN2B etc. The impairment in pre-mRNA splicing was accompanied with the increase of gamma-H2AX, a marker for DNA damage. The analysis of mRNA-sequencing revealed that p105 depletion-induced intron retention occurs at specific loci of intron sequences. Collectively, these data suggest that p105 is required for removal of specific intron in pre-mRNAs necessary for the survival of cancer cells.

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## **618 Single cell sequencing of preimplantation embryos reveals early dynamic of alternative splicing independent of transcription change**

*Jiefu Li, Zefeng Wang*

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The journey of a life starts from fertilized egg. The process of a fertilized egg transit into a totipotent zygote, and subsequently undergoes synchronous cell divisions are one of the most complicated cell transformations in biology. The very early stage of this transformation occurs in the absence of transcription and therefore is often regulated through mRNA processing and degradation. Alternative splicing (AS) is a key mechanism of gene regulation at RNA level, with most mammalian genes containing multiple isoforms of distinct activities. Splicing regulation can also occur in a dynamic fashion, with thousands of AS events changes temporally during cell cycle. To systematically study the dynamics of splicing regulation, we developed a computational pipeline to analyze several RNA-seq datasets of single cell profiling for early embryos. We found that the changes of splicing pattern in different cells occur synchronously before 4 cell stages. In addition, we identified >500 common AS events in all stages from oocytes to morula, which are enriched for genes involved in RNA binding, translation, and RNA decay. Surprisingly, there are dramatic changes in ratios of different splicing isoforms at early embryos, with 380 genes containing altered AS events between oocytes and zygote. Since the cells remain transcriptionally quiescent during this time, such observation suggested an isoforms-specific mRNA degradation and/or splicing changes independent of RNA transcription. The genes with early changes of splicing isoforms are enriched for functions in intracellular protein transport and protein location, however, the mechanism and functional implication are unclear. Genes with splicing changes from zygote to 2-cell are mainly associated with cell cycle regulation, which is consistent with recent findings that temporal regulation of splicing plays key roles in cell cycle. This study generates a detailed splicing map during early embryo development at single cell resolution, which enables discrimination of every embryonic stage by AS patterns. The early alteration of AS isoforms proceed to any known changes at RNA level during preimplantation embryos, suggesting an early RNA dynamic prime for the transcriptome adjustment to embryo development.

**619 Cryptic exons are repressed by a family of dinucleotide repeat binding splicing factors***Jonathan Ling<sup>1</sup>, Kristen Lynch<sup>2</sup>, Philip Wong<sup>1</sup>*<sup>1</sup>Johns Hopkins University, Baltimore, MD, USA; <sup>2</sup>University of Pennsylvania, Philadelphia, PA, USA

The fidelity of RNA splicing is regulated by a network of splicing enhancers and repressors, although the rules that govern splice site selection are not yet fully understood. With recent advances in RNA sequencing (RNA-seq), it is now possible to explore alternative splicing across the entire transcriptome at a base-pair level of resolution. Such datasets have provided many insights into the role of splicing in human disease and developmental biology.

Through the analysis of previously unannotated transcripts in RNA-seq data, our lab has recently identified novel functions for the RNA binding proteins TDP-43, PTBP1 and PTBP2. TDP-43 has been implicated in the pathogenesis of several diseases, including amyotrophic lateral sclerosis, frontotemporal dementia, and inclusion body myositis. In contrast, PTBP1 and PTBP2 have been identified as key regulators of neuronal differentiation. Our findings indicate that TDP-43, PTBP1 and PTBP2 belong to a family of splicing factors that repress cryptic exons by utilizing a dinucleotide repeat consensus sequence. TDP-43 binds to 'UG' repeats whereas PTBP1 and PTBP2 bind to 'CU' repeats.

Here, we report the novel finding that hnRNP L also belongs to this family of cryptic exon repressors. HnRNP L has been implicated in the development of the mammalian immune system. In contrast to TDP-43, PTBP1 and PTBP2, hnRNP L binds to 'CA' repeats for splicing repression. Interestingly, cryptic exon repressors appear to show distinct position preference in relation to the 3'SS and 5'SS. Together, our work demonstrates that despite having diverse functions across unique cell types, TDP-43, PTBP1, PTBP2, and hnRNP L appear to be linked by a shared mechanism of cryptic exon repression.

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2. Ling JP, Chhabra R, Merran JD, et al. PTBP1 and PTBP2 Repress Nonconserved Cryptic Exons. *Cell Rep*. 2016;17(1):104-113.

**620 Y14 governs p53 expression and modulates DNA damage sensitivity***Chia-Chen Lu<sup>1,2</sup>, Chi-Chieh Lee<sup>2</sup>, Ching-Tzu Tseng<sup>2</sup>, Woan-Yuh Tarn<sup>1,2</sup>*<sup>1</sup>Taiwan International Graduate Program in Molecular Medicine, National Yang-Ming University and Academia Sinica, Taipei, Taiwan; <sup>2</sup>Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

Y14 is a core component of the exon junction complex (EJC), while it also exerts cellular functions independent of the EJC. Depletion of Y14 causes G2/M arrest, DNA damage and apoptosis. Here we show that knockdown of Y14 induces the expression of an alternative spliced isoform of p53, namely p53 $\beta$ , in human cells. Y14, in the context of the EJC, inhibited aberrant exon inclusion during the splicing of p53 pre-mRNA, and thus prevent p53 $\beta$  expression. The anti-cancer agent camptothecin specifically suppressed p53 $\beta$  induction. Intriguingly, both depletion and overexpression of Y14 increased overall p53 protein levels, suggesting that Y14 governs the quality and quantity control of p53. Moreover, Y14 depletion unexpectedly reduced p21 protein levels, which in conjunction with aberrant p53 expression accordingly increased cell sensitivity to genotoxic agents. This study establishes a direct link between Y14 and p53 expression and suggests a function for Y14 in DNA damage signaling.

## 621 Study of the ageing-related alternative pre-mRNA splicing of the LMNA gene

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Two proteins, lamin A and lamin C, are the important components of the nuclear lamina, and are produced from one gene, LMNA, via alternative splicing. The premature ageing of Hutchinson Gilford Progeria Syndrome (HGPS) patients originates from a single nucleotide mutation in exon 11 of LMNA gene that activates a cryptic site driving the production of truncated lamin A protein, named progerin, that functions in a dominant-negative manner. Thus, the LMNA pre-mRNA splicing can be used as a tool to investigate at least some of the molecular mechanisms that regulate physiological ageing. To identify the splicing factors that play crucial roles in whether lamin A or progerin is produced, we developed the in vitro splicing system which recapitulates this alternative splicing event. The early (E) spliceosomal complexes assembled in HeLa nuclear extracts on either the wild type or the HGPS-mutated model pre-mRNA were purified using immunoaffinity selection and gradient density ultracentrifugation, and their protein compositions were identified by mass spectrometry. The proteins that are found in one spliceosomal complex and are absent in the other are considered as the modulators of the alternative splicing outcome and they will be discussed.

Which of two proteins, lamin A or lamin C, is produced depends on whether intron 10 is removed or retained, respectively. Lamin A appeared to be dispensable as the LMNA-knockout mice expressing Lamin C only were healthy. Since the lamin A vs Lamin C alternative splicing is an upstream event compared to the lamin A vs progerin splicing, the regulation of the former event in favour of the preferential selection of the lamin C splice sites, would reduce a possibility of progerin production. To investigate cis-acting regulatory sequences in intron 10, we carry out transfection assays using the LMNA minigene constructs containing a set of the deletions in intron 10. Two cis-acting elements were identified. Deletion of one element dramatically increases production of lamin C at the expense of lamin A; the deletion of the other element triggers skipping of the entire exon 11. Implication of these sequences in regulation of the LMNA pre-mRNA splicing will be discussed.

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## 622 Withdrawn

## 623 Systematic survey of RNA binding proteins in human genome for their activity to regulate alternative splicing

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Alternative splicing is generally regulated by *trans*-acting factors that specifically bind *cis*-elements in pre-mRNA to activate or inhibit the splicing reaction. This regulation is important for normal gene expression, and closely associated with human diseases. Canonical splicing factors usually have a modular domain configuration, containing RNA-binding domains to recognize pre-mRNA targets and functional domains to affect splicing. To explore the activity of potential splicing factors, we engineered a series of artificial RNA binding factors by combining a sequence-specific RNA-binding domain of human *Pumilio1* (PUF domain) with various putative functional domain from all human RNA binding proteins. We then co-express the engineered RNA binding factors with three splicing reporters inserted with the PUF target sequences in different pre-mRNA contexts. When applying this system to test characteristic protein domains in various RNA binding proteins, we found that many domains in endogenous RNA binding proteins have activity to modulate splicing, expanding the repertoire of potential splicing factors. Furthermore we used statistical modeling and machine learning approaches to analyze the experimental data for the connection between sequences of these RNA binding proteins and their activities in regulating splicing. We were able to classify the splicing regulatory activities of these RNA binding protein based on their sequence compositions using partial least squares regression. We further validated our results and developed a classification model to predict the splicing regulation activity of unknown functional domains. In addition to discovery novel splicing factors, such survey can help to optimize the design of artificial splicing factors that may serve as a new gene manipulation tool.

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## 624 Flipping the switch on Sex-lethal expression: Sister of Sex-lethal antagonizes Sxl-dependent alternative splicing to maintain a male-specific gene expression pattern in *Drosophila*

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In *Drosophila* female development is governed by a single RNA-binding protein, Sex-lethal (Sxl), that controls the expression of key factors involved in dosage compensation, germline homeostasis and the establishment of female morphology and behaviour. Functional Sxl protein is only synthesized in female flies. Deletion or mutation of the *Sxl* locus results in female-specific lethality, while forced expression kills male flies. Its expression is established in the pre-cellular, female embryo by an X-chromosome counting mechanism and maintained by an autoregulatory, positive feedback loop. For this, Sxl associates with its own primary transcript to suppress inclusion of a poison exon with a premature termination codon during RNA splicing. Conversely, in male flies expression of functional Sxl protein is prevented by inclusion of the poison exon during splicing, resulting in a shortened, non-functional open reading frame. Production of a few functional Sxl molecules by stochastic aberrant splicing in male flies could however trigger the self-sustaining feedback loop, resulting in Sxl protein production snowballing out of control. Hence, one would predict additional backup mechanisms to operate in males, protecting against sustained Sxl production.

We have identified Sister of Sex-lethal (Ssx) as a protein that antagonizes Sxl in autoregulatory splicing by competition for the same RNA elements. Male flies mutant for *ssx* exhibit a low level of productive Sxl mRNA splicing and in cultured *Drosophila* cells, Sxl-induced changes in alternative splicing can be reverted by the simultaneous expression of Ssx. In sum, our data suggest that Ssx helps to establish a stable, male-specific gene expression pattern by protecting male flies against Sxl initiating the autoregulatory, positive feedback loop.



## 625 Function of ubiquitin-fold containing Sde2 as an intron-specific pre-mRNA splicing regulator

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*Schizosaccharomyces pombe* Sde2 protein is reported to play a role in heterochromatin silencing and pre-mRNA splicing. We show that Sde2 is a ubiquitin fold containing splicing regulator which supports splicing of a subset of pre-mRNAs in an intron-specific manner in *S. pombe*. Sde2 in *S. pombe* and human is translated as an inactive precursor harbouring the ubiquitin fold (referred to as Sde2<sub>UBL</sub>), an invariant GGKGG motif, and a C-terminal domain (referred to as Sde2-C). The precursors undergo processing at GG<sup>A</sup>K by ubiquitin specific proteases to separate the two polypeptides. Activated Sde2-C begins with lysine and functions as a component of the spliceosome. Lack of Sde2 or defects in processing results in inefficient excision of selected introns from target pre-mRNAs. Thus ubiquitin-like processing generates <sup>Lys</sup>Sde2-C to maintain genomic integrity in *S. pombe* through specific pre-mRNA splicing.

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## 626 A system to study rate-limiting regulation by the minor spliceosome in healthy cells and cell culture models of minor spliceosomal diseases

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U12-type introns are a group of rare introns with highly conserved core splicing signals. They are spliced by a dedicated machinery, the minor spliceosome. The splicing of U12-type introns is thought to act as a rate-limiting regulatory mechanism for the expression of their host genes. However, the mechanistic basis of this regulation has remained unclear. As originally proposed by Patel and Steitz [1], the splicing of U12-type introns may be slower than that of U2-type introns; alternatively, it may be inefficient, meaning that a subset of transcripts fails splicing altogether [2]. Although one study [3] has lent some support to the slow splicing hypothesis, several more recent studies suggest that splicing occurs significantly faster than previously thought [4,5]. To differentiate between the two possibilities we are using imaging methods to investigate intron retention and splicing kinetics in the genes containing U12-type introns. For measuring splicing kinetics, we have adopted the single-molecule resolution live-cell imaging system developed by Martin et al. [4], and created reporter cell lines for visualizing splicing of U12-type and U2-type introns. In conjunction, we are using single-molecule FISH to study the nuclear fate and localization of transcripts with unspliced U12-type introns. The same methods will be used to investigate minor spliceosomal diseases. Recently, several mutations in the specific snRNA and protein components of the minor spliceosome have been shown to cause human diseases. We have used genome editing to generate cell line models of these diseases and will use imaging and biochemical methods to investigate the impact of various disease mutations on the rate-limiting regulation, splicing kinetics and nuclear mRNA turnover.

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**627 Development of RNA G-quadruplex Stabilizers as Splice-Switching Inducers of Apoptosis***Olivia I. Paisley<sup>1</sup>, Carika Weldon<sup>2</sup>, Cyril Dominguez<sup>2</sup>, Ian C. Eperon<sup>2</sup>, Laurence H. Hurley<sup>3</sup>, Glenn A. Burley<sup>1</sup>*<sup>1</sup>Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, UK; <sup>2</sup>Department of Biochemistry, University of Leicester, Leicester, UK; <sup>3</sup>College of Pharmacology and Toxicology, University of Arizona, Arizona, USA

The Bcl-2 family of proteins is the primary regulator of the intrinsic apoptotic pathway.<sup>1</sup> The splicing isoforms of one such member of the family, Bcl-x are Bcl-xS (pro-apoptotic) and Bcl-xL (anti-apoptotic). These isoforms exhibit antagonistic functions with the anti-apoptotic Bcl-xL isoform over-expressed in a variety of cancers. The redirection of alternative splicing of Bcl-x towards the expression of the pro-apoptotic Bcl-xS isoform thus provides a novel target for cancer therapy.<sup>2</sup> Previous work by Bauman *et al.*<sup>3</sup> have demonstrated initial proof of concept of altering the outcome of splicing patterns using splice-switching oligonucleotides. However at present, a small molecule strategy that can control splice site selection of Bcl-x has not been reported.

Herein we report a new class of ellipticine analogues that induces a switch in the pre-mRNA splicing pattern of Bcl-x in favour of the pro-apoptotic Bcl-xS isoform *in vitro* and *in cellulo*.<sup>4</sup> We hypothesise that the splice-switching behaviour of GQC-05 is due to the stabilization of RNA G-quadruplexes located close to the 5' splice sites of Bcl-x. Structure-Activity-Relationship profile reveals structural features essential for this splice-switching activity. In parallel, a one-pot, two-step modular route towards the ellipticine scaffold has been established which will allow a larger library of compounds to be synthesised for further investigation.

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**628 Characterization of splicing modulator DDU40800***Andrea Pawellek<sup>1</sup>, Ursula Ryder<sup>1</sup>, Marek Gierlinski<sup>2</sup>, Andrew Woodland<sup>3</sup>, David Gray<sup>3</sup>, Angus Lamond<sup>1</sup>*<sup>1</sup>Centre for Gene Regulation & Expression, College of Life Sciences, Dundee, UK; <sup>2</sup>Computational Biology, College of Life Sciences, Dundee, UK; <sup>3</sup>Drug Discovery Unit, College of Life Sciences, Dundee, UK

During the EU-funded Eurasnet project (see; [www.eurasnet.info](http://www.eurasnet.info)), we, together with the group of Reinhard Lührmann and the University of Dundee Drug Discovery Unit (DDU), used a high throughput *in vitro* splicing assay (adapted from Samatov et. al., 2012), to screen a curated library of >75,000 small, drug-like compounds. This identified several novel classes of small molecule splicing inhibitors, including madrasin (Pawellek et al., J.Biol. Chem. 2014). In collaboration with the DDU we have now analysed further molecules related in structure to a subset of the original hits. This has identified further drug-like, small molecules that both inhibit splicing *in vitro* and modulate the pattern of pre-mRNA splicing of endogenous transcripts in multiple human cancer cell lines. One of these new compounds, DDU40800, interfered with one or more early steps of spliceosome formation *in vitro* and disrupted Cajal bodies *in cellulo*. RNA-seq analysis of both HeLa and HEK293 cells treated with DDU40800 for 24h identified ~3,000 altered splicing events. GO term analysis showed an enrichment of transcripts in this data set that encode proteins involved in RNA processing, cell cycle and DNA damage and repair. Treatment with DDU40800 inhibited cell cycle progression, arresting cells in S/G2&M phase, as shown by FACS analysis. We are currently using a combination of thermal proteome profiling (TPP), drug affinity responsive target stability (DARTS) and IP analyses to identify the direct targets of DDU40800 and characterise its mechanism of action.

**629 The Splicing Program of SRSF1 transcripts is finely tuned by cell metabolism***Daniela Pignataro<sup>1</sup>, Morena Catillo<sup>1</sup>, Antonina Di Maio<sup>1,2</sup>, Giulia Brenna<sup>1</sup>, Giuseppe Biamonti<sup>1</sup>*<sup>1</sup>Istituto di Genetica Molecolare del CNR, Pavia, Italy; <sup>2</sup>Dipartimento di Biologia e Biotecnologie, Pavia, Italy

Splicing factor SRSF1 has an essential role in gene expression regulation and in cancer progression. Moreover, it modulates the epithelial to mesenchymal cell transition (EMT). The SRSF1 level is finely tuned in response to cell density through a complex alternative splicing (AS) program involving intron 3, the last coding exon (Ex4) and 2 introns in the 3'UTR. In this manner, six SRSF1 transcripts (called Is1 to Is6) are generated. Is1, which retains both introns in the 3'UTR, encodes for SRSF1. Is2, Is3 and Is4 are exclusively nuclear. Is5 and Is6 are exported to the cytoplasm and degraded by the non-sense mediated RNA decay (NMD) pathway. While Is5 can encode a protein that differs from SRSF1 for the last 5 aminoacids, Is6 is translated into a protein variant that lacks the entire RS domain involved in protein-interactions. This variant, however, is barely detectable under standard growth conditions. The relative abundance of the six transcripts is also modulated in a tissue specific manner. Concerning cell density, Is1 prevails in Low-Density cells (LD) while the abundance of remaining 5 molecules, particularly of Is3, drastically increases in High-Density cells (HD). This switch occurs in all the cell lines tested so far, from mouse to man.. We have investigated the signals and mechanisms underlying this regulation. We have found that the glucose metabolism is crucial in determining the splicing profile of SRSF1 transcripts. In particular, the effect of cell density can be reproduced by modulating the level of specific Krebs cycle intermediates that impact protein stability. Our findings unveil a link between cell metabolism and alternative splicing programs with possible implications in cancer progression.

**630 Global analysis of alternative splicing upon differentiation of human monocytes reveals MBNL1 as a major regulator***Hongfei Liu<sup>1</sup>, Paolo Lorenzini<sup>1,2</sup>, Fan Zhang<sup>3</sup>, Jie Zheng<sup>3</sup>, Xavier Roca<sup>1</sup>*<sup>1</sup>School of Biological Sciences, Nanyang Technological University, Singapore, Singapore; <sup>2</sup>Nanyang Institute of Technology, Health and Medicine Interdisciplinary Graduate School, Nanyang Technological University, Singapore, Singapore; <sup>3</sup>School of Computer Science and Engineering, Nanyang Technological University, Singapore, Singapore

Alternative splicing (AS) plays important roles in immune cells such as lymphocytes, but are virtually unexplored in monocytes. Myelodysplastic syndromes are frequently caused by mutations in splicing-factor genes, suggesting that AS plays a key role in myeloid and monocyte physiology. Here we use RNA-Sequencing (RNA-seq) to detect gene-expression and AS changes between human primary monocytes and GM-CSF-induced pro-inflammatory and M-CSF-induced wound-healing macrophages. We also performed RNA-seq on the human monocytic cell line THP-1 and its derived macrophages upon Vitamin D3 and PMA treatment. Most differentially expressed genes (DEGs) are shared between GM-CSF- and M-CSF-induced macrophages, but these stimuli regulate many specific genes in clustered in different pathways. Many RNA-binding proteins (RBPs) change expression upon monocyte-to-macrophage differentiation, and twenty of these changes are shared between primary cells and THP-1. Furthermore, we identified over three thousand AS events that change upon macrophage differentiation by M-CSF and/or GM-CSF. Compared to monocytes, macrophages exhibit twice as many increases in exon inclusion versus skipping, indicating that splicing activators are dominant in this process. The genes with AS changes cluster in pathways that are relevant for the immune function of these cells, and are different from those enriched in DEGs. During monocyte differentiation, the splicing factors MBNL1 and RBFOX2 change their expression at RNA and protein levels and regulate a fraction of the AS events. We identified known and new MBNL1 splicing targets in the context of monocyte differentiation, and we are now testing the physiological significance of MBNL1-mediated AS regulation in these cells.

### 631 Exonization of an intronic Alu element alters the critical C-terminus of Survival Motor Neuron protein

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Spinal muscular atrophy (SMA), a leading genetic disease of children and infants, is caused by mutations or deletions of *Survival Motor Neuron 1* (*SMN1*) gene. *SMN2*, a nearly identical copy of *SMN1*, fails to compensate for the loss of *SMN1* due to skipping of exon 7. *SMN2* predominantly produces SMND7, an unstable protein. Here we report exon 6B, a novel exon, generated by exonization of an intronic Alu-like sequence of *SMN*. We validate the expression of exon 6B-containing transcripts *SMN6B* and *SMN6BD7* in human tissues and cell lines. We confirm generation of *SMN6B* transcripts from both *SMN1* and *SMN2*. We detect expression of SMN6B protein using antibodies raised against a unique polypeptide encoded by exon 6B. We analyze RNA-Seq data to show that hnRNP C is a potential regulator of *SMN6B* expression and demonstrate that *SMN6B* is a substrate of nonsense-mediated decay. We show interaction of SMN6B with Gemin2, a critical SMN-interacting protein. We demonstrate that SMN6B is more stable than SMNΔ7 and localizes to both the nucleus and the cytoplasm. Our finding expands the diversity of transcripts generated from human *SMN* genes and reveals a novel protein isoform predicted to be stably expressed during conditions of stress.

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### 632 High-throughput screening for splicing regulatory regions

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Alternative splicing (AS) is a co- or post-transcriptional process by which one gene gives rise to multiple isoforms. This 'split and combine' step has increased eukaryotic proteome diversity manifold and has been implicated in several diseases given its pervasive impact. Many proteins recognize distinct splicing regulatory regions across the transcriptome and their combinatorial interplay results in a complex 'splicing code'. However, the implication of these splicing regulatory regions in shaping the splicing outcome remains poorly understood.

Here, we have established a high-throughput method to screen splicing regulatory regions to comprehensively identify and characterize all such regions that determine a particular splicing decision. As a proof-of-principle, we performed the screen with a minigene harboring a disease-relevant alternative exon of the *RON* receptor kinase gene, whose skipping isoform is implicated in cancer progression. A library of thousands of randomly mutagenized minigene variants was transfected as a pool into human HEK293T and MCF7 cell lines, and the spliced isoforms were subsequently analyzed by RNA sequencing. Importantly, a barcode sequence was used to tag the minigene variants and thereby linked mutations to their corresponding spliced products. The effects of the mutations on alternative splicing were pinpointed by a linear regression-based model. Using this approach, we characterised the regulatory landscape of *RON* exon 11 splicing and identified known and new regulatory regions, especially in introns to a detailed extent.

In summary, this novel screening approach introduces a tool to study the relationship of RNA sequence variants and their impact on splicing outcome, offering new insights into the fundamental workings of alternative splicing regulation as well as the relevance of mutations in human disease. This will help in reconstructing the underlying regulatory networks and will provide a blueprint for ultimately cracking the splicing code.



### 633 The regulation of alternative mRNA splicing by CDK12 supports the tumorigenic potential of breast cancer cells

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CDK12 (cyclin-dependent kinase 12) is a regulatory kinase with evolutionarily conserved roles in modulating transcription elongation. Recent tumor genome studies of breast and ovarian cancers highlighted recurrent *CDK12* mutations, which have been shown to disrupt DNA repair in cell-based assays. In breast cancers, *CDK12* is also frequently co-amplified with the *HER2* (*ERBB2*) oncogene. The mechanisms underlying functions of CDK12 in general and in cancer remain poorly defined. Based on global analysis of mRNA transcripts in normal and breast cancer cell lines with and without *CDK12* amplification, we demonstrate that CDK12 primarily regulates alternative last exon (ALE) splicing, a specialized subtype of alternative mRNA splicing, that is both gene- and cell type-specific. These are unusual properties for spliceosome regulatory factors, which typically regulate multiple forms of alternative splicing in a global manner. In breast cancer cells, regulation by CDK12 modulates ALE splicing of the DNA damage response activator *ATM*, creating a novel short mRNA isoform. Further evidence suggests that ALE regulation of *ATM* is distinct from transcriptional regulation. While CDK12 kinase activity can affect the expression of the *ATM* gene, the entire CDK12 protein is required to modulate splicing of the novel *ATM* isoform. These observations suggest disparate functions of CDK12 in the regulation of transcription and alternative splicing. In addition to *ATM*, CDK12 also modulates ALE splicing of a *DNAJB6* isoform that influences cell invasion and tumorigenesis in xenografts. We found that there is a direct correlation between CDK12 levels, *DNAJB6* isoform levels, and the migration capacity and invasiveness of breast tumor cells. Taken together, these results suggest mechanisms by which *CDK12* mutations and gene amplifications can contribute to the pathogenesis of the cancer.

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### 634 PTBP2 is regulated by U11/U12 di-snRNP

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Here, we have investigated alternate splicing regulation by the U11/U12 di-snRNP, which is predominantly responsible for the initial recognition of U12-type introns. However, we have earlier shown that it can also regulate alternative splicing using exon definition interactions that provide feedback/cross-regulation for the 48K and 65K proteins of the U11/U12-di-snRNP (Verbeeren et al., 2010; Niemelä et al., 2015). To ask if there are additional alternative splicing events regulated by U11/U12 di-snRNP, we knocked down U11-48K and U11-35K proteins, both integral components of this complex. RNAseq followed by alternative splicing analysis with SUPPA (Alamancos et al., 2015) revealed 232 statistically significant alternative splicing events shared between the 48K and 35K knockdowns - all located in the genes not carrying U12-type introns.

Alternative splicing targets included PTBP2, a major splicing repressor required for many brain or neuronal specific alternate splicing. Both the 48K and 35K knockdowns lead to reduced PTBP2 exon 10 inclusion, translational frameshifting and ~2-fold downregulation of the PTBP2 mRNA levels. RNAseq analysis of PTBP2 knock-down cells indicated that ~50% of the alternative splicing changes specific to 48K/35K knockdowns were also shared with the PTBP2 knock-down. Native gel analysis suggests that U11/U12 di-snRNP binds to pyrimidine-rich sequences upstream of exon 10 that is also a known target of PTBP1, a negative regulator of PTBP2. We provide further evidence that U12 snRNA within the U11/U12 di-snRNP can compete with PTBP1 binding thus participating in PTBP2 regulation via exon 10 inclusion/exclusion. Additionally, we found that alternative splicing of muscleblind-like proteins 1 and 2 are also regulated by U11/U12 di-snRNP via PTBP2. Together, our data suggests that U11/U12 di-snRNP level can have non-canonical roles in alternative splicing regulation, with the most prominent role being regulation of PTBP2 mRNA levels in conjunction with PTBP1 (Spellman et al., 2007) and miRNAs.

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### 635 Hypoxia induces changes in alternative pre-mRNA splicing that may lead to tumour cell survival

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Eukaryotic cells sense oxygen and adapt to hypoxia by strict regulation of a number of genes. The biological responses to hypoxia involve induction of transcription of a network of target genes, a process which is co-ordinately regulated by hypoxia inducible transcription factors (HIFs).

RNA splicing takes place in the nucleus and occurs either co- or post-transcriptionally. Noncoding sequences (introns) in nuclear mRNA precursors (pre-mRNA) are removed by dedicated splicing machinery. Coding sequences (exons) are joined to generate the mature mRNA that is exported to the cytoplasm and translated into protein. The most of human genes pre-mRNAs undergo alternative splicing, which is a very precise process and plays a major role in the regulation of gene expression and the generation of proteomic and functional diversity. It is now clear, that the splicing machinery heavily contributes to biological complexity especially to the ability of cells to adapt to different developmental stages and altered cellular conditions.

We demonstrate that SR proteins isolated from hypoxic cells are more phosphorylated than those isolated from normoxic cells. We show that expression of SR protein kinases (CLK1, SRPK1, SRPK2) in hypoxic cells is elevated at mRNA and protein levels and that increased expression of CLK1 kinase is regulated by HIFs. Reduction of cellular CLK1 level affects hypoxia-dependent endogenous CAIX and Cyr61 gene pre-mRNA splicing. Our primary data also shows that the localization of splicing associated proteins is not changed depending on oxygen conditions thus confirming that probably other factors are involved in such regulation.

These findings provide insights to how the splicing of hypoxia dependent genes is regulated in hypoxic tumour cells and contributes to their survival.

### 636 Regulation of alternative splicing of *U2AF1*

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The auxiliary factor of U2 small nuclear ribonucleoprotein (U2AF) is a heterodimer critical for 3' splice site (3'ss) recognition. The gene for the 35-kD subunit of U2AF gives rise to two protein isoforms (termed U2AF35a and U2AF35b) that are encoded by alternatively spliced *U2AF1* exons 3 and Ab, respectively. The splicing recognition sequences of exon 3 are less favourable than exon Ab, yet U2AF35a expression is higher than U2AF35b across tissues. We show that U2AF35b repression is facilitated by weak, closely spaced branch points (BPs) just upstream of a long polypyrimidine tract (PPT) of exon Ab. Each BP lacked canonical uridines at position -2 relative to the BP adenines, with efficient U2 base-pairing interactions predicted only for shifted registers, reminiscent to those previously described for non-canonical contacts between U1 and a subset of 5' splice sites. The weak BP cluster was compensated by interactions involving unpaired cytosines in an upstream, EvoFold-predicted stem-loop structure (termed ESL) that binds FUBP1/2. Exon Ab inclusion in mature transcripts correlated with predicted free energies of a series of mutant ESLs. We also show that the isoform-specific U2AF35 expression differences are U2AF65-dependant and require interactions between the U2AF-homology motif (UHM) and the  $\alpha 6$  helix of U2AF35. These results expand the repertoire of exon repression mechanisms that control alternative splicing and provide new insights into tissue-specific subfunctionalization of duplicated exons in vertebrate evolution.

**637 Investigating the role of SR proteins in nuclear surveillance***Marius Wegener, Camila Freitags Stahl, Michaela Mueller McNicoll***Goethe University, Frankfurt am Main, Germany**

Prior to their cytoplasmic export pre-mRNAs undergo extensive processing to reach maturity. Splicing is an essential processing step in gene expression and is tightly regulated by SR proteins. During transcription these splicing factors bind to pre-mRNAs and recruit the spliceosome to splice sites via interactions between phosphorylated serine-arginine rich domains. Despite their interference with several mRNA maturation processes such as 3'end processing, mRNP packaging and mRNA export only little is known about their role in nuclear surveillance. To investigate splicing quality control through SR proteins, we inhibited splicing by isoginkgetin and performed RNA-Seq and identified several transcripts with retained introns that escape the nuclear surveillance machinery in HeLa as well as in pluripotent P19 cells. Using immunofluorescence microscopy, we observed the formation of distinct nuclear bodies upon splicing stress, which are distinct from nuclear stress bodies (SNBs). All SR proteins accumulate in these bodies and co-localise with co-factors of the nuclear exosome, which is not observed in control cells. To test whether exosome cofactors are recruited by SR proteins upon splicing stress and test their phosphorylation state, we performed co-IPs and found that RBM7, ZC3H18 and ZC3H14 are strong RNA-independent interaction partner of several SR proteins in stressed and control cells. Because quality control also involves the controlled export of properly matured mRNAs, we decided to first investigate ZC3H14, a conserved zinc-finger poly(A) binding protein with reported roles in mRNA export in yeast and *Drosophila*. To probe the function of ZC3H14 in higher Eukaryotes we depleted ZC3H14 and observed that the protein level of several highly expressed genes strongly decreased, while mRNA levels remained stable. Subcellular fraction revealed that ZC3H14 depletion lead to retention of mature mRNAs in the nucleus and co-IP experiments showed that ZC3H14 co-purifies with the nuclear export factor NXF1 and with hypophosphorylated SR proteins in an RNA-independent manner. Based on these data we speculate that SR proteins are important determinants in a dynamic splicing control mechanism acting at the interface between mRNA export by controlling ZC3H14 activity and exosomal decay by recruiting or stabilizing exosomal cofactors to aberrant transcripts.

**638 hnRNP DL, or: the forgotten brother***Katrin Kemmerer, Sandra Fischer, Julia E. Weigand***Technical University Darmstadt, Darmstadt, Germany**

HnRNP DL is the paralog of the well-known mRNA destabilizing factor AUF1 (AU-rich element binding factor or hnRNP D). Albeit hnRNP DL shares over 70% sequence homology in the RNA binding domains with AUF1, is ubiquitously expressed and thus might be as important for mRNA decay as its 'famous brother', its function has hardly been studied so far.

Studying alternative splicing in endothelial cells, we found that hnRNP DL is downregulated in response to hypoxia by increased inclusion of a poison exon into its 3'UTR. Exon inclusion introduces an exon junction downstream of the canonical stop codon, tagging the resultant mRNA for nonsense mediated decay (NMD). Downregulation of hnRNP DL expression in endothelial cells leads to reduced cell proliferation. In accordance, transcriptomics after hnRNP DL knockdown show a reduction of cell cycle related genes. HnRNP DL knockdown also alters the abundance and splicing pattern of membrane-receptors and cytoskeleton-associated genes, hinting at additional roles in endothelial cell function, e.g. angiogenic processes.

Similar to other RNA binding proteins (RBPs), hnRNP DL controls its own expression in a negative feedback loop. Overexpression of hnRNP DL increases the inclusion of the poison exon, resulting in degradation via the NMD pathway and a reduced protein level, ensuring homeostasis. Studying this autoregulatory feedback loop in hnRNP DL, we identified two novel ways to regulate AUF1 expression: (i) AUF1, like hnRNP DL, autoregulates its own expression by inclusion of a poison exon in its 3'UTR; (ii) both proteins crossregulate each other's expression using alternative splicing coupled to NMD. Thereby the expression of the two RBPs is intricately coupled, with a negative regulation observed in both directions. This double negative feedback loop directly links hnRNP DL to mRNA degradation, adding an additional layer of complexity to ARE-mediated decay.

**639 Molecular basis of aberrant CFTR exon 9 splicing driven by TDP-43 and hnRNP A1***Maria Zlobina, Christelle Beaumont, Peter Lukavsky***CEITEC-Masaryk University, Brno, Czech Republic**

The majority of human genes can produce multiple protein isoforms with distinct functions by alternative splicing. This process is highly regulated and even small malfunction causes diseases. TAR DNA-binding protein 43 (TDP-43) inhibits splicing of exon 9 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which in turn results in severe forms of cystic fibrosis. Mutation in the 3' splice site (3'ss) of CFTR exon 9 causing extension of a UG-rich region and polypyrimidine tract shortening, creates a high affinity binding site for TDP-43. After RNA binding TDP-43 recruits hnRNP A1 and thus formed complex prevents the recognition of the 3'ss of exon 9 by the spliceosomal machinery and causes exon 9 skipping resulting in a non-functional CFTR protein. Although RNA recognition and binding by TDP-43 alone and in complex with other hnRNPs has numerous functional implications, molecular details of such interactions remained elusive.

Our structural studies combined with biophysical approaches reveal that two copies of TDP-43 RBD create a new protein-protein interface with a salt bridge upon binding to the extended UG-rich sequence. Site-directed mutagenesis of amino acids involved in salt bridge formation reveals the functional significance of this protein-protein interface. Mutation at the interaction site of the two TDP-43 RBD copies reduces exon 9 skipping almost to the same extent as completely abolishing UG-rich RNA binding. This functional complex recruits two copies of hnRNP A1 to the intron 8 - exon 9 junction where they interact with 3'ss RNA *via* their RRM2 domains while RRM1 domains presumably participate in protein dimerization. NMR monitoring of the TDP-43 - hnRNP A1 complex assembly shows that their interaction with CFTR exon 9 3'ss RNA is not masking the polypyrimidine tract on the pre-mRNA for binding of the canonical splicing factor U2AF65. Shortening of the polypyrimidine tract, on the other hand, lowers the binding affinity of U2AF65 twofold. At the same time, hnRNP A1 binding at the 3'ss blocks access of U2AF35. Thus CFTR exon 9 skipping is driven by a network of interactions formed by TDP-43 and hnRNP A1 at the CFTR exon 9 3'ss which competes for the formation of the canonical splicing complex

**640 Induction Of Secreted Soluble Decoy EGFR Variants By Splicing Interference Inhibits Signaling And Tumor Growth In Treatment-Refractory Lung Cancer***Jeong Eun Park<sup>1</sup>, Lee Spraggon<sup>2</sup>, Elisa de Stanchina<sup>2</sup>, Luca Cartegni<sup>1</sup>***<sup>1</sup>Rutgers University, Piscataway, NJ, USA; <sup>2</sup>Memorial Sloan-Kettering Cancer Center, New York, NY, USA**

Many cancer-associated proteins can express pathogenic or non-pathogenic variants, depending on the differential inclusion of functional domains by alternative splicing or polyadenylation.

We developed a novel therapeutic approach which employs antisense manipulation of RNA processing to induce negative dominant variants of oncogenes, to reverse pathological signaling in cancer. Here, we describe a number of novel secreted soluble decoy isoforms produced by an U1-snRNP-dependent alternative intronic polyadenylation (IPA) mechanism.

Activation of the epidermal growth factor receptor (EGFR) kinase plays a central role in several cancers, in particular lung cancer. Despite recent advances in cancer therapy, a majority of non-small cell lung cancer (NSCLC) patients eventually develop secondary resistance to TK inhibitors and other EGFR-targeted drugs. Our strategy effectively induces the expression of potent natural inhibitors of EGFR signaling, which encompass the extracellular ligand-binding, dimerization domain, but lack the intracellular kinase domain necessary for activation. These soluble decoy EGFR (sdEGFR) isoforms are generated at the expense of the oncogenic full-length receptor, and block signaling of pathogenic EGFR in treatment-refractory cancer *in vitro* and in animal models. Importantly, their activity is independent of the specific mechanism of resistance involved (e.g. gateway resistance mutation T790M), and thus represents a potential alternative to treatment of refractory NSCLC.



## 641 Antisense oligonucleotides delivered to the amniotic cavity *in utero* modulate gene expression in the postnatal mouse

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Antisense oligonucleotides (ASOs) are quickly becoming an established tool for the therapeutic modulation of gene expression, though their utility in treating different types of conditions must still be determined. Congenital diseases account for a sizeable portion of illness. Prenatal screening and diagnosis allow for the detection of many genetic diseases early in gestation. Fetal therapeutic strategies aimed at managing disease processes as early as possible is an important goal. A safe and effective *in utero* pharmacotherapy designed to modulate gene expression would ideally avoid direct mechanical engagement of the fetus while presenting an external reservoir of drug. The amniotic cavity could serve as an ideal reservoir. We find that transuterine microinjection of ASOs into the amniotic cavity of mouse embryos modulates target mRNA expression for up to a month after birth. Our results show that ASO delivery to the amniotic cavity is well tolerated and produces a lasting effect on postnatal gene expression. Amniotic cavity delivery of ASOs introduces new opportunities for the fetal treatment of conditions where prenatal intervention can maximize therapeutic efficacy.

## 642 IL-8 as a Pharmacodynamic Biomarker of Trabectedin (OT-101) - An anti-TGF-β2 Antisense Drug - Results of a Phase I/II Study in Pancreatic Cancer Patients

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**Introduction:** Overexpression of transforming growth factor-beta 2 (TGF-β2) has been implicated as a pivotal factor for malignant progression of pancreatic carcinogenesis. OT-101 (Trabectedin) is an 18-mer phosphorothioate antisense specific for human TGF-β2 mRNA. In a Phase I/II study, pancreatic cancer (PAC) patients treated 2<sup>nd</sup>-line and beyond showed significantly improved overall survival (OS) when OT-101 was followed with chemotherapy. Here we examined the relationship between plasma levels of cyto-/chemokines with OS and identified IL-8 as a pharmacodynamic (PD) biomarker for improved OS.

**Methods:** 37 PAC patients were treated with continuous IV infusion of OT-101 in escalating doses for 7 days (40, 80, 160, 240 mg/m<sup>2</sup>/day) or 4 days (140, 190, 250, 330 mg/m<sup>2</sup>/day) in 2 week cycles. Plasma levels from 12 patients (140 mg/m<sup>2</sup>/day) were measured for 31 cyto-/chemokines on Days 1, 2, and 5 of 3 cycles of OT-101. Maximum levels of IL-8 on Day 5 were subtracted from levels on Day 2 and these standardized scores were correlated with OS values. Interactions with PK parameters were also investigated utilizing an ANCOVA model.

**Results:** Median OS of 14.5 months and 2.6 months were observed in patients treated with and without subsequent chemotherapies, respectively (p = 0.0009). Peak IL-8 levels were apparent after 2 days of OT-101 treatment. Significant regression and 95% CI was evident, whereby increasing difference of IL-8 following Day 2 vs Day 5 was positively correlated with OS (R<sup>2</sup> = 0.58, F<sub>1,9</sub> = 12.32, P = 0.0066). R-squares for all patients (0.4995, p < 0.0001) increased when sub-grouping patients without (0.8524, p < 0.0001) and with (0.9744, p < 0.0001) subsequent chemotherapy. Similar analyses for other cytokines of the 31 cytokine panel, especially IL-6, did not show the same results as IL-8.

**Conclusion:** Peak IL-8 response on Cycle 1 Day 2 of OT-101 treatment was correlated with OS in PAC patients. The correlation persisted regardless of treatment with or without subsequent chemotherapy suggesting that the observed IL-8 spike could be considered to be a PD biomarker for OT-101. The IL-8 specific response defines a subset of immune response and not a global immune response.

### 643 Preclinical Proof of Concept for Treatment for Inflammatory Bowel Disease (IBD) with Bacterial Delivery of Therapeutic Short Interfering RNAs (siRNAs)

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The balance between pro-inflammatory and anti-inflammatory cytokines in the mucosa regulates the development and potential perpetuation of mucosal inflammation in patients with IBD. We evaluated live attenuated bacterial delivery of siRNAs against IBD gene targets to achieve specificity, efficacy, and safety. An optimum pair of sequences for 8 relevant IBD epithelial cell gene targets (IL - 6Ra, IL - 7, IL - 13Ra1, IL - 18, Chitinase 3 - like - 1, Claudin - 2, MIP3a, and TNF-a) were selected from 169 mouse - specific siRNAs and screened for efficacy at the mRNA level. Two siRNA sequences for each target were encoded into cDNA for producing a short hairpin (shRNA) in a *transkingdom* RNA interference (*tkRNAi*) bacterial delivery platform. These cDNAs were cloned into an expression vector that also encodes for invasins and listeriolysin and subsequently transformed into the appropriate carrier strain of *E. coli* (CEQ600 strains).

The *in vitro* efficacy was assessed by an invasion assay using the CMT-93 mouse colon epithelial cells (or RAW264.7 macrophages for TNF-a) and qRT-PCR measurement of mRNA reduction vs  $\beta$ -actin control. Three gene targets (IL - 6Ra, Claudin - 2, and MIP3a) and two *tkRNAi* strains were tested *in vivo* using an oxazolone or dextran sulfate sodium (DSS) acute murine colitis model. The primary endpoint was to target and silence the gene of interest in the colonic mucosa (reduction in mRNA and/or protein). Secondary endpoints were improvement in the disease phenotype (body weight loss, colon length and weight, endoscopy and histologic scoring).

Oral delivery of IL - 6Ra *tkRNAi* strains (CEQ608/CEQ609) led to a significant reduction in colon length and abolished IL - 6Ra message in proximal ileum in DSS exposed groups. Claudin - 2 strains (CEQ621/CEQ626) caused a significant reduction in Claudin - 2 mRNA expression and protein levels in the colon as well as attenuation of the disease phenotype. Treatment with MIP3a therapeutic strains CEQ631/CEQ632 also resulted in a significant reduction in sum pathology scores and reduction in MIP3a mRNA expression. These findings suggest that *tkRNAi* - mediated gene silencing of pro-inflammatory targets represents a potential therapeutic development avenue for IBD therapy.

### 644 *tkRNAi*-mediated $\beta$ -catenin knockdown in the Gastrointestinal Mucosa Familial Adenomatous Polyposis Patients. Results of START-FAP trial.

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**Background:** Familial Adenomatous Polyposis (FAP) is an inherited gastrointestinal (GI) disorder that predisposes patients to early-onset of colorectal cancer. In FAP patients, mutations in the Adenomatous Polyposis Coli (APC) gene result in dysregulation and accumulation of  $\beta$ -catenin which leads to formation of adenomatous polyps in the GI mucosa. CEQ508 produced by *transKingdom* RNA interference (*tkRNAi*<sup>TM</sup>) technology, is a novel therapeutic agent using live-attenuated *Escherichia coli* genetically engineered to produce and deliver  $\beta$ -catenin suppressing short-hairpin RNA into the GI mucosal epithelia.

**Methods:** START-FAP is a Phase I dose-escalating study to evaluate safety and tolerability of single daily doses of CEQ508 in adult patients with FAP. Six patients with FAP were orally administered (3 each in Cohort 1 and 2) with CEQ-508 (108 and 109 colony forming units [CFU]/day for 28 days). The primary objective was to establish general safety for orally administered CEQ508 and to determine the maximum tolerated dose. The secondary objective was the effectiveness of CEQ508 on the gene expression of the target gene  $\beta$  - catenin. A mixed Nested-ANOVA model was used to evaluate  $\beta$ -catenin knockdown in normal mucosa and polyps.

**Results:** Daily oral dosing of 10<sup>8</sup> and 10<sup>9</sup> CFU of CEQ508 for 28 days was well-tolerated. Histological evaluation of polyps and normal mucosa at baseline and EOT indicated no changes in tissue morphology or inflammation within GI tissues in CEQ508-treated cohort 1 patients. A slight inflammation (from score of 0 to 1 at EOT) was noted in colon normal mucosa tissue of cohort 2 patients. Daily oral dosing of 10<sup>9</sup> CFU of CEQ508 for 28 days was well-tolerated with targeted  $\beta$ -catenin knockdown in polyps. Significant reduction was observed in overall  $\beta$ -catenin expression in polyps at EOT (P = 0.0005). Patient to patient variation accounted for 8.2% of the total variation. Reduction was observed primarily in the duodenum (39.3% decrease, P < 0.0001) and ileum (28.8% decrease, P = 0.012).

**Conclusion:** This Phase I trial of bacterial delivery of RNAi investigational agent CEQ508 in FAP patients demonstrated an acceptable safety profile and was well-tolerated at the two bacterial dose levels tested, with no MTD having been identified.

## 645 Modulation of *MKNK2* Alternative Splicing by Antisense Oligonucleotides as Novel Cancer Therapy

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In many cancers the Ras-MAPK signaling pathway is dysregulated and hyperactivated. Most notably, Ras is mutated in ~30% of all cancers. We have reported that cells transformed by mutant Ras up-regulate the alternative splicing factor SRSF1 and alter *MKNK2* splicing. The kinase Mnk2, which phosphorylates the translation initiation factor eIF4E, is a substrate of the Ras-MAPK pathway. The human *MKNK2* gene encodes for two alternatively spliced isoforms with differing last exons - Mnk2a and Mnk2b. We found that Mnk2a is down-regulated in several types of cancer and acts as a tumor suppressor by directly interacting with, phosphorylating and activating p38 $\alpha$ -MAPK. p38 $\alpha$ -MAPK activation by Mnk2a leads to activation of its target genes, induction of apoptosis and suppression of Ras-induced transformation in culture and in vivo. In contrast, Mnk2b does not activate p38 $\alpha$ -MAPK, while still increasing eIF4E phosphorylation, functioning as a pro-oncogenic factor. Thus, the switch between the production of the tumor suppressive isoform Mnk2a and the pro-oncogenic isoform Mnk2b, is instrumental in countering the oncogenic properties of cancer cells.

In order to manipulate levels of the Mnk2 isoforms in cancer cells, we designed Mnk2-specific 2'-O-Methyl modified antisense RNA oligonucleotides (ASOs) that induce a switch in Mnk2 alternative splicing. The ASO that causes a switch toward increased Mnk2a expression, inhibited anchorage-independent growth, colony formation and proliferation of high grade glioma, hepatocellular carcinoma (HCC) and metastatic breast carcinoma cells and re-sensitized drug resistant high grade glioma cells to chemotherapy; while the reciprocal switch toward increased Mnk2b expression had the opposite effect. Our preliminary data shows that ASO that enhances Mnk2a inhibits glioblastoma *in vivo*. These results suggest that manipulation of *MKNK2* alternative splicing by ASOs is a novel approach to inhibit specific types of cancer.

## 646 Targeting GRHL2 and CDH1 simultaneously using small artificial RNAs induces an epithelial-mesenchymal transition in MCF7 human breast cancer cells

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The epithelial-mesenchymal transition (EMT) is a program by which epithelial cells lose polarity and adhesion to gain migratory and invasive features and become multipotent mesenchymal stromal cells. The EMT is essential to development, but it has also been shown to occur in wound healing and in metastatic cancer progression. The inverse transition (MET) has however also been shown to accelerate tumor cell growth and establish deadly metastatic lesions. Various microRNAs (miRNAs) were reported to directly target EMT transcription factors and reverse the EMT process. Here, we assessed whether conversely EMT can be induced in MCF7 mammary epithelial cells by use of small artificial RNAs (smart RNAs) targeting simultaneously two genes involved in the maintenance of the epithelial phenotype, the transcription factor GRHL2 and the adhesion molecule CDH1 (E-cadherin). We used the miRBooking algorithm, which we developed to determine the stable set of miRNA::mRNA interactions in given cellular conditions, to simulate the overexpression of all 4<sup>7</sup> possible miRNA seeds into the MCF7 cell line, and selected those predicted to efficiently silence our targets, while having little or no effects on non-target genes. We completed to full miRNA sequences fifteen selected seeds by optimizing the base pairing on each target site. We introduced nine of them in lentiviral vectors that we used to infect MCF7 cells. We then monitored the effects on these infected cells on target expression at both the protein and RNA levels using Western Blot and RT-qPCR, respectively, on cell morphology using immunofluorescence assays, and, on cell migration using wound-healing assays. We observed for several infected cell populations a marked decrease (> 30%) in the expression of our targets, the appearance of cytoplasmic extensions, which is a characteristic of the mesenchymal state, and, increased migration. These results support the use of rationally designed smart RNAs to trigger the EMT program in human mammary epithelial MCF7 cells and investigate its role in tumor progression.

**647 Targeted inhibition of WRN helicase, replication stress and cancer***Natalie Orlovetskie, Nayef Jarrous***Hebrew University of Jerusalem, Jerusalem, Israel**

Human WRN helicase, which is encoded by the Werner syndrome gene, belongs to a family of RecQ helicases that are implicated in genome maintenance, including replication, recombination, excision repair and DNA damage response. These processes are often found upregulated in human cancers, many of which also display increased levels of WRN. Therefore, targeted destruction of WRN could be useful for elimination of malignant cells. The poster I would present at the conference, provide a proof of concept for applying the external guide sequence (EGS) approach in directing an RNase P RNA to efficiently cleave the WRN mRNA in cultured human cell lines, thus abolishing translation and activity of this distinctive 3'-5' DNA helicase-nuclease. Remarkably, the EGS-directed knockdown of WRN leads to inhibition of cell viability and changes in RNA polymerase III and RNase P activities, thus implicating two prominent chromatin binding apparatuses in early stages of WRN-related stress response. Hence, further assessment of this targeting system could be beneficial for selective cancer therapies, particularly in the light of the recent improvements introduced into EGSs.

**648 Readthrough inducing potential of glutamine isoacceptor tRNAs lies in their primary sequence outside of the anticodon***Zuzana Pavlíková<sup>1,2</sup>, Petra Beznosková<sup>1,2</sup>, Jakub Zeman<sup>1,2</sup>, Leoš Shivaya Valášek<sup>1</sup>***<sup>1</sup>Laboratory of Regulation of Gene Expression, Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic; <sup>2</sup>Faculty of Science, Charles University, Prague, Czech Republic**

Glutamine was repeatedly observed to incorporate almost exclusively into a few viral and cellular polypeptides during programmed stop codon readthrough. In some other cases, however, almost no incorporation of glutamine was detected. Here we asked what factors are responsible for the selective incorporation of the glutamine amino acid in these observed cases. Our recent findings suggested that one specific glutamine isoacceptor tRNA, bearing the CUG anticodon, manifests a great readthrough potential at the UAG-N stop tetranucleotide when compared to the low readthrough potential of all other tested glutamine isoacceptors bearing the UUG anticodon instead to base pair with the UAA stop. Interestingly, all glutamine isodecoder genes are sequentially otherwise very similar varying only in up to 4 nucleotides. We took a closer look at a contribution of the differing anticodon *versus* single nucleotide changes in the primary sequence of the Glu-tRNA backbone with respect to their varying readthrough potential. Additionally, we also analyzed the readthrough potential of individual glutamine tRNAs and their chimeric variants on all remaining stop codon tetranucleotides. Strikingly, we observed that the backbone of glutamine tRNA (*tQ(CUG)M*) is the major contributor to its high readthrough potential and thus it could be used as an exceptionally potent readthrough inducing tRNA (rti-tRNA) also for UAA and UGA stop codons when its anticodon is mutated to UUG or C/GCA, respectively. Our comprehensive analysis of the readthrough inducing potential of glutamine tRNA isoacceptor tRNAs hence brings new insights into stop codon decoding with the unexpected role of the tRNA structure in this process. It is also noteworthy that this still ongoing development of a “superpower” rti-tRNA could have a great application in a medical research by contributing to the common effort of many labs to specifically increase readthrough at premature termination codons, which are the major cause of more than 15% of genetic diseases and inherited cancers.



## 649 Control of breast cancer metastasis by microRNAs

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**Introduction-** Breast cancer is one of the most frequently diagnosed malignancy worldwide, accounting for over a million cases each year. It is also a leading cause of cancer death in women worldwide. The majority of deaths from breast cancer are not due to the primary tumor itself, but are the result of metastasis to other organs in the body. We recently noted a connection between miR-96 and miR-182 overexpression and reduction in breast cancer metastasis.

**Aims-** Decipher the cellular and biological mechanisms behind miRNA-metastases regulation, gain deeper understanding of the metastatic process and to shed light on the pathways involved in metastasis regulation.

**Methods-** In vitro breast cancer cell lines and functional assays (cell proliferation, migration and invasion), primary and metastases formation in an orthotopic breast cancer mouse model followed by in vivo and ex vivo imaging and high-throughput experiments (exome sequencing, RNA and small RNA sequencing, relative proteomics).

**Results-** miR-96 and miR-182 reduce metastatic burden in breast cancer model by targeting several pathways involved in cell movement and cytoskeleton organization, cell metabolism and immune regulation.

**Conclusions-** Breast cancer related mortality is frequently resulted from metastases formation. miR-96 and miR-182 reduce metastases formation by regulating several key pathways in the cancer progression cascade and may serve as candidates for novel translational studies.

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## 650 Prophylactic and therapeutic potential of 5'PPP-NS1-shRNA against influenza infection

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Influenza virus nonstructural protein 1 (NS1) suppresses host innate immune defense by targeting cytosolic pathogen sensor, Retinoic acid-inducible gene-I (RIG-I). In this study we show that silencing NS1 with *in vitro* transcribed 5'-triphosphate containing NS1 shRNA designed using the conserved region of a number of Influenza viruses not only prevented NS1 expression but also induced RIG-I activation and type I interferon expression. This strategy inhibited Influenza virus replication and also showed therapeutic potential in *in vitro* and *in vivo* experimental animal models.

**651 In a search for an ideal IVT mRNA to engineer human mesenchymal stem cells***Justyna Rozycka<sup>1</sup>, Adam Nowakowski<sup>1</sup>, Piotr Walczak<sup>2,3</sup>, Barbara Lukomska<sup>1</sup>, Miroslaw Janowski<sup>1,3</sup>*<sup>1</sup>NeuroRepair Department, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland; <sup>2</sup>Russell H. Morgan Dept. of Radiology and Radiological Science, Division of MR Research, Baltimore, USA; <sup>3</sup>Vascular Biology Research Program, Institute for Cell Engineering, The Johns Hopkins University School of Medicine, Baltimore, USA

**INTRODUCTION** The delivery of mesenchymal stem cells (MSCs) to the brain is still challenging. The intra-arterial route is very promising as it is minimally invasive and allows for precise delivery to the desired regions of the brain. However, to reach the tissue infused cells must dock to the endothelium and cross the blood-brain barrier. The underlying processes are not dissolved yet but some key molecules were identified to participate in this mechanism. Similar to leukocytes ITGA4 may be involved in MSC adhesion and transmigration thus our goal is to induce production of ITGA4 by human bone marrow MSCs (hBM-MSC) in a clinically suitable manner using IVT mRNA-ITGA4. Nevertheless, this method still needs to be improved in order to increase IVT mRNA stability and to restrain its cytotoxicity. One of the approach to overcome these obstacles involves incorporation of 5'cap analogues and chemically modified nucleotides into the mRNA strand during the *in vitro* transcription. Our aim was to compare two different IVT mRNA variants focusing on discrepancies in effectiveness in synthesis of exogenous protein and their cytotoxicity.

**METHODS** A pSP72 plasmid (P2191-Promega) with itga4 gene cDNA was used as a template for IVT mRNA-ITGA4(I) production with mMessage-mMachine®T7UltraKit (AM1345-Ambion) containing an anti-reverse-cap-analog (ARCA). Chemically modified IVT mRNA-ITGA4(II) was purchased from AmpTec, containing ARCA, 5-methylcytidine, and pseudouridine. Different doses of mRNA-ITGA4 were delivered to hBM-MSCs with Lipofectamine2000. Immunocytochemistry confirmed transfection efficacy. Cell Counting Kit-8 (Dojindo) was employed for cell proliferation rate assay.

**RESULTS** In all experimental variants tested, ITGA4 protein synthesis occurred with similar efficacy. The ITGA4 protein presence was of transient nature. Both IVT mRNA-ITGA4 variants negatively affect cell proliferation compared to control cells. The dose escalation negatively affected cell proliferation of mRNA-ITGA4(I) modified hBM-MSCs, while such effect was not visible in case of mRNA-ITGA4(II) engineered cells. Interestingly, there was no significant difference in cell proliferation rate between both mRNA variants at the lowest dose.

**CONCLUSION** IVT ARCA-capped mRNA is sufficient for effective synthesis of ITGA4 protein in hBM-MSCs.

**652 Protecting brain after stroke by modulating noncoding RNAs***Raghu Vemuganti<sup>1,2</sup>, Suresh Mehta<sup>1</sup>, Mary Lopez<sup>1</sup>, TaeHee Kim<sup>1</sup>, Robert Dempsey<sup>1</sup>*<sup>1</sup>University of Wisconsin, Madison, WI, USA; <sup>2</sup>William S. Middleton Memorial VA Hospital, Madison, WI, USA

Altered expression of many genes defines the secondary neuronal death that promotes the long-term neurologic dysfunction after stroke (focal cerebral ischemia). As post-stroke brain damage is rapid and completes within days, it is critical to provide therapies quickly. We show that stroke changes the cerebral expression profiles of microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) during the 1 day period in rodents subjected to transient focal ischemia. We evaluated the functional significance of some of these ncRNAs in post-stroke outcome. We tested the role of 2 pro-survival miRNAs (miR-7a and miR-21) that are highly expressed in mammalian brain. Following stroke, miR-7a was significantly down-regulated and resulted in de-repression of its major target  $\alpha$ -synuclein which is a promoter of ischemic brain damage. Replenishing miR-7a levels with a miR-7a mimic decreased the ischemic brain damage and resulted in better recovery of sensory-motor functions at 7 days after stroke. The miR-7a mimic treated rats also showed a significantly curtailed  $\alpha$ -synuclein protein induction indicating that the benefits might be due to its action on the target. The miRNA miR-21 is known to promote neuronal survival under adverse conditions. When rodents treated with a miR-21 mimic to increase its brain levels by ~40 fold were subjected to stroke, post-ischemic inflammation and apoptosis were curtailed and the survival of animals improved significantly. Furthermore, miR-21 mimic treated rodents showed significantly smaller ischemic infarcts and better functional recovery without any toxicity. We also tested an lncRNA that is transcribed downstream to Fos gene called FosDT which was induced in the post-ischemic brain. FosDT scaffolds the chromatin-modifying proteins Sin3A and coREST that are the essential cofactors of the transcription factor REST. When FosDT was knocked-down with a siRNA, there was a significant improvement in the motor and sensory functional recovery and a decrease in the ischemic neuronal death. FosDT knockdown also de-repressed REST downstream neuroprotective genes GRIN1 and GRIA1 indicating that its action are by modulating REST. Overall, our studies show that ncRNAs are promising therapeutic targets for developing drugs to mitigate post-stroke neurologic dysfunction. These studies were funded by NIH, Veterans Administration and American Heart Association.

## 653 RNA Dendrimer Platform for Nanomedicine

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RNA nanotechnology has been rapidly advancing due to its increasing application in nanomedicine. Its functional versatility and ease of assembly allow efficient preparation of homogeneous nanoparticles with defined shape, size and stoichiometry. In therapeutics, RNA nanoparticles have shown favorable pharmacokinetic and biodistribution properties by avoiding accumulation in healthy organs and tissues with efficient retention in tumors. In order to evaluate the effects size, shape, and payload have on the therapeutic efficacy of RNA nanoparticles, we designed five generations (G0-G4) of RNA dendrimers built with the ultrastable three-way junction (3WJ) motif of bacteriophage phi29 as building block. This series of dendrimers is assembled around a central, square-shaped RNA core surrounded by an increasing number of 3WJ layers. All five generations can be efficiently self-assembled using one-pot synthesis into monodisperse globular particles with diameters ranging from 2.8 nm (G0) to 26 nm (G4). Thermal gradient fluorescence and gel electrophoresis (TGGE) reveal a bi-phasic assembly/disassembly mechanism in which the outer most layers form around a preformed thermodynamically stable core. This feature allows thermodynamic control over release and degradation of the external 3WJ strands that can be functionalized with targeting and therapeutic moieties. Upon incorporation of folate on the peripheral branches, RNA dendrimers show high binding and internalization into cancer cells. RNA dendrimers are envisioned to have a major impact in targeting, disease therapy, molecular diagnostics and bioelectronics in the near future.

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## 654 Antisense oligonucleotide mediated rescue of aberrant *COL17A1* splicing in junctional epidermolysis bullosa

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Junctional epidermolysis bullosa (JEB) is a devastating disease of the skin and mucous membranes, characterized by blistering and erosions upon minor mechanical friction. Genotypically, mutations within the collagen 17 gene (*COL17A1*) underlie this phenotype.

Two antisense oligonucleotides (ASOs) targeting a cryptic acceptor splice site were tested for their ability to restore a wild type *COL17A1* splice pattern in keratinocytes of a patient harboring a mutation at the intron 6 / exon7 junction. The mutation leads to aberrant splicing loss of 16 nucleotides and as a consequence of the reading frame shift to nonsense-mediated mRNA decay.

We found that both tested ASOs were capable of restoring the wild type splice pattern in JEB patient keratinocytes. In addition to that, both ASOs induced skipping of either the 36 nucleotide long exon 7 alone or both exon 6 (48 nts) and 7. Interestingly, skipping of exon 6 alone was also observed in healthy control keratinocytes and therefore seems to be a naturally occurring splicing event. Either of the two exon skipping events will potentially result in the generation of a slightly shortened *COL17A1* mRNA and protein. Additionally, semi-quantitative real-time PCR showed that the overall expression level of *COL17A1* was significantly increased upon ASO treatment compared to non-treated patient keratinocytes.

In summary, we showed that ASOs can restore type XVII collagen expression *in vitro* and are regarded to be promising tools for future clinical applications in JEB.

**655 RiboLace: a highly sensitive method for active ribosomes profiling**

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Ribosome profiling, or Ribo-Seq, consists in the massive sequencing of RNA fragments protected from nuclease digestion by ribosomes. This method provides genome-wide insights into the mechanistic aspects of protein expression, thanks to its unique ability to provide positional information on ribosomes flowing along transcripts. Despite its many accomplishments, ribosome profiling still faces several challenges. RiboSeq cannot distinguish between fragments protected either by ribosomes in active translation or by stalled ribosomes, potentially miscalculating translation levels for many transcripts. In addition, current protocols require large amounts of starting material, impeding their use in specific tissues or sub-cellular compartments such as axons. By means of an antibody-free and tag-free pull-down, based on a new puromycin-containing molecule, we developed a new method (called RiboLace) for the isolation of active ribosomes. RiboLace is fast, it requires low input material and it can be rapidly used to obtain a global snapshot on the active translato

**656 The IRES<sub>5'UTR</sub> of the dicistrovirus Cricket Paralysis Virus contains an essential pseudo-knot structure for its translational activity**

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RNA viruses contain highly structured regions located upstream of their coding sequences called Internal Ribosome Entry Site (IRES). The cellular translational machinery is hi-jacked by these IRESs in a cap-independent and highly efficient manner, therefore allowing viral proteins synthesis at the expense of host translation. The Cricket Paralysis Virus (CrPV), a member of the *dicistroviridae* family, contains a single positive-stranded RNA genome that encodes two non-overlapping ORFs separated by a short intergenic region (IGR). The 5'UTR contains the IRES<sub>5'UTR</sub> that directs the expression of non structural proteins encoded by ORF1, and the intergenic region contains a second IRES, called IGR, that drives synthesis of structural proteins from ORF2. Whereas IGR has been extensively studied over the last two decades, the IRES<sub>5'UTR</sub> remains largely unexplored. Here, we mapped, for the first time, the minimal IRES element required for efficient translation initiation in the 760-nucleotide-long 5'UTR of CrPV genome. Using chemical probing (SHAPE, DMS and CMCT), we established the secondary structure of the entire 5' UTR. We discovered that IRES<sub>5'UTR</sub> contains a pseudo-knot structure that is essential for proper folding of the IRES and for translation initiation both *in vitro* and *in vivo*. Using cell-free translation extracts, we demonstrated that IRES<sub>5'UTR</sub> promotes direct recruitment of the ribosome on the cognate viral AUG start codon without any scanning step, using a HCV-related translation initiation mechanism suggesting that it belongs to IRES type III class. By mass spectrometry, we also showed that IRES<sub>5'UTR</sub> directly recruits eIF3 like other type III IRES. It has been shown that the two CrPV IRES are temporally expressed at distinct stages of the infection. Therefore, our results pave the way for studies addressing the synergy and the interplay between the two IRESes from CrPV and the molecular mechanism responsible for differential expression of non-structural and structural proteins during the infectious process.



## 657 Consecutive elongation of D-amino acids in translation

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In ribosomal translation, only 20 kinds of proteinogenic amino acids, namely 19 L-amino acids and glycine, are exclusively utilized for polypeptide synthesis. However, a reconstituted cell-free translation system in combination with an artificially reprogrammed genetic code has successfully been used to introduce various nonproteinogenic amino acids into polypeptides. To date, amino acids with artificial sidechains as well as *N*-methyl- $\alpha$ -amino acids,  $\beta$ -amino acids, and D-amino acids have been successfully introduced into peptide chains. Incorporation of certain *N*-methyl- $\alpha$ -amino acids and *N*-alkyl- $\alpha$ -amino acids into the nascent peptide chain has been accomplished not only singly but also consecutively. In contrast, even two incorporations of D-amino acids in a row is extremely inefficient. Fujino *et al.* classified the 19 D-amino acid counterparts into three groups based on their single incorporation efficiency, and they reported that even the amino acids classified in the best efficiency group, such as D-Phe and D-Ala, could not be introduced into a peptide consecutively. Here we report achievement of continuous d-amino acid elongation by the use of engineered tRNAs and optimized concentrations of translation factors, enabling us to incorporate up to ten consecutive D-Ser residues into a nascent peptide chain. We have also expressed macrocyclic peptides consisting of four or five consecutive D-amino acids consisting of D-Phe, D-Ser, D-Ala, or D-Cys closed by either a disulfide bond or a thioether bond.

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## 658 Ribosomal protein L9 in translational bypassing

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Each time a peptide bond is formed, the ribosome moves along the mRNA by one codon. These step size is maintained very accurately to avoid non-programmed recoding during translation and thus to minimize errors in polypeptide sequences. *In vivo* data implement the ribosomal protein L9 in reading frame maintenance during translation and in the regulation of translational bypassing and frameshifting. L9 is known to take part in polysome interactions; however, the mechanism of L9 action in reading frame maintenance and recoding is completely unclear. Here we studied the effect of L9 on the ribosomal bypassing of a 50-nt segment of gene 60 mRNA *in vitro* used a minimal reconstituted *E. coli* translation system. We compare the bypassing efficiency for ribosomes with and without ribosomal protein L9 under condition of mono - and polyribosome formation and estimated the importance of this protein while bypassing signals are weakened or eliminated. We show that polysome formation reduces bypassing efficiency, whereas the deletion of the L9 has no effect on bypassing.

## 659 Elongation of the Nascent Peptide in the Ribosomal Exit Tunnel

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The ribosome is a stochastic nanomachine responsible for protein synthesis in all cells. Ribosomes translate the genetic information from the messenger RNA into a sequence of amino acids. The catalytic center is buried in the large (50S) subunit, so the newly synthesized nascent peptide chain (NC) has to exit the ribosome through a tunnel. There has been evidence emerging that the tunnel is a functional environment that regulates ribosomal function and facilitates protein (pre)-folding. Moreover, the tunnel has strong pharmaceutical relevance because it binds a clinically important class of antibiotics. Nevertheless, only a little information has been gathered about the NC structure and dynamics so far.

For example, it is not known what force actually drives the NC through the tunnel, or how the tunnel environment affects the NC conformations. To address these questions, we have performed all-atom molecular dynamics simulations (MD) of the prokaryotic ribosome under physiological conditions. We have developed a unique MD protocol to mimic the elongation process. In a series of non-equilibrium simulations, amino acids are added one by one to the growing peptide chain.

We focus on a few model NC sequences and study the role of amino acid side chains on i) the rate of NC relaxation to the equilibrium, and ii) the NS progress throughout the tunnel.

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## 660 Building a reporter system to assess repeat-associated translation: Application to screen genetic modulators of microsatellite expansion disorders

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Several neuromuscular disorders are characterized by microsatellite expansions. In particular, hexanucleotide repeat expansion of GGGGCC (G4C2) in the first intron of *C9orf72* is the most commonly observed mutation associated with familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Several mechanisms have been proposed for the contributions of G4C2 expansion in pathogenesis. As such, the aberrant expansion of repeated G4C2 sequences can not only interfere with the endogenous function of *C9orf72*, but also gives rise to toxic RNA and dipeptides repeats (DPRs). Different DPR species are generated from all frames of G4C2 repeats through a recently discovered unconventional mode of RNA translation termed, repeat-associated non-ATG (RAN) translation. However, the mechanism of RAN translation is still poorly understood, and the specific role of RAN biology in the development of disease is not fully determined.

To address these questions, we set up a cell-based reporter system for RAN translation. We show that our inducible G4C2 repeat-containing constructs can efficiently stimulate RAN translation without inducing cytotoxicity in cultured human cells. We further demonstrate that our reporters are compatible with several cell-based assays and show that we can detect the resulting DPR proteins from different translating frames. Interestingly, the subcellular accumulation and aggregation pattern of the DPRs generated are reminiscent of the phenotypes observed in patient-derived samples, proving that this system can be used to accurately model microsatellite expansion disorders. Thus, our reporter assays can now be adapted to high throughput discovery approaches, including small molecule and genetic screens, to identify modulators of RAN translation. Further experiments will be performed to validate the hits obtained in different cellular models and determine their role in the translation of other repeat expansions.

Taken together, our assays establish novel tools that will help elucidate the mechanism of RAN translation and define the contributions of repetitive RNAs and peptides in disease. Ultimately, our screens will provide new insights into the pathology of microsatellite expansions in the context of several debilitating Human disorders.

## 661 Structure of ABCE1 in the context of a native mammalian 48S initiation complex

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For many years initiation and termination of mRNA translation have been studied separately. However, a direct link between these two isolated stages has been suggested by the fact that some initiation factors also control termination and can even promote ribosome recycling; i.e. the last stage where post-terminating 80S ribosomes are split to start a new round of initiation. Notably, it is now well established that, among other factors, ribosomal recycling critically requires the NTPase ABCE1. However, several earlier reports have proposed that ABCE1 also somehow participates in the initiation complex assembly. Based on an extended analysis of our recently published late-stage 48S initiation complex from rabbit (Simonetti et al., 2016), here we provide a new interpretation of our structure and new structural data indicating a role of ABCE1 in the initiation process. Taken together, our previous structure (Simonetti et al., 2016), and this new analysis, they represent a strong structural argument for the regulatory role of the recycling factor ABCE1 in the initiation process and attempt to explore the interplay between ABCE1 and several initiation factors during the transition from ribosomal recycling to formation of competent 48S initiation complexes.

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## 662 Characterization of an Internal Ribosome Entry Site (IRES) in p53 mRNA

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The tumour suppressor *p53* gene is one of the most studied cancer-related genes. So far, many *p53* isoforms have been identified either resulting from alternative splicing or from non-canonical translation mechanisms. It is known that cap-dependent translation is repressed under stress conditions to preserve energy. Therefore, other translational mechanisms are required to keep the synthesis of vital proteins. Internal Ribosome Entry Sites (IRESes) were first discovered in viruses, and then observed in eukaryotes, as secondary structures present in RNA that were capable of recruiting ribosomes to the vicinity of an initiation codon inserted in an optimal environment allowing cap-independent translation of mRNAs. Translation of *Δ40p53*, a *p53* isoform, is one example of this non-canonical mechanism due to the presence of an IRES near an alternative initiation codon (AUG<sub>40</sub>). Here, we will present and characterize a new IRES in *p53* mRNA. We present details on the localization, structure, function and regulation of this IRES under normal and stress conditions. Importantly, our data reveals that the function of this IRES is required for cell survival and proliferation under certain cell conditions. This finding can have grave implications for understanding *p53* function dynamics and cancer progression in specific environments.

## 663 Selective recognition and transport of misfolded polypeptides toward aggresome via the CED complex

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In mammalian cells, misfolded polypeptides are degraded by the ubiquitin-proteasome system (UPS). When the UPS is malfunctioned, misfolded polypeptides are sequestered into aggresome and ultimately degraded by autophagy. Here, we show that CBP80/20-dependent translation initiation factor(CTIF)-containing complex functions in protein surveillance mechanism. Through LC-MS/MS, we found two CTIF-interacting factors: dynactin 1 (DCTN1) and eukaryotic translation initiation factor 1 alpha 1 (eEF1A1). DCTN1 involves in the retrograde movement of cargoes by the interaction with dynein motor protein. Previous reports showed that eEF1A1 directly binds pre-existing or newly synthesized misfolded polypeptides and could generate signal for aggresome formation. Our data shows CTIF-eEF1A1-DCTN1 (CED) forms complex and connects selective recognition of misfolded polypeptides and targeting toward aggresome. These events reduce apoptosis level of cells which can be caused by proteotoxic stresses. Interestingly, we observed CTIF is enriched in the Lewy bodies of cerebellar molecular layer in PD patient brain sections. Considering that aggresome has similarities to misfolded protein-containing inclusion bodies which are observed in neuronal cells, this study can shed light on neurodegenerative proteinopathies.

\*These authors contributed equally to this work.

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## 664 Analyses of cell type specific translation from IRES mRNA derived from two different poliovirus strains

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Protein synthesis of Poliovirus (PV) is governed by the internal ribosome entry site (IRES) located in the 5' untranslated region (5' UTR) of its RNA genome. During PV infection, viral proteases cleave the cap-binding complex component eIF4G, leading to inhibition of host protein synthesis. PV IRES enables efficient viral translation by directly binding to a complex composed of eIF4A and eIF4G to recruit the 40S ribosome subunit. There are three serotypes of wild poliovirus type 1, type 2, and type 3. The Mahoney virulent strain of type 1 can propagate in the gut epithelial cells and in neuronal cells, followed by degeneration and lysis of cells leading to paralytic poliomyelitis. In contrast, the Sabin vaccine strain of type 1 also propagates in gut, but not in neuronal cells. Although it is well known that a major attenuating mutation for the Sabin strain in neuronal cells is located in the IRES, the relationship between attenuation and IRES-driven translation remains unclear. An intriguing possibility is that the attenuation of the Sabin strain is due to an effect on the efficiency of IRES-driven ribosome recruitment specifically in neural cells.

To test this possibility, we monitored the *in vitro* translation activity of both PV IRES mRNAs utilizing HeLa (non-neuronal cell line) and SK-N-SH (neuronal cell line) cell lysates. We found that the Sabin IRES-mediated translation was defective in SK-N-SH cell lysates, but not in HeLa cell lysates. Next, we divided HeLa or SK-N-SH cell lysates into two fractions: ribosome complexes and ribosome-free lysates. We then monitored the *in vitro* translation activity of both PV IRES mRNAs when ribosome complexes from one cell were combined with lysates from the other cell-type. This revealed that SK-N-SH ribosome complexes contribute to the Sabin IRES-mediated translational repression in extracts derived from this cell line. To identify specific ribosome-associated factors mediating this effect, we analyzed SK-N-SH ribosome complexes by mass spectrometry. This revealed several candidates which we are validating in functional assays. The status of these experiments will be presented. Collectively, our results suggest a new mode for viral cell tropism, based on altered translation activity of the IRES.



**665 The Impact of N1-Methyladenosine on Translation***Erica Thomas, Hani Zaher***Washington University in St. Louis, St. Louis, Missouri, USA**

Nucleic acids are consistently experiencing damage from both exogenous and endogenous agents, which can result in a disruption of their function and ultimately pose a challenge to the cell. Additionally, intentional modifications to RNA have recently emerged as regulators of gene expression through mRNA splicing, stability, and translation. Alkylation is a type of adduct that is known to impact the function of nucleic acids, either as a cellularly driven modification or damage mark. For example, the repair of alkylated DNA is known to be important for preventing mutagenesis, and DNA methylation plays a vital role in the epigenetic control of gene expression. However, little is known about the cellular responses to the alkylation of RNA. Emerging evidence suggests that mechanisms to dealkylate RNA exist in virtually all organisms; however, whether ribosome mRNA surveillance also plays a role in quality control is not well understood. In order to investigate the impact of alkylation on the decoding of mRNA, we introduced a single alkylation adduct, N1-Methyladenosine (m1A), into mRNA and examined its effects on translation using a well-defined bacterial *in vitro* translation system. We find that m1A has a detrimental impact on the speed of the decoding process, causing at least a 250-fold decrease in the rate of translation. Additionally, the presence of m1A causes either increased or decreased rates of miscoding for a subset of near-cognate aminoacyl-tRNAs or for release factors, which appear dependent on the position of the mismatch. Our data suggests that m1A can either inhibit or promote mispairing of neighboring nucleotides with the anticodon. These data demonstrate the ability of alkylation to severely disrupt the proper decoding of mRNA, and support the hypothesis that cells must remove alkylative damage from RNA to maintain proper homeostasis.

**666 Eukaryotic translation initiation factor 3 undergoes dramatic structural changes prior to its binding to the 40S ribosomal subunit***Jakub Zeman, Michal Rosulek, Petr Novák, Leoš Valášek***Institute of Microbiology of the CAS, v. v. i., Prague, Czech Republic**

Eukaryotic translation initiation factor 3 (eIF3) is a multiprotein complex serving as an essential scaffold promoting binding of several other factors to the 40S subunit, where it coordinates their actions during the initiation phase of translation. It comprises 12 proteins in mammals, whereas only 5 subunits in the budding yeast. Due to a high degree of flexibility of multiple eIF3 subunits/domains, a detailed crystal structure of neither mammalian nor yeast eIF3 complexes has been solved till now. However, high resolution models of eIF3 in complex with the 40S subunit plus some other eIFs were published based on short-length cross-linking, negatively stained EM reconstruction and cryo-EM imaging.

We previously examined the protein-protein interactions among yeast eIF3 subunits and based on these biochemical experiments supported by various genetic analyses we built the eIF3 subunit interaction map, where we inserted all known crystal structures of eIF3 subunits or their domains. Here we purified all individual subunits of yeast eIF3 from bacteria and reconstructed the whole protein complex *in vitro* to determine an overall shape of the free 5-subunit eIF3 in solution. The reconstituted eIF3 was cross-linked with four different cross-linkers and the trypsin-digested samples were analyzed using Mass spectrometry. The obtained cross-links not only support our aforementioned eIF3 subunit interaction map but also reveal a completely new geometry of eIF3. The whole complex seems to be very compactly packed when free in solution, which contrasts with the published eIF3-40S models, where eIF3 appears to wrap around the 40S head with its extended arms. To understand the process of the eIF3 rearrangement/unpacking during Multifactor complex (eIF3, eIF1, eIF5, and eIF2 ternary complex) formation and its binding to the 40S subunit, we analyzed the eIF3-eIF1-eIF5 complex as well as the eIF3-40S preinitiation complex using the same methodology. The obtained results support our idea that binding of other factors slightly opens compactly packed eIF3 complex prior to its binding to the 40S subunit, where it spreads its arms to literally embrace it. Our data thus suggests a robust structural rearrangement of the overall shape of eIF3 prior to its 40S-binding with functional implications that will be discussed.

**667 DDX3 regulates translation of ATF4 during stress**

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Accumulation of unfolded and potentially toxic proteins in the endoplasmic reticulum (ER) activates a cell stress adaptive response, which involves a reprogramming of general gene expression. ATF4 is a master stress-induced transcription factor that orchestrates gene expression during various ER stresses including those involved in cancer. ER stress-induced ATF4 expression occurs mainly at the translational level involving the activity of the phosphorylated translation initiation factor eIF2 $\alpha$  (P-eIF2 $\alpha$ ). While it is well established that under ER stress P-eIF2 $\alpha$  drives ATF4 expression through a specialised mode of translation re-initiation, factors (e.g. RNA-binding proteins and specific translation initiation factors) involved in P-eIF2 $\alpha$ -mediated ATF4 translation remain unknown. Here we identified the RNA-binding protein named DDX3 as a promotor of ATF4 expression in cancer cells treated with sorafenib, an ER stress inducer used as a chemotherapeutic. Depletion experiments showed that DDX3 drives ATF4 expression independently of eIF2 $\alpha$  phosphorylation. Luciferase and polyribosomes translational assays showed that DDX3 drives sorafenib-induced ATF4 mRNA expression at the translational level. Protein-interaction assays identified DDX3 as a component of the translation initiation complex eIF4F. The interaction between DDX3 and the eIF4F complex is potentially mediated by eIF4GI, which we found to be required for sorafenib-induced ATF4 expression. Thus, this study identified a novel DDX3-based mechanism that regulates P-eIF2 $\alpha$ -mediated ATF4 mRNA translation that occurs during stress.

**668 Ribosome rearrangements at the onset of translational bypassing**

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Bypassing is a recoding event that leads to the translation of two distal open reading frames into a single polypeptide chain. Here, we present the structure of a translating ribosome stalled at the bypassing take-off site of *gene 60* of bacteriophage T4. The nascent peptide in the exit tunnel anchors the P-site peptidyl-tRNA<sup>Gly</sup> to the ribosome and locks an inactive conformation of the peptidyl transferase center (PTC). The mRNA forms a short dynamic hairpin in the decoding site. The ribosomal subunits adopt a rolling conformation in which the rotation of the small subunit around its long axis causes the opening of the A-site region. Together, PTC conformation and mRNA structure safeguard against premature termination and read-through of the stop codon, and reconfigure the ribosome to a state poised for take-off and sliding along the non-coding mRNA gap.

## 669 The application of *in vitro* translation assays to study the regulation by small bacterial RNAs and the Hfq protein

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The bacterial small noncoding RNAs (sRNAs) contribute to the regulation of bacterial cell adaptation to changing environmental conditions. sRNAs regulate gene expression by binding to partly or perfectly complementary sequences in target mRNAs, which leads to changes in their translation and/or stability. In this process they are assisted by a matchmaker protein Hfq. The small RNA binding to mRNA may lead to either negative or positive regulation of gene expression. Numerous small RNAs affect mRNA translation by binding to mRNAs at the canonical sites necessary for the initiation of translation, i.e. in the area of Shine-Dalgarno sequence and the start codon. However, others bind upstream of the ribosome binding site, where their effect on translation may be indirect. There is also a growing number of small RNAs known, which bind within the coding sequence of mRNAs. Some of them pair with sequences within the so-called five-codon window and affect translation initiation, while others bind more deeply in the coding sequence and are thought to affect mRNA stability.

To reconstitute *in vitro* the effect of small RNAs on mRNA translation regulation the S30 extracts from *Escherichia coli* cells were used. The translationally active cell extracts were prepared from wildtype *E. coli* BL21 cells, and BL21 ΔHfq mutant cells. The translation progress was monitored by fluorescence of fused GFP protein and by S<sup>35</sup> labeling. Preliminary studies allowed reproducing the effect of RybB sRNA on the repression of the initiation step of translation of *S. enterica ompD* mRNA fragment in fusion with GFP protein. In further studies, we plan to compare sRNAs binding at different sites within mRNAs to dissect their effects on translation, and to better elucidate the contribution of Hfq to translation regulation.

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## 670 Translational regulation in embryonic development and homeostasis

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Precision in gene expression is essential to establish and maintain a cellular identity in embryonic development. Therefore, key regulatory mechanisms are in place at every step of developmental cell-fate transitions, ranging from transcriptional control to proteostasis. Interestingly, recent studies suggest that up to 60% of gene expression is regulated at the level of translation. Highlighting its importance in developmental gene regulation, slight alterations in mechanisms regulating translation lead to severe developmental defects. However, the role of translational regulation in shaping developmental gene expression landscape is poorly understood. Since pluripotent stem cells (PSCs) display robust mechanisms to maintain their molecular integrity from genome to proteome, we reasoned that this could be an ideal platform to unravel hidden layers in translational regulation. For this, first we performed translational state mass spectrometry (TS-MS) in PSCs to identify novel translational regulators. This led to the identification of an RBP that associates exclusively with polysomes in a stress dependent manner, suggesting its direct role in regulating translation. In addition, our live imaging and localization studies revealed that this RBP localizes to RNA stress granules. Moreover, it interacts with RNA-degradation machinery in a stress dependent manner. Loss of function studies indicate that this RBP is essential for survival of PSCs. eCLiP analysis revealed that this RBP specifically binds a wide range of transcripts, including critical components of translation machinery, core pluripotency factors and specific sno-RNAs. Localization and loss of function studies in developing zebrafish embryos revealed that this RBP and its paralog are essential for early cell-fate decisions during gastrulation and in the maturation of cardiomyocytes *in vivo*. Based on these data, we hypothesize that this RBP acts as a translation-decision hub, binding to specific mRNAs and deciding their translation state, localization and stability, depending on external stimuli. Here we identified a novel set of translational regulators potentially important in managing cellular stress as well as mediating embryonic development.

**671 Cellular adhesion is regulated by mRNA translation in Spreading Initiation Center***Jonathan Bergeman<sup>1</sup>, Marc-Étienne Huot<sup>1,2</sup>*<sup>1</sup>Centre de recherche sur le cancer de l'Université Laval, Quebec,, Canada; <sup>2</sup>Centre de Recherche du CHU de Québec (axe Onconlogie) , H@tel-Dieu de Québec, Quebec,, Canada

RNA-binding proteins (RBPs) have been shown to participate in different steps of tumor progression, such as cell adhesion and migration. As such, our results suggest that cancer-related dysregulation of RNA-binding protein activity, can affect cancer cell dissemination and alter tissue homing.

In order to understand the role of RBPs in these processes, we investigate the mechanisms taking place in spreading initiation centers (SICs), a transient structure found above in nascent and immature adhesion complexes. We found that numerous essential mechanisms were shared by SIC positive cells and mesenchymal-like cancer cell lines (highly metastatic), but were absent in those that were identified as endothelial-like (non-invasive). Furthermore, SIC-positive cells share different characteristics with amoeboid migrating cells, a morphological state known to greatly increase migration and invasion potential.

Our previously work showed a robust enrichment of RBPs (FMRP, G3BP1, Sam68) in SICs. These RBPs are known for their ability to control mRNA translation. We also observed a significant increase of neosynthesized protein into SICs upon cellular adhesion. Concomitantly, we discovered that inhibiting translation induced a delay in cell adhesion kinetics, maintained SICs formation and decrease cell adhesion capacity. These results clearly indicate that mRNA translation regulation acts as a novel checkpoint consolidating cell adhesion. Using a puromycin based method, we identified proteins specifically translated in SICs during the early stages of adhesion. mRNAs corresponding to these newly translated proteins were validated for their sub-localization within SICs, using RNA FISH.

Hence, we postulate that localized translation within SICs can modulate adhesion consolidation and seems to recapitulate events observed in transitions between the amoeboid and mesenchymal morphologies. These changes in adhesion strength allow metastatic cells to modulate their morphology in order to facilitate their dissemination.

**672 *In vivo* translome profiling reveals early defects in ribosome biology underlying SMA pathogenesis***Paola Bernabò<sup>1</sup>, Toma Tebaldi<sup>2</sup>, Ewout JN Groen<sup>3,4</sup>, Fiona M Lane<sup>3,4</sup>, Elena Perenthaler<sup>1</sup>, Francesca Mattedi<sup>1</sup>, Helen J Newbery<sup>3,4</sup>, Haiyan Zhou<sup>5</sup>, Paola Zuccotti<sup>2</sup>, Valentina Potrich<sup>2</sup>, Francesco Muntoni<sup>5</sup>, Alessandro Quattrone<sup>2</sup>, Thomas H Gillingwater<sup>3,4</sup>, Gabriella Viero<sup>1</sup>*<sup>1</sup>Institute of Biophysics -CNR, Trento, Italy; <sup>2</sup>Centre for Integrative Biology, University of Trento, Trento, Italy; <sup>3</sup>Euan MacDonald Centre for Motor Neurone Disease Research, University of Edinburgh, Edinburgh, UK; <sup>4</sup>Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK; <sup>5</sup>Dubowitz Neuromuscular Centre, Great Ormond Street Institute of Child Health, University College London, London, UK

Genetic alterations impacting on ubiquitously expressed proteins involved in mRNA metabolism often result in neurodegenerative conditions, with increasing evidence suggesting that translational defects can contribute to disease. Spinal Muscular Atrophy (SMA) is a neuromuscular disease caused by low levels of SMN protein, whose role in disease pathogenesis remains unclear. By determining in parallel the *in vivo* transcriptome and translome in SMA mice we identified a robust decrease in translational efficiency, arising during early stages of disease. Translational defects affected translation-related transcripts, were cell autonomous, and were fully rescued after treatment with antisense oligonucleotides to restore SMN levels. Defects in translation were accompanied by a decrease in the number of ribosomes in motor neurons *in vivo*. Our findings suggest that neuronal tissues and cells are particularly sensitive to perturbations in translation during SMA, and identify ribosome biology as an important, yet largely neglected, factor in motor neuron degeneration.



### 673 Cellular levels of readthrough inducing tRNAs and their consequences in yeast and mammals

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Stop codon readthrough is an important recoding event during protein synthesis. The stop codon (i.e. nonsense codon) is read as a sense codon by a tRNA, translation continues to the next stop codon, resulting in production of specific amounts of C-terminally extended proteins that have specific physiological roles in the cell. Recently, we observed that only a few nearcognate tRNAs have the capacity to decode the stop codon tetranucleotide and started to call them readthrough inducing tRNA (rti-tRNA). We have also noted that a basal readthrough is highly dependent on the cellular levels of rti-tRNAs suggesting that the amounts of C-terminally extended proteins might vary a strain to strain or a cell type to cell type. Because nonsense mutations are a primary cause of many cancers and inherited genetic diseases, many labs are engaged in designing and producing small drugs that will help a ribosome to bypass the premature termination codon (PTC). However, encouraging results have been obtained only in a few cases, suggesting that the success of this effort still requires identification and a better understanding of all factors that are involved in this process. Here, we will demonstrate that cellular levels of readthrough inducing tRNAs not only contribute to the cell type diversification, but also play a critical role in an inducibility of stop codon readthrough by small molecule drugs. Our data bring a new insight into the mechanism of translation termination and recoding, and may open up novel therapeutic avenues for the PTC-derived diseases.

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### 674 Structure of Ty1 internally initiated RNA influences restriction factor expression

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The long-terminal repeat retrotransposon Ty1 is the most abundant mobile genetic element in many *S. cerevisiae* isolates. Ty1 retrotransposons contribute to the genetic diversity of host cells, but they can also act as an insertional mutagen and cause genetic instability. Interestingly, retrotransposition occurs at a low level despite a high level of Ty1 RNA, even though *S. cerevisiae* lacks intrinsic defense mechanisms other eukaryotes use to prevent transposon movement. p22 is a recently discovered Ty1 protein that inhibits retrotransposition in a dose-dependent manner. p22 is a truncated form of Gag encoded by internally initiated Ty1i RNA that contains two closely-spaced AUG codons.

We showed that two p22 initiation codons on Ty1i RNA are embedded in structural domain I, which is formed by an interaction between the 5'UTR and the coding sequence. *In vitro* translation experiments revealed that both p22 initiation codons can be utilized but that AUG1 is used preferentially. We demonstrated that the structural integrity of Ty1i RNA is critical for efficient expression of p22 from AUG1. Even small changes in sequence of domain I disrupt RNA secondary and tertiary structure resulting in strong inhibition of p22 synthesis. Our studies have mapped two high affinity Ty1 Gag binding sites in Ty1i RNA. Deletion of one of the binding sites leads to a decrease in p22 level *in vivo* by destabilizing Ty1i RNA. Our work supports the hypothesis that structural motifs of domain I are not only important for efficient translation of p22 protein but may also contribute to the stability of Ty1i RNA *via* interactions with Gag. Such interactions raise the possibility of an autogenous control loop where Gag positively controls the synthesis of p22, which in turn inhibits Gag function and mediates Ty1 copy number control mechanism.

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## 675 Cryo-EM structure of ABCE1/40S complex reveals new insights on translation in *Trypanosoma cruzi*

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*Trypanosoma cruzi* (*T. cruzi*) is a kinetoplastid parasite responsible for Chagas disease, which affects about 7 million people worldwide, mostly in Latin America. The translational machinery in kinetoplastids is markedly different from that in mammals, which suggests regulatory mechanisms that are kinetoplastid-specific. Noteworthy, the ribosomal RNA (rRNA) comprises expansion segments (ESs) in both large and small subunits (LSU, SSU) that are longer and structured differently than that in other eucaryotes. In addition, the LSU rRNA is cleaved into 6 pieces. Here, we purified native and GMP-PNP stalled 40S complexes from *T. cruzi* and analyzed them by cryo-electron microscopy. The structure of the stalled complex reveals that the ribosome-recycling factor ABCE1 interacts with 40S, but that it adopts a different conformation from that within the recycling complex. Furthermore, our analysis through molecular docking reveals that the binding of ABCE1 to the 40S is fully compatible with the assembly of the pre-initiation complex. Finally, we discover an uncharacterized factor at the platform of the 40S/ABCE1 and native 40S complexes, termed provisionally  $\eta$ F. This factor seems to play an important role on translation regulation in kinetoplastids. Overall, our work offers structural insights on kinetoplastids-specific aspects of translation regulation and highlights a recycling-independent role of ABCE1.

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## 676 Withdrawn

## 677 The novel function of PABP interacting protein 1 (Paip1) in translation initiation

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Translation initiation is a rate-limiting step of translation and one of the major targets for translational control in eukaryotes. Cellular mRNAs have the cap structure at the 5' end and the poly(A) tail at the 3' end. The cap binding protein, eIF4E, the RNA helicase, eIF4A, and the scaffolding protein, eIF4G, form the cap-binding eIF4F complex, which associates with the cap structure, leading to translation initiation by facilitating recruitment of the 40S ribosomal subunit to mRNAs. Poly(A) binding protein (PABP) interacts with the poly(A) tail and plays the essential roles in both translational control and mRNA stability. The interaction between PABP and eIF4G results in circularization of the mRNA to facilitate translation. PABP interacting protein 1 (Paip1) has been found as a positive regulator in translation by supporting the formation of circularized mRNA. However, the molecular mechanism underlying translational regulation caused by Paip1 is still obscure.

In this study, we explored the influence of Paip1 on the activity of translation utilizing the cell free translation system that reconstitutes the synergism between the 5' cap structure and the 3' poly(A) tail. Reporter mRNAs are incubated in micrococcal nuclease-treated cell extracts that are supplemented with Paip1 or GFP. Surprisingly, we observed significant translation repression by Paip1 in comparison to the negative control GFP when translation occurs on transcripts that contain both a physiological m<sup>7</sup>G cap structure and a poly(A) tail. Our results strongly indicate that Paip1 acts as a negative regulator for translation initiation at least *in vitro*. To confirm the role of Paip1 as a negative translational regulator, we checked the Paip1 effect on translation with purified recombinant Paip1 and several mammalian Paip1 KO cell line. Moreover, we identified a functional domain of Paip1 for translation repression. Here, we present the novel molecular function of Paip1 as a negative regulator in translation initiation.

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## 678 tFRAP: A FRAP-based technique to monitor protein translation in living cells

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Traditionally, studies on protein translation rely on systems, in which cells have been lysed prior determination of levels of the protein of interest. However, these assays do not reflect the protein synthesis in living cells in real time, but analyze protein levels after a given incubation time, leading to limitations in results based on experimental parameters. To overcome this problem, we have previously established a novel Fluorescence recovery after photobleaching (FRAP)-based technique to monitor protein translation in living cells. For this, the protein of interest fused to green fluorescent protein (GFP) is expressed in cell lines. After bleaching the entire cell, the fluorescent signal of the protein of interest is lost, allowing to capture the signal recovery of newly translated GFP-tagged protein over time. Here we present an improved version of this technique using two fluorescent dyes: tFRAP (translational FRAP). For tFRAP we have inserted a second fluorescent dye, red fluorescent protein (RFP), into the same expression vector, which is used to correct for different transfection rates of individual cells. This advanced technique is a new powerful tool for quantifying translation rates in living cells and will be useful in future studies on mRNA translation.

### 679 **The *Schizosaccharomyces pombe* Ppr10-Mpa1 complex plays a general role in mitochondrial protein translation**

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The pentatricopeptide repeat (PPR) proteins characterized by tandem repeats of a degenerate 35-amino-acid motif function in all aspects of organellar RNA metabolism, many of which are essential for organellar gene expression. In this study, we report the characterization of a fission yeast *Schizosaccharomyces pombe* PPR protein, Ppr10, and a novel Ppr10-associated protein, designated Mpa1. The ppr10 deletion mutant exhibits growth defects in respiratory media. Deletion of ppr10 perturbs iron homeostasis and leads to apoptotic cell death. Deletion of ppr10 also affects the accumulation of specific mitochondrial mRNAs and severely impairs mitochondrial protein synthesis, suggesting a general role for Ppr10 in mitochondrial gene expression. Ppr10 interacts with Mpa1 in vivo and in vitro and the two proteins colocalize in the mitochondrial matrix. The ppr10 and mpa1 deletion mutants exhibit very similar phenotypes. One of Mpa1's functions is to maintain the normal protein level of Ppr10 protein by protecting it from degradation by the mitochondrial matrix protease Lon1. Our findings suggest that the Ppr10-Mpa1 complex functions as a general mitochondrial translational activator, likely through interaction with mitochondrial mRNAs and mitochondrial translation initiation factor Mti2.

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### 680 **hnRNP Q1 translationally increases the expression of Aurora-A and promotes tumorigenesis in colorectal cancer**

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Heterogeneous nuclear ribonucleoprotein (hnRNP) Q1, a RNA binding protein, has been implicated in many post-transcriptional regulatory processes including mRNA splicing, RNA metabolism and translation. Our previous study has demonstrated that Aurora-A can be translational up-regulated in colorectal cancer. By biotin pull-down assay and in vivo translational assay, we found that Aurora-A mRNA can be translational up-regulated by hnRNP Q1. In this study, we further clarified the regulatory mechanism of hnRNP Q1 in translational regulating Aurora-A mRNA, as well as investigated the clinical role of hnRNP Q1 in colorectal carcinogenesis. Our results indicated that hnRNP Q1 can bind to the Aurora-A mRNA 5'-untranslated region (5'-UTR). Ribosomal protein S6-IP assay further indicated that hnRNP Q1 can enhance the translational efficacy of Aurora-A mRNA. Ribosomal profiling assay further confirmed the translational regulation of Aurora-A mRNA by hnRNP Q1. Overexpression of hnRNP Q1 promotes cell proliferation and tumor growth. The expression level of hnRNP Q1 is positively correlated with Aurora-A in colorectal cancer. Taken together, our data indicate hnRNP Q1 is a novel trans-acting factor that binds to Aurora-A mRNA 5'-UTRs and regulates its translation, which increases cell proliferation and contributes to tumorigenesis in colorectal cancer.



**681 Processing bodies regulate selective translation in photomorphogenic Arabidopsis**Geng-Jen Jang<sup>1,2</sup>, Shu-Hsing Wu<sup>1</sup><sup>1</sup>Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan; <sup>2</sup>Institute of Plant Biology, National Taiwan University, Taipei, Taiwan

Light is a vital environmental signal for most of mammals and plants. Seeds of many land plants are buried in soil and need to protrude from the soil to reach light after germination. The transition process for plants from dark to light is named photomorphogenesis or de-etiolation. Transcriptome and translome analyses revealed that light can enhance both the transcription and translation in de-etiolated Arabidopsis seedlings. Despite thousands of genes showed light-triggered translational enhancement, their corresponding mRNAs have no significant changes in abundance before and after light treatment. This implies a selective translation regulation in photomorphogenesis. Processing bodies (p-bodies), the cytoplasmic granules that function in the storage, degradation and translational repression of mRNAs. However, the functions of p-bodies in translation control remain largely obscure in plants. We provide the evidence that p-bodies contribute to the selective translation by sequestering specific mRNAs under dark. First, dynamic monitoring of p-bodies revealed that light triggers the reduction of p-bodies accumulated in etiolated seedlings. Second, through transcriptome and translome comparisons, we found increased translation for thousands of genes in dark-grown p-bodies mutant *Decapping 5 (dcp5-1)*, including genes known to regulate the transition from dark to light. Finally, *dcp5-1* is hypersensitive to light, indicating p-bodies attenuate photomorphogenesis. Taken together, our study indicates that, upon the seedlings protruding from soil, light triggers the dis-assembly of p-bodies to release specific mRNAs for active translation in generating proteins needed for photomorphogenesis.

**682 The human CCHC-type Zinc Finger Nucleic Acid Binding Protein binds G-rich elements in target mRNA coding sequences and promotes translation**Daniel Benhalevy<sup>1</sup>, Sanjay Gupta<sup>2</sup>, Charles Danan<sup>1</sup>, Suman Ghosal<sup>1</sup>, Hong-Wei Sun<sup>3</sup>, Hinke Kazemier<sup>4</sup>, Katrin Paeschke<sup>4</sup>, Markus Hafner<sup>1</sup>, Stefan Juranek<sup>2,4</sup>

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The CCHC-type Zinc Finger Nucleic Acid Binding Protein (CNBP/ZNF9) is conserved in eukaryotes and essential for embryonic development in mammals. It has been implicated in transcriptional as well as post-transcriptional gene regulation; however, its nucleic acid ligands and molecular function remain elusive. Here, we use multiple systems-wide approaches to identify CNBP targets and document the consequences of CNBP binding. We established CNBP as a cytoplasmic RNA-binding-protein and used Photoactivatable Ribonucleoside Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP) to identify 8420 CNBP binding sites on 4178 mRNAs. CNBP preferentially binds G-rich elements in the target mRNA coding sequences, the majority of which were previously found to form G-quadruplex and other stable structures *in vitro*. Functional analyses, including RNA sequencing, ribosome profiling, and quantitative mass spectrometry, revealed that CNBP binding does not influence target mRNA abundance but rather increases their translational efficiency. CNBP constitutes the first example of a sequence-specific RNA-binding protein that promotes translation of its mRNA targets.

### 683 Ribosomal dysfunction and defective erythropoiesis in a zebrafish model of Diamond-Blackfan anemia

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Mutations in genes involved in ribosome biogenesis have been identified in patients with specific disease conditions, called ribosomopathies. Diamond-Blackfan anemia (DBA) is one of such disorders, characterized by hypoplastic anemia with reduced erythroid progenitors in bone marrow and associated physical deformities. To date, heterozygous mutations in 17 ribosomal protein (RP) genes have been identified in ~60% of the DBA patients. However, it remains unknown how mutations in such ubiquitous genes specifically affect erythropoiesis.

To investigate the molecular pathogenesis of DBA, we developed a zebrafish model of DBA by knocking down the zebrafish ortholog (*rps19*) of the human *RPS19*, which is the most frequently mutated gene in the patients. The knockdown embryos displayed a drastic reduction of red blood cells, whereas differentiation of other myeloid and endothelial cells seemed to be normal. The anemia phenotype was almost completely rescued by injection of wild-type *rps19* mRNAs, but not by mRNAs with patient-type mutations. To evaluate the impact of RP deficiency on translation, we carried out RNA-seq analysis of the polysomal mRNAs from *Rps19*-deficient and control embryos, and deduced the changes of translational rate of respective mRNAs. We found that the translational efficiency of 75 genes decreased more than 50%, whereas that of 182 genes increased more than twice from the control. Interestingly, 9 genes out of the top 40 down-regulated genes were found to associate for erythropoiesis. One of these genes expresses in ICM, which is the region for embryonic erythroid production. Injection of the mRNA of this gene into the *Rps19*-deficient embryos partially rescued the anemia phenotype. These data should provide an important clue to the pathogenesis of DBA.

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### 684 Structural determination of the human RISC-Loading Complex

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In organisms from all kingdoms of life, a tight regulation of translation is required for adaptation to ambient conditions and to respond to various signaling cues. In all metazoan species, a prominent way of regulating translation is by means of microRNA-mediated translational repression of a messenger-RNA. MicroRNAs are processed from a pre-form to the mature microRNA by action of the RISC-Loading complex (RLC). In humans, this complex is a heterotrimeric complex of the proteins Dicer, Argonaute2 and TRBP. Dicer is the responsible RNaseIII enzyme processing the pre-microRNA to a duplex microRNA of which one strand is loaded into Argonaute2. The microRNA-loaded Argonaute2 protein is capable of binding to mRNAs through complementarity between the microRNA and sites within the mRNAs. Such a binding event entails translational repression or mRNA degradation, both affecting the process of translation. Structural information regarding the RLC is limited to low resolution models from negative stain EM studies, but higher resolution structures are needed in order to fully understand the processing of the pre-microRNA into mature microRNA. Especially the process of strand selection with Argonaute2 loading and the role of TRBP in this regard are very interesting for understanding the microRNA pathway. My Ph.D.-project is concerned with obtaining a structural model of the human RLC by means of cryo-EM. Results on purification and preliminary results from EM experiments will be presented.

## 685 p53 mutations influence IRES-dependent expression of p53 isoforms

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Full-length p53 (FLp53) is a tumour suppressor protein that has been considered a master regulator of many cellular functions. Several isoforms have been described so far for p53 and some of the functions of shorter p53 isoforms have been elucidated and they are different from and complement FLp53 activity. p53 is the most commonly mutated gene in cancer and depending on its mutation status p53 may act as a tumour suppressor or a proto-oncogene. Recently, we have shown that the most common p53 cancer mutants express a larger number and higher levels of shorter p53 protein isoforms that are translated from the mutated FLp53 mRNA (Candeias *et al.* EMBO R., 2016). Also, we found that cells expressing these shorter p53 isoforms exhibit mutant p53 "gain-of-function" cancer phenotypes, such as enhanced cell survival, proliferation, invasion and adhesion, altered mammary tissue architecture and invasive cell structures. Here, we found that some of these mutations affect the function of an Internal Ribosome Entry Site (IRES) in p53 mRNA. Using bicistronic constructs, luciferase assays and FACS, we investigated which mutations influence IRES-dependent translation of shorter p53 isoforms and to what extent this may lead to the onset or progression of some types of tumours.

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## 686 T-cell-restricted intracellular antigen 1 facilitates mitochondrial fragmentation by enhancing translation of mitochondrial fission factor mRNA

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Mitochondrial morphology is dynamically regulated by the formation of small fragmented units or interconnected mitochondrial networks, and this dynamic morphological change is a pivotal process in normal mitochondrial function. In the present study, we identified a novel regulator responsible for the regulation of mitochondrial dynamics. An assay using CHANG liver cells stably expressing mitochondrial-targeted yellow fluorescent protein (mtYFP) and a group of siRNAs revealed that T-cell intracellular antigen protein-1 (TIA-1) affects mitochondrial morphology by enhancing mitochondrial fission. The function of TIA-1 in mitochondrial dynamics was investigated through various biological approaches and expression analysis in human specimen. Downregulation of TIA-1 enhanced mitochondrial elongation, while ectopic expression of TIA-1 resulted in mitochondria fragmentation. Additionally, TIA-1 increased mitochondrial activity, including the rate of ATP synthesis and oxygen consumption. Further, we identified mitochondrial fission factor (MFF) as a direct target of TIA-1, and showed that TIA-1 promotes mitochondrial fragmentation by enhancing MFF translation. TIA-1 null cells had a decreased level of MFF and less mitochondrial Drp1, a critical factor for mitochondrial fragmentation, thereby enhancing mitochondrial elongation. Taken together, our results indicate that TIA-1 is a novel factor that facilitates mitochondrial dynamics by enhancing MFF expression and contributes to mitochondrial dysfunction.

## 687 Conserved non-AUG uORFs revealed by a novel regression analysis of ribosome profiling data

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Upstream open reading frames (uORFs), located in transcript leaders (5'UTRs), are potent *cis*-acting regulators of translation and mRNA turnover. Recent genome-wide ribosome profiling studies suggest that many uORFs initiate with non-AUG start codons. While intriguing, these non-AUG uORF predictions have been made without statistical control or validation, thus the importance and identity of these elements remains to be demonstrated. To address this, we took a comparative genomics approach to study AUG and non-AUG uORFs. We mapped transcription leaders in multiple *Saccharomyces* yeast species and applied a novel machine learning algorithm (uORF-seqr) to ribosome profiling data to identify and validate statistically significant uORFs. We found that AUG and non-AUG uORFs are equally frequent in *Saccharomyces* yeasts. Hundreds of predicted non-AUG uORFs are conserved within *Saccharomyces*, and are implicated in regulating cell wall regulation and biogenesis. uORFs initiating with UUG are particularly common, and are conserved at rates similar to that of AUG uORFs. These results suggest that non-AUG uORFs play critical roles in regulating gene expression, and may be particularly important in regulating cell growth during stress.

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## 688 Widespread presence of internal translation initiation sites in *Escherichia coli*

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Bacteria can diversify their proteome by extending the genetic code using non-canonical paths during protein synthesis such as programmed frameshifting, stop codon readthrough, translational bypassing or codon redefinition. Another clearly existing but widely unexplored way to expand the genetic code is internal initiation of translation. Although internal initiation events have been reported in few genes of *E. coli* such as *infB*, *clpB* and *cheA*, the functions of internal translation products have been unclear. Despite the presence of Shine-Dalgarno-like sequences in the vicinity of potential start codons within the coding regions of some other genes, their ability to start internal initiation remains mostly undiscovered. Therefore, to systematically search for the internal initiation sites in vivo, we pursued to conduct ribosome profiling experiment in *E. coli*. To efficiently capture the ribosomes at internal start sites, we performed ribosome profiling in the presence of the antibiotic retapamulin. Retapamulin has been shown in vitro to inhibit translation initiation by competing with initiator tRNA binding. Our ribosome profiling data revealed that retapamulin indeed causes a strong arrest on initiating ribosomes, locations of which map to the canonical start sites of actively expressed genes. Strikingly, we also observed retapamulin-arrested ribosomes at sites within the coding regions of a small subset of genes. Computational analysis of these arrest sites revealed that they are preceded by Shine-Dalgarno-like sequences and therefore constitute putative internal initiation regions, either in frame or out-of-frame relative to the primary start codon of those genes. We are currently analyzing the possible functions of these alternative products and their contributions to the cell fitness. Our findings using antibiotic retapamulin as a precise tool to locate alternative initiation sites might suggest that internal initiation is an unorthodox way to enrich bacterial proteome by generating alternative proteins.



## 689 Vga(A)LC from *Staphylococcus haemolyticus* directly protects ribosome against antibiotics in an ATP-dependent manner

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Protein biosynthesis - translation - is one of the core cellular processes, reading and deciphering information coded in genes to produce proteins. Being essential for bacteria, translation is targeted by approximately half of the antibiotics used in clinical practice. Multiple ATPases belonging to ABCF superfamily protect bacterial from antibiotics either acting as molecular pumps excreting antibiotics or by interacting with the ribosome directly, such as staphylococcal ABCF Vga(A) (Sharkey et al., 2016).

We have applied biochemical assays to investigate the molecular mechanism of action of *Staphylococcus haemolyticus* Vga(A)LC (Novotna and Janata, 2006). The enzyme protects ribosomal transpeptidation from antibiotic lincomycin in an ATP-dependent manner. Both non-hydrolysable ATP analogue ADPNP or ATP added to the ATPase-deficient EQ2 mutant fail to support the protective activity, suggesting that the ATP hydrolysis, rather than ATP binding per se is necessary for the activity. Moreover, EQ2 mutant inhibits transpeptidation in a strictly ATP-dependent manner, suggesting a model of Vga(A)LC re-setting the transpeptidation activity of the ribosome via direct interaction with the peptidyltransferase center.

### References:

Sharkey L.K., Edwards T.A. and O'Neill A.J. (2016) ABC-F Proteins Mediate Antibiotic Resistance through Ribosomal Protection. *MBio* 7(2):e01975

Novotna G. and Janata J. (2006) A new evolutionary variant of the streptogramin A resistance protein, Vga(A) LC, from *Staphylococcus haemolyticus* with shifted substrate specificity towards lincosamides. *Antimicrobial Agents and Chemotherapy* 50(12):4070-6

## 690 Dicer-2 is a cytoplasmic polyadenylation factor

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Cytoplasmic polyadenylation is a widespread mechanism of translation control involved in germ cell and early development, among other processes. Canonical CPE (Cytoplasmic Polyadenylation Element)-mediated mechanisms of cytoplasmic polyadenylation have been thoroughly studied in vertebrate models. Two sequence elements in the 3' UTR of substrate mRNAs, the U-rich CPE and the AAUAAA polyadenylation hexanucleotide (Hex), mediate this mechanism. CPE and Hex elements are also found in the 3' UTRs of *Drosophila* mRNAs, but have not been functionally characterized.

Our group has identified sequences distinct from the CPE and Hex that drive polyadenylation of *Toll* mRNA in early *Drosophila* embryos<sup>1</sup>. Using these sequences in RNA affinity chromatography experiments, we have identified the siRNA processing factor Dicer-2 as a potential cytoplasmic polyadenylation factor. Indeed, depletion and co-immunoprecipitation experiments indicate that Dicer-2 interacts with the cytoplasmic poly(A) polymerase Wispy and is necessary for polyadenylation and translation of at least two cytoplasmic polyadenylation substrates. These results uncover a novel function of Dicer-2 in activation of mRNA translation through cytoplasmic polyadenylation.

To further our studies, we are currently mapping the Dicer-2 interactome in early embryos. Interestingly, the majority of Dicer-2 interactors are not siRNA-associated factors. Sixty percent of the interactors bind to Dicer-2 in an RNA-independent fashion, which further strengthens the role of Dicer-2 in RNA regulatory mechanisms beyond siRNA processing. We are also identifying the mRNA targets of Dicer-2 by RIP-Seq and PAR-Clip. We will present our efforts to dissect the functions of Dicer-2 outside the siRNA machinery.

(1) Coll et al. A novel, non-canonical mechanism of cytoplasmic polyadenylation operates in *Drosophila* embryogenesis. *Genes Dev.* 24: 129-134 (2010).

## 691 eRF1 translation termination factor is controlled by an autoregulatory circuit involving readthrough and nonsense-mediated decay in plants

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Eukaryotic Release Factor 1 (eRF1) is the key component of translation termination, it recognizes the stop codon and promotes translation termination by stimulating peptide release and ribosome disassembly. eRF1 concentration has to be strictly controlled. Low eRF1 protein level reduces the efficiency of termination, thereby increasing the frequency of both frameshift and readthrough (RT), while eRF1 overexpression might cause premature termination at coding codons. It is well known for decades that in prokaryotes, RF2 expression is stabilized by a negative autoregulatory circuit, however the regulation of eukaryotic eRF1 remains to be unraveled.

By combining various transient assays and transgenic approaches, we show that in plants, eRF1 protein level is regulated by a complex autoregulatory circuit, in which both translational readthrough and Nonsense-mediated decay (NMD), a translation termination coupled mRNA degradation system, play important role. The regulatory circuit is based on the special 3'UTR structure of eRF1-1 mRNAs that can sense eRF1 protein level. Notably, the stop codon of eRF1-1 mRNA is in a readthrough promoting context, while its 3'UTR induces NMD. We demonstrate that readthrough partially protects the eRF1-1 mRNA from NMD. We also found that enhanced eRF1 level reduces readthrough frequency in plants. Thus elevated eRF1 protein level inhibits readthrough on the eRF1-1 mRNA, which in turn stimulates NMD, thereby reducing eRF1-1 transcript levels. Similarly, low eRF1 protein level leads to increased eRF1-1 mRNA expression, as more frequent readthrough can more efficiently protect the eRF1-1 mRNA from NMD.

This specific autoregulatory circuit of eRF1 is evolutionary conserved among all seed plants. We propose that this eRF1 autoregulatory mechanism controls fluctuations in eRF1 protein expression thus it finely balances general translation termination efficiency.

## 692 Functional relation of hnRNP K - rpS19 crosstalk in erythroid cell maturation

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In erythropoiesis post-transcriptional regulation is crucial for the structural and functional re-organization in circulating enucleated reticulocytes to safeguard their terminal maturation to erythrocytes. Timely mitochondria degradation is initiated by the newly synthesized reticulocyte 15-lipoxygenase (r15-LOX). R15-LOX mRNA translation is under the control of hnRNP K that constitutes a silencing complex at the 3'UTR differentiation control element (DICE), in concert with hnRNP E1 and DDX6<sup>1,3</sup>. To investigate how DICE-associated hnRNP K restrains r15-LOX mRNA translation initiation we employed three independent interaction screens to identify proteins, which facilitate hnRNP K interference with 80S ribosome formation. These assays combined RNA chromatography from translational active K562 cell extracts with hnRNP K-immunoprecipitation<sup>3</sup>. Furthermore, we enriched hnRNP K interacting proteins from cytoplasmic extracts of non-induced K562 cells and in induced erythroid maturation<sup>2</sup>. Based on the differential hnRNP K arginine dimethylation in erythroid maturation we performed protein affinity purification from RNase A-treated K562 cell extracts employing recombinant asymmetrically dimethylated or non-methylated hnRNP K<sup>4,5</sup>. Besides hnRNP K, hnRNP E1 and DDX6, all purification strategies identified rpS19 that is localized at the 40S ribosomal subunit surface and extends into its functional center<sup>6</sup>. RpS19 mutations have been functionally linked to impaired erythropoiesis in Diamond Blackfan Anemia (DBA)<sup>7</sup>. Interestingly, in K562 cells the interaction between hnRNP K and rpS19 declines during erythroid maturation. To address its potential functional relevance, we evaluated the rpS19-hnRNP K interaction *in vitro* and *in vivo*. We identified arginine residues in interaction-related hnRNP K domains, which are differentially dimethylated in the course of erythroid maturation and analyzed their impact on rpS19 binding. Additionally we applied thermal shift assays to characterize DBA-related rpS19 variants for their influence on hnRNP K binding. To analyze a functional relation of rpS19 DBA variants to erythropoiesis and hnRNP K-mediated r15-LOX mRNA silencing, we generated inducible K562 cell lines, which enable shRNA-dependent regulation of rpS19 expression.

<sup>1</sup>Ostareck, D., et al., (1997) Cell; <sup>2</sup>Naarmann, I.S. et al., (2008) J. Biol. Chem.; <sup>3</sup>Naarmann, I.S., et al., (2010) RNA;

<sup>4</sup>Ostareck-Lederer, A. et al., (2006) J. Biol. Chem.; <sup>5</sup>Moritz, B. et al., (2014) Biol. Chem.; <sup>6</sup>Anger, A.M., et al., (2013) Nature;

<sup>7</sup>Draptchinskaya et al., (1999) Nat. Gen.

### **693 Structure of modified mRNAs modulates protein expression**

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The efficacy of mRNA therapeutics critically depends on the ability of an exogenously-introduced mRNA to simultaneously evade the innate immune system while efficiently engaging the translation machinery. Immune system evasion can be accomplished by incorporating chemically modified nucleotides, but the impact of these modified nucleotides on translation efficiency has not been fully explored. To determine how primary mRNA sequence and nucleotide chemistry combine to affect functional protein expression, we performed extensive functional characterization on a set of synonymous mRNA sequences containing different nucleotide modifications. Importantly, by altering the chemical modification pattern, we can substantially alter secondary structure and global thermodynamic stability without changing sequence. Contrary to expectation, we find that more structured ORFs lead to greater protein output. This increased protein output is primarily due to increased translation efficiency, not increased mRNA half-life. Thus secondary structure (determined jointly by primary sequence and nucleotide chemistry) confers an advantage for translation, but not stability, of therapeutic mRNAs.

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### **694 Regulatory mechanisms modulating the function of eukaryotic translation repressors**

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Control of translation plays a key role in various cellular processes. Translation repressors are an important class of proteins that regulate translation largely at the initiation step. These proteins upon inhibiting translation lead to accumulation of repressed mRNPs in RNA granules. RGG-motif containing translation repressors have recently emerged as key regulators of mRNA fate in cytoplasm. Unraveling the regulatory mechanisms that influence activity of these repressors would provide insight into mRNA movements in and out of translation. Our group's first publication recently reported that arginine methylation of one such repressor protein Scd6, promotes its repression activity (Poornima et al., 2016, Nucleic Acids Research). We observe that arginine methylation of Scd6 promotes its binding to eIF4G1. We have been exploring other modes of regulation of translation repressor proteins. Latest unpublished results arguing for an alternative and exciting mode of regulation of RGG-motif proteins will be presented. Using a combination of genetic, biochemical and imaging approaches we demonstrate that this regulatory mechanism could be a common feature of RGG-motif containing translation repressors.

## 695 Probing a translational switch involved in tumor angiogenesis

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As a tumor proliferates, it rapidly outgrows its blood supply, creating an oxygen-starved environment. Low oxygenation (hypoxia) suppresses the canonical biochemical pathway through which the translation of messenger RNAs (mRNAs) into proteins by the ribosome is typically initiated. Despite shutting down this general mechanism for initiating protein synthesis, the tumor can continue to initiate translation of a subset of mRNAs that are involved in angiogenesis and tumor survival. To explain this observation, it has been proposed that, in addition to the eukaryotic mRNA-specific 7-methyl guanosine cap at the 5' end, these pro-angiogenic mRNAs, of which Fibroblast Growth Factor-9 (FGF9) and Hypoxia Inducible Factor 1 $\alpha$  (HIF1 $\alpha$ ) are prototypical members, contain highly stable structures in their 5' untranslated regions. These structures, also referred to as Cap Independent Translation Enhancers (CITEs), form part of a switch that activates a non-canonical pathway for initiating translation, presumably by binding directly to eukaryotic initiation factor 4G (eIF4G), thereby recruiting ribosomes in a manner distinct from the canonical 5' cap-dependent mechanism. To test this hypothesis, here we report the binding affinities of pro-angiogenic mRNAs, as well as control mRNAs lacking CITEs, to eIF4G as a measure of their ability to initiate translation in the hypoxic environment of a tumor. Understanding the dynamics of eIF4G-CITE interactions can inform the design and development of novel angiogenesis inhibitors that function by targeting these interactions.

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## 696 Coherent mRNA Assemblies of Ion Channel Transcripts Underlying the Cardiac Action Potential

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How excitable cells regulate the precise balance of different ion channels mediating electrical signaling is poorly understood. In the heart, perturbation of this balance can lead to dangerous arrhythmias and sudden cardiac death. We previously reported that alternate transcripts of the *hERG* (*KCNH2*) gene, which encode two subunits of repolarizing  $I_{Kr}$  channels, are physically associated. The proximity of the transcripts promotes cotranslational assembly and favors the proper subunit constituency required for normal cardiac repolarization. We now report that the *hERG* transcripts also associate with transcripts encoding other ion channels whose coordinate expression underlies the cardiac ventricular action potential. Using an antibody specifically directed against the nascent hERG 1a subunit, we co-purified *hERG 1a*, *hERG 1b*, *SCN5A*, *CACNA1C* and *KCNQ1* but not *KCNE1*, *RYR2* or *KCNJ2* mRNA transcripts from human cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs) and from human heart tissue. Using fluorescent *in situ* hybridization (FISH), we have so far observed co-localization of single *hERG* and *SCN5A* transcripts in iPSC-CMs. The same transcripts shown to interact with *hERG* transcripts exhibited a co-knockdown effect in RT-qPCR assays when either *hERG 1a* or *hERG 1b* was silenced, while those that did not interact were unaffected. Whole-cell patch-clamp recordings performed in iPSC-CMs revealed that *hERG 1b*-specific silencing reduced the magnitude of corresponding ion currents involved in the ventricular action potential including  $I_{Kr}$ ,  $I_{Na}$  and  $I_{Ca}$ , but not  $I_{K1}$ , as predicted from the RT-qPCR results. This coherent assembly of transcripts, or *microtranslatome*, represents a novel mechanism coordinating the activities of a subset of channel types underlying the ventricular action potential.



## 697 Translation of human mTOR can be mediated by a cap-independent mechanism to insure its expression and function in conditions of global translation inhibition

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The 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. Major advances are emerging regarding the regulators and effects of mTOR signaling pathway; however, regulation of mTOR gene expression is not well known. Here, we show that the 5' untranslated region of the human mTOR transcript forms a highly folded RNA scaffold capable of binding directly to the 40S ribosomal subunit. We further demonstrate that this cis-acting RNA regulon is active both in normal and stress conditions, and that its activation status in response to translational adverse conditions parallels mTOR protein levels. Moreover, our data reveal that the cap-independent translation of mTOR is necessary for its ability to induce cell cycle progression into S-phase. These results suggest a novel regulatory mechanism of mTOR gene expression that integrates the protein profile rearrangement triggered by global translation inhibitory conditions.

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## 698 The mTOR pathway regulates expression of synaptic cell adhesion proteins in neurons

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Local mRNA translation in neuronal dendrites is a key mechanism behind long-term memory formation. The serine/threonine protein kinase mechanistic target of rapamycin (mTOR) is a central regulator of such mRNA translation. It activates local translation of mRNAs encoding proteins required for changes in synaptic efficacy thereby modifying synaptic connections and enabling memory formation.

Although it has been known for some time that mTOR regulates local mRNA translation in dendrites only few targets have been identified and specific mechanisms remain largely unexplored. Identifying these targets and mechanisms will be important to understand neuronal function as well as the pathogenesis of neuropsychiatric disorders associated with changes in mTOR activity.

Our studies suggest that mTOR activation upregulates expression of the synaptic cell adhesion proteins N-cadherin (Ncad) and  $\beta$ -catenin. We could show that an inhibition of the mTOR signalling pathway leads to a downregulation of Ncad mRNA and protein in primary cortical neurons. The Ncad transcript contains a highly conserved 3'untranslated region (3'UTR). Upon mTOR inhibition we observed decreased reporter activity when the coding sequence of the luciferase reporter was fused to the 3'UTR of the Ncad transcript suggesting that mTOR regulates Ncad mRNA stability or translation. Currently, we are screening the 3'UTR of the Ncad transcript for *cis* acting sequence motifs that interact with microRNAs or RNA binding proteins and that may be involved in the mTOR-mediated regulation of Ncad expression. Furthermore, we are studying whether mTOR regulates Ncad expression in response to neuronal activation locally at the synapse.

Altogether, our results suggest that mTOR controls synaptic cell adhesion by regulating Ncad and  $\beta$ -catenin expression. As dynamic changes in synaptic cell adhesion are required for changes in synaptic efficacy we expect this to be an important mechanism during long-term memory formation. Its dysregulation may contribute to the pathogenesis of neuropsychiatric disorders typically associated with changes in mTOR activity (e.g. autism and ADHD).

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## 700 Regulation of the selenoproteins by microRNAs

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Selenoproteins are a diverse group of proteins whose functions depend on the trace element selenium. Twenty-five human and twenty-four mouse selenoproteins, containing selenium as selenocysteine, have been identified. Most of them are involved in oxidative stress protection, maintaining cellular redox balance, thyroid metabolism, male reproduction, and many other aspects of human health.

Selenoprotein expression is regulated via unique translational mechanism and recoding of the UGA from stop codon into selenocysteine (Sec), methylation of the Ser/Sec tRNA, and selenium status. Therefore, efforts in the selenium field were focused on understanding the mechanisms of Sec biosynthesis and incorporation while the regulation by microRNAs is underrepresented.

We are testing the hypothesis that miRNAs regulate selenoproteins and may contribute to their spatiotemporal expression. Our research covers computational analysis of more than 49 genes (all selenoproteins in human and mouse) for regulation by miRNAs and identification of co-regulatory patterns. Our in-silico analysis indicates that at least three miRNAs are predicted to regulate subsets of 4 selenoprotein genes, four miRNAs predicted to target the subsets of 3 selenoproteins, and eight miRNAs appear in subsets of 2 selenoprotein genes, indicating potential co-regulation. To achieve comprehensive analysis and predict miRNA regulations for selenoproteins, we are expanding our in-silico analysis by using three additional platforms for miRNA target identification.

We are currently experimentally validating the predicted miRNA/gene interactions for two selenoprotein genes (SelH and SepW1) by miRNAs using cell culture model, luciferase reporter assays, quantitative RT-PCR and competition assays. We aim to describe the mechanism of regulation of selenoproteins by micro RNAs targeting 3'UTR of the gene.

## 701 HnRNPM Functions as an IRES-binding Protein to Selectively Activate Hypoxia-induced Translation for Colon Cancer Tumorigenesis

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The key regulators involved in IRES-mediated translational reprogramming in the cancer cells remain largely unknown. This study aims to elucidate the missing piece of mechanism underlying the hypoxia-induced, IRES-mediated translational activation. We identified hnRNPM as a novel IRES interacting factor to promote translation in hypoxia. Transcriptomic- and translatomic-wide analyses revealed a unique set of hnRNPM-targeted mRNAs with increased ribosome occupancy during hypoxia. Clinical samples and animal model showed that elevation of hnRNPM accompanies with the development of colorectal cancer likely due to hypoxic microenvironment created by aberrant cell proliferation. Collectively, these data highlight the critical role of hnRNPM-IRES-mediated translation in transforming cancer cells toward malignancy and demonstrate significant implications for targeting hnRNPM in future anticancer therapy.

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## 702 A Global Functional Survey for Translational Regulators

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Post-transcriptional gene regulation is now recognized as a major checkpoint where the expression of genetic information is controlled to modulate protein production. This regulation involves a highly-integrated network of proteins which controls the translation of messenger RNA (mRNA) to generate a dynamic proteome that meets the changing needs of the cell. This network involves an intricate interplay between cis-acting elements encoded on the mRNA and trans-acting factors which interact with the mRNA: RNA-binding proteins (RBPs). The influence of RBPs can determine whether a given mRNA is translationally activated or repressed, localized to a specific region or compartment within the cell, or degraded. Motivated by the important role RBPs play in post-transcriptional gene regulation, several groups have worked to identify over 1000 human RBPs and their mRNA targets, and the RNA-binding domains (RBDs) of these proteins have been revealed on a proteome-wide scale. However, these studies have focused primarily on identifying RBPs and providing evidence for their physical interactions with RNA without focusing on their regulatory functions. To answer the outstanding question of how RBPs regulate mRNA translation, we have adapted the tethering assay to a broad and powerful survey for regulatory activity on a genome-wide scale. In particular, we hypothesize that RBPs, many of which are currently uncharacterized, play a central role in regulating translation, thus our global functional analysis will reveal missing links and allow a better understanding of translational control.

**703 Translational Control of Invasion in Melanoma - A novel role for LARP6?***Jennifer Thomas, James Chettle, Paola Falletta, Sarah Blagden, Colin Goding***University of Oxford, Oxford, UK**

Malignant cells within solid tumors exist in a hostile microenvironment characterised by stressors such as hypoxia and nutrient deprivation. Under these conditions, canonical 'cap-dependent' mRNA translation is shut down following phosphorylation of the translation initiation factor eIF2 $\alpha$ . Tumour cells employ poorly understood adaptive mechanisms to survive stress, continuing to translate pro-survival proteins by cap-independent mechanisms. In melanoma, translational reprogramming following eIF2B inhibition under conditions of cell stress can in itself enhance tumorigenicity, drive invasion and metastasis and impose a drug-resistant phenotype. EIF2B inhibition also drives invasiveness in yeast cells, suggesting an evolutionarily conserved migratory response to starvation, co-opted by malignant cells to drive metastasis.

An important candidate mediator of cap-independent translation under stress is the RNA binding protein La-related protein 6 (LARP6). A member of the evolutionarily conserved LARP family of RNA binding proteins that bind specific subsets of mRNAs to promote or inhibit their translation, LARP6 was initially identified for its ability to recruit alternative translation machinery to collagen-encoding mRNAs to promote their cap-independent translation during inflammation, wound healing and fibrosis. The demonstration that ectopic expression of LARP6 in cancer cells can drive proliferation, lamellipodia formation and invasiveness, suggests a role beyond collagen synthesis.

We show that in melanoma, LARP6 is up-regulated by the intra-tumoral stresses that push cells to become invasive. Moreover, its expression is closely correlated with both an invasive and drug-resistant gene expression signature. We hypothesise that LARP6 facilitates the translation of pro-invasion mRNAs to drive melanoma invasion and therapy resistance.

**704 Functional sites on Ribosome Modulation Factor (RMF) for 100S ribosome formation***Hideji Yoshida<sup>1</sup>, Hideki Nakayama<sup>2</sup>, Yasushi Maki<sup>1</sup>, Shou Furuike<sup>1</sup>, Masami Ueta<sup>3</sup>, Chieko Wada<sup>3</sup>, Akira Wada<sup>3</sup>***<sup>1</sup>Osaka Medical College, Osaka, Japan; <sup>2</sup>HORIBA Ltd., Kyoto, Japan; <sup>3</sup>Yoshida Biological Laboratory, Kyoto, Japan**

In some gram-negative bacteria such as *Escherichia coli*, protein synthesis is suppressed by the formation of 100S ribosomes under several stress conditions. The 100S ribosome, a dimer of 70S ribosomes, is mainly formed by Ribosome Modulation Factor (RMF) binding to the 70S ribosomes. During the stationary phase, most of the 70S ribosomes turn to 100S ribosomes which have lost translational activity. When the cells in the stationary phase are transferred to rich nutritious culture media, the RMF is immediately released from the 100S ribosome, and the 100S ribosome dissociates back into 70S ribosomes. This process is rapid and is completed within 1 min. After this process, the cells reinitiate protein synthesis and proliferation within 6 min. The *E. coli* mutant strain with *rmf* gene deletion cannot form 100S ribosomes, and the lifetime of this mutant is shorter than that of the wildtype. These phenomena indicate that the interconversion between the 70S and 100S ribosomes is an important strategy for survival under stress conditions. This 100S formation is called 'Hibernation process' in the ribosome cycle of stationary phase.

Here, we report the mutational effects of RMF on the formation of 100S ribosome. The 12 conserved amino acid residues were found in the *rmf* gene by a homology search for 50 bacteria. As the results of single amino acid substitutions and domain deletions, it is elucidated that some amino acid residues are critical for the ribosomal dimerization.



## 705 Temperature-dependent adaptation of tRNA-nucleotidyltransferases

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CCA-adding enzymes are highly specific RNA polymerases that synthesize and maintain the sequence C-C-A at the tRNA 3'-end. For a proper and efficient CCA-addition at high fidelity, the CCA-adding enzymes execute specific and coordinated structural rearrangements in each polymerization step. These domain movements require a certain flexibility of the enzyme body, including the catalytic core. As an increased flexibility also represents a hallmark for the adaptation of proteins to low temperature environments, we investigated the impact of cold adaptation on the reactivity and specificity of CCA-adding enzymes from psychrophilic bacteria. A comparative study of the corresponding enzymes from closely related psychro-, meso-, and thermophilic *Bacillales* indicates that both the N-terminal catalytic core as well as the C-terminal tRNA binding region are equally important for cold adaptation. In addition, the psychrophilic enzymes exhibit a broad temperature tolerance that matches the temperature growth range of the corresponding organisms. Surprisingly, these enzymes show a considerable error rate during CCA synthesis *in vitro* as well as *in vivo*, resulting in additional incorporations of C and A residues. It seems that the activity of psychrophilic CCA-adding enzymes is not only achieved at the expense of structural stability, reaction velocity and substrate affinity, but also results in a reduced polymerization fidelity.

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## 706 tRNAscan-SE 2.0 and GtRNAdb 2.1: Improved resources for analysis of transfer RNA genes

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tRNAscan-SE has been widely used for whole-genome transfer RNA gene prediction for nearly two decades. It was initially designed to detect tRNA genes with domain-specific covariance scoring models (one each for Archaea, Bacteria, and Eukarya), with all tRNA isotypes combined in each model for maximum sensitivity. However, we have found numerous cases where tRNAscan-SE may not classify the tRNA's isotype appropriately when relying on the anticodon prediction. With the increased availability of new genomes, a vastly larger training set has enabled creation of nearly one hundred specialized isotype-specific models, improving the ability to identify and accurately classify both cytosolic and mitochondrial tRNAs.

Using the latest version of the Infernal covariance model search software, we developed a new multi-model annotation strategy for tRNAscan-SE 2.0, where predicted tRNAs are assessed with the full set of isotype-specific covariance models. Comparative analysis among these models enables better annotation, particularly of atypical tRNAs, some of which may produce "recoding" events due to mutations in the anticodon. The new tRNAscan-SE also enables better recognition of tRNA-derived SINEs that are abundant in many eukaryotic genomes. Moreover, new mt-tRNA covariance models integrated with tRNAscan-SE 2.0 provide accurate mitochondrial tRNA identification in vertebrates, including previously unannotated nuclear-encoded mt-tRNAs that often number in the hundreds across nuclear genomes.

To provide access to this more complete, better annotated collection of tRNA genes, we have expanded the Genomic tRNA Database to include both cytosolic tRNA genes and mt-tRNAs in vertebrates. These and other new enhancements of tRNAscan-SE and the Genomic tRNA Database will provide researchers more accurate detection and more comprehensive annotation for all tRNA genes.

**707 The rich spectrum of C/D box snoRNA interactions in human cells***Tatyana Dudnakova, Hywel Dunn-Davies, Rosie Peters, David Tollervey***WTCCB, University of Edinburgh, Edinburgh, UK**

The C/D box small nucleolar RNAs (snoRNAs) are a class of abundant, small stable RNAs, most of which act as guides for site-specific, ribose 2'-O-methylation of target rRNAs. However in human cells there is a large group of "orphan" snoRNAs not shown to interact with rRNA whose targets and functions are unknown. Recent studies implicated changes in the expression of the box C/D class of snoRNAs in several human diseases. Here we report the identification of novel RNA targets for box C/D snoRNAs in human HEK293 cells. We identified hundreds of novel snoRNA interactions with rRNA, including many predicted to block known methylation sites. Interactions were also identified with other snoRNAs and, most intriguingly, mRNAs. In HEK cells snoRNA-mRNA interactions comprised 30% of reproducible snoRNA intermolecular hybrids; 9260 reproducible mRNA hybrids represented 1301 interactions between 144 snoRNAs and 888 mRNAs. Relatively few mRNA interactions are predicted to guide methylation, suggesting a functional role for snoRNA binding to the target RNA. Notably, snoRNAs were preferentially bound in conserved regions of pre-mRNA introns. We hypothesize that mammalian C/D box snoRNAs have acquired novel functions - regulating rRNA methylation efficiency and pre-mRNA processing and/or stability.

**708 Non-coding RNA is required for DNA double-strand break repair***Dominika Hrossova, Christos Coucoravas, Marianne Farnebo***Department of Oncology-Pathology, Cancer Centrum Karolinska (CCK), Karolinska Institutet, Stockholm, Sweden**

Among the most cytotoxic DNA lesions are double-strand breaks (DSBs), which arise upon collapse of the replication fork, during processing of interstrand crosslinks, or through exposure to ionizing radiation (IR), which is widely used to treat cancer. Variable classes of non-coding RNAs, including microRNAs, long noncoding RNAs, double-strand break-induced RNAs, small interfering RNAs and piwi-interacting RNAs, have emerged as potential regulators of DSB repair and genome stability<sup>1</sup>. However, a deeper mechanistic insight into the RNA-dependent DNA repair is still missing. By manipulating a sub-class of small nucleolar (sno)RNAs referred to as small Cajal body-associated (sca)RNAs in cancer cells, we have generated preliminary data supporting a novel view on RNA-mediated DNA repair. The scaRNAs are targeted to Cajal bodies by the WRAP53 $\beta$  protein and are necessary for guiding site-specific modifications of snRNAs. Notably, WRAP53 $\beta$  localizes to the site of DNA breaks and help in DNA repair<sup>2</sup>. We show that knockdown of scaRNAs results in inefficient DNA double-strand break repair, impaired recruitment of DNA repair factors to sites of damage and massive heterochromatinization of the nucleus. Furthermore, we confirmed that they bind specifically to DNA damage repair factors, histone modifiers and modified histones. Importantly, we were able to visualize scaRNA at the site of DNA breaks induced either by laser micro-irradiation or using localized cleavage by the Fok-I enzyme combined with the single molecule RNA FISH. Interestingly, tethering of MS2-tagged scaRNA to the chromatin without damage was sufficient for recruitment of downstream repair factors from the DNA damage-signaling cascade. Thus, we propose the direct involvement of scaRNAs in the DNA damage response most probably by mediating chromatin relaxation enabling recruitment of downstream repair factors.

Reference:

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## 709 Towards structural characterization of the mammalian tRNA ligase complex

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The 3'-5' RNA ligation pathway is a fundamental process involved in tRNA splicing and unfolded protein response in metazoa. The tRNA ligase complex, a ~200 kDa assembly consisting of the ligase HSPC117, the DEAD-box helicase DDX1 and additional subunits FAM98B, CGI-99 and Ashwin, was only recently identified as the enzyme catalyzing this process. Subunits of the complex have additionally been implicated in other cellular functions including nucleocytoplasmic RNA transport, homologous recombination at DNA double-strand breaks and transcription modulation, underscoring the central role of the tRNA ligase complex in RNA metabolism. Our aim is to obtain structural insights into the molecular mechanism of the tRNA ligase complex. To this end, we have succeeded in reconstituting the catalytic core of the complex by coexpression in insect cells and analyzed its inter-subunit interactions by cross-linking and mass spectrometry. We also determined the crystal structure of the N-terminal domain of CGI-99, revealing that it adopts a calponin homology (CH) fold and suggesting that it might mediate actin or microtubule binding. We are currently investigating this putative activity of CGI-99, in light of the putative function of the tRNA ligase complex in RNA transport.

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## 710 Queuosine tRNA modification is essential for the virulence of the bloodstream stage of *Trypanosoma brucei*

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A general feature of tRNAs is a high number of nucleotide chemical modifications that are introduced post-transcriptionally. Queuosine (Q), one of the most complex tRNA modifications, is found at the first position of the anticodon (wobble base) of several tRNAs. Despite its omnipresence in bacteria and eukaryotes, the function of Q in tRNA is not completely clear. In this study, we have used the protozoan parasite *Trypanosoma brucei* as a model for a comprehensive analysis of the tRNA guanine transglycosylase (TGT), the enzyme responsible for Q-tRNA formation in eukaryotes. Unlike its bacterial counterpart, in most eukaryotes TGT predominantly functions as a heterodimeric enzyme. In order to investigate the composition and function of the trypanosomal TGT, we used the sequence of the human TGT to search for potential homologues in the *T. brucei* genome. Analogous to humans, is the presence of two homologues of the TGT enzyme (TbTGT1 and TbTGT2) in *T. brucei*. We showed using RNAi knock-down strategy that both subunits are responsible for Q-tRNA formation. However, only TbTGT1 resulted in a strong growth phenotype, while TbTGT2 did not affect growth of *T. brucei* cells in culture. In order to determine the functional significance of Q-tRNA modification in trypanosomes, we generated a gene knock-out of the TbTGT2 and performed additional phenotypic in vivo characterization directly in the bloodstream of the mammalian host with the goal to simulate actual conditions associated with parasite infection. After infecting mice using these mutant parasites, we observed that it takes significantly longer for the trypanosome cells to appear in the blood, and eventually to kill the animals, as compared to WT parasites. Our data lead to the conclusion, that queuosine plays an important physiological role during survival of the parasites inside the mammalian host and may be at the heart of virulence.

**711 Identifying novel targets for the snoRNA class of stable ncRNAs**

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Small nucleolar RNAs (snoRNAs) have long been known to function as guides for rRNA modifying enzymes. Most box C/D snoRNAs direct the 2'-O-methylation of rRNA substrates, but some lack known targets and are therefore termed 'orphan snoRNAs'. *Saccharomyces cerevisiae* had two orphan snoRNAs, snR4 and snR45, with no known function in ribosome synthesis. CLASH analyses of RNA-RNA interactions showed that these snoRNAs bind multiple mRNAs, while RNA sequencing revealed that snoRNA deletion alters mRNA abundance. Synthetic gene array screens with deletions of the SNR4 and SNR45 genes identified multiple positive and negative genetic interactions. In particular, deletion of either snoRNA gene was synthetic-lethal with mutation of the snoRNA-associated methyltransferase Nop1 (Fibrillarin in human). Associations were identified between snR4 and snR45 and multiple sequences within the rRNA, including two recently identified sites of 18S rRNA acetylation. We now aim to determine the direct consequences of snoRNA binding to target RNAs.

**712 FUS/TLS mediates the processing of snoRNAs to shorter RNA fragments that can regulate gene expression**

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FUS/TLS is a multifunctional protein involved in many pathways of RNA metabolism in human cells, including transcription, splicing, alternative splicing, RNA transport, miRNA processing and replication-dependent histone gene expression. Several FUS/TLS mutations have been also found in familial forms of ALS and FTLN diseases, implicating a pathogenic role of this protein in neurodegenerative disorders.

Interestingly, we have recently found that FUS binds small nucleolar RNAs in human cells. Using RIP-seq we identified both C/D and A/HCA box snoRNAs in immunoprecipitated fraction. The interaction of FUS with snoRNA fragments was further confirmed by EMSA. Surprisingly, when we measured the level of mature snoRNAs in cell with FUS depletion (FUS KD) or FUS overexpression (FUS OE) we observed that FUS negatively influences the level of selected snoRNAs. However, the splicing efficiency of introns encoding these snoRNAs was not altered suggesting, that FUS does not affect snoRNAs biogenesis.

Further scanning of available human small RNAs databases revealed existence of RNA fragments (19-33 nt), called sdRNAs, that can derive from selected snoRNAs. As FUS also interacts with snoRNP protein components (confirmed by IP and PLA assay) we hypothesize that FUS might compete with snoRNP proteins and induce synthesis of sdRNAs resulting in decreased level of mature snoRNAs. Such sdRNAs could be involved in regulation of gene expression. Indeed, using in silico approach we predicted putative targets for our sdRNAs. Some of them may hybridize to the 3'UTRs of target mRNAs suggesting their role in posttranscriptional regulation of transcript stability and/or protein synthesis, that will be further tested. Moreover, we have found an interesting group of 104 sdRNAs that can interact with noncoding, processed ZDHHC11B-002 transcript. The RNA duplex is formed between the sdRNA and the ZDHHC11B-002 unique 3'end region, that distinguishes it from protein coding mRNA transcribed from the same gene. As we observed elevated level of ZDHHC11B-002 transcript in FUS KD cells, we suggest that 104 sdRNAs prevent the synthesis of noncoding RNA that might stabilize the level of coding transcript. The mechanism of such regulation and its biological significance will be discussed.

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### 713 Sm proteins target spliceosomal snRNPs into Cajal bodies

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Cajal bodies (CBs) are nuclear non-membrane bound organelles containing small nuclear ribonucleoprotein particles (snRNPs) that undergo in CBs final maturation steps. However, signals that target and retain snRNPs in CBs have yet to be described. Here, we utilized microinjection and ectopic expression of various snRNA deletion mutants and provide evidence that Sm and SMN binding sites are together necessary and sufficient for CB localization of snRNAs. In addition, a chimeric 7SK RNA containing both Sm and SMN sites was targeted to CBs. We further show the essential role for Sm proteins in targeting and accumulation of snRNPs in CBs. Finally, we determined that the low-complexity GR rich domains are essential for localization of Sm proteins into the CB. We propose a model in which the accessibility of the Sm ring represents a key determinant in quality control of the final snRNP maturation and sequestration of immature particles in CBs.

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### 714 The peculiar case of CCA adding enzyme in *T. brucei*

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tRNAs are transcribed in their precursor form and then processed through series of enzymatic reactions to generate a mature tRNA which can then be used for protein synthesis. Therefore, in most of the eukaryotes, apart from the cytosol, tRNA processing enzymes are also present in the mitochondria to specifically process the mitochondrially encoded tRNAs. *Trypanosoma brucei* which is a single cell eukaryote, presents a unique situation where all the tRNA genes have been thrown out of the mitochondrial genome and tRNAs instead are imported from the nuclear encoded mature cytosolic pool of tRNAs. Therefore, the tRNA processing enzymes are not required in its mitochondria. However, recent results from our lab shows that CCA adding enzyme which is a tRNA processing enzyme that adds ubiquitous CCA tail to the tRNAs is present in the mitochondria.

In trypanosomes, there is only one known CCA adding enzyme gene which is postulated to give rise to both cytosolic and mitochondrial form of the enzyme. When this gene was knocked down, a growth retardation could be observed already after day 1. But, contrary to expectations one could not see a general accumulation of shorter CCA-less tRNAs in the total cell RNA when visualised on a denaturing polyacrylamide gel. Moreover, when individual tRNAs were probed using northern blot, several tRNAs remained unaffected. However, northern blot analysis revealed that one of the tRNAs, i.e., cysteine tRNA showed an accumulation of shorter tRNA species in a time dependent manner after knockdown of CCA adding enzyme. This is peculiar since this enzyme is not known to be more specific or sensitive for certain tRNAs. Also, this accumulation of shorter tRNA-Cys could be found in the digitonin extracted mitochondrial enriched fraction of *T. brucei*. But it is not very clear whether the CCA-less tRNAs are imported into the mitochondria because of RNAi or the lack of enzyme in the mitochondria leads to accumulation of CCA-less tRNAs during internal tRNA turnover in the mitochondria. This is something to be looked at in future with respect to the role of CCA adding enzyme in the mitochondria of *T. brucei*.

## 715 The Molecular Mechanisms Underlying t6A Biosynthesis in Bacteria

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Modifications of the anticodon stem and loop (ASL) of tRNA are the most distinct and chemically complex of all RNA modifications. ASL modifications are required by at least one third of all bacterial tRNA species for cognate and/or wobble codon recognition and translocation, they enhance aminoacylation properties of tRNA, and prevent ribosomal frameshifting. The hypermodified nucleoside threonylcarbamoyladenine (t<sup>6</sup>A), found in the ASL at position 37 has been the subject of intense research in the last decade due to its essentiality in bacteria and potential for drug target discovery. The t<sup>6</sup>A biosynthesis enzymes have been identified in all three domains of life, revealing kingdom-specific variations. The four protein subunits TsaB, TsaC/TsaC2, TsaD, and TsaE are responsible for t<sup>6</sup>A biosynthesis in bacteria. TsaC catalyzes the formation of the pathway intermediate threonylcarbamoyl adenylate (TC-AMP) from L-Thr, ATP and CO<sub>2</sub>, but the exact roles of the remaining subunits and their concerted interactions during catalysis remain an enigma. We combined biochemical, crystallographic and small-angle X-ray scattering analyses to characterize the molecular events and dynamics underlying t<sup>6</sup>A biosynthesis in *Thermotoga maritima*. We show that an extended 2:2 stoichiometric heterocomplex of TsaD and TsaB (TsaBD 2:2), in which TsaB acts as a homodimerization module, constitutes the minimal scaffold for binding to one tRNA molecule. Binding of a single TsaE subunit (TsaBDE 2:2:1 complex) increases the affinity for tRNA whereas binding of a second TsaE subunit (TsaBDE 2:2:2 complex) abolishes tRNA binding, indicating that TsaE and tRNA occupy the same/overlapping site. A previously unreported TsaE/TsaC2 complex forms only in the absence of TsaBD2:2 and ATP. SAXS solution structures of TsaBD 2:2, TsaBDE 2:2:2, and of TsaC2 reveal structural plasticity indicative of a highly dynamic system. Based on this data and a high resolution crystal structure of TsaBDE 2:2:2 bound to ATP, we propose a mechanism catalytic cycle in which channeling of TC-AMP to the TsaD active site triggers ATP hydrolysis and release of TsaE, thus freeing up its site for tRNA binding and subsequent transfer of the TC moiety to A<sub>37</sub> of tRNA.

## 716 Computational analysis of tRNA-derived fragments in tumor, tumor-adjacent and normal tissues from breast cancer patients

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In recent years non-canonical roles of tRNAs have sparked interest within the research community as it was demonstrated that they are a source of a new class of small RNAs. These are a product of specific cleavage within either the anticodon loop generating tRNA-halves or D and T-loop leading to formation of smaller tRNA-derived fragments (tRFs). Regulatory potential of these fragments has been implicated upon cellular stress, in various neurodegenerative diseases as well as during tumorigenesis and shown to inhibit translation or target mRNAs for degradation.

We performed a computational analysis of a set of publicly available sRNA-Seq data from biopsied samples from tumor (T), tumor-adjacent (TN) and normal (N) tissues of five breast cancer patients. We detected tRFs derived from 5' and 3' ends of both pre-tRNAs and mature tRNAs. Our results of differential expression analysis of 17-35 nt long fragments show that although there are clear differences in expression between T, TN and N samples, the number and sets of differentiating fragments largely vary from patient to patient. We suspected that tRNA modifications affecting secondary structure may lead to variable tRF species. Thus, we investigated modification status of tRNAs and detected changes in fraction of modified bases in both pre-tRNA and mature tRNAs at specific positions. Our results indicate that Arg, Cys, Ala and Glu-tRNAs give rise to most substantial differences in modification level between tissue types with most aberrantly modified bases occurring at positions: 4, 25 and 56/57. These changes although detectable in all sample sets, again are not uniform between patients.

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## 717 Elimination of A'-A0 pre-rRNA processing byproduct in human cells is dependent on the consecutive action of two nuclear exosome-associated nucleases: RRP6 and DIS3

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Pre-rRNA processing in higher eukaryotes is a complex process, involving endonucleolytic cleavages of the large precursor molecule within external and internal spacer sequences, and their further trimming by exonucleases. Eventually, the series of coordinated nucleolytic events leads to separation and maturation of 18S, 5.8S and 28S rRNA species, which are incorporated into ribosomes during their biogenesis. In addition, quality control systems based on the action of nucleases ensure that the excised spacer sequences do not accumulate in the cells during pre-rRNA processing, and are instead efficiently removed. The identity of endonucleases, such as UTP24 or LAS1, responsible for several steps of rRNA maturation in human cells has been revealed relatively recently. For other cleavages, endonucleolytic activities remain to be identified. On the other hand, the role of exonucleases in trimming precursor molecules and quality control has not been studied in detail. For instance, exonucleolytic activities of the catalytic subunits of the major human 3'-5' exoribonuclease - the exosome complex, have been demonstrated so far to participate in the 3'-end maturation of 18S (hRRP6) and 5.8S (hDIS3 and hRRP6) molecules. hXRN2 5'-3' exoribonuclease was in turn shown to be responsible for trimming the 5'-ends of 5.8S and 28S precursors.

Here, we present the results of our studies concerning the role of hDIS3, hRRP6 and hXRN2 exoribonucleolytic activities in the removal of 5'-ETS fragments, arising after cleavages at sites A' and A0. To this end, we utilized cell lines with silenced expression of the respective endogenous protein and exogenously producing WT or catalytically-dead enzyme variants. RNA isolated from these cells was analyzed by northern-blot, primer extension, cRT-PCR and 3'-RACE techniques. Altogether, our data indicate that hDIS3, hRRP6 and hXRN2 contribute differentially to the degradation of +1-A' and A'-A0 fragments of the 5'-ETS. Furthermore, elimination of the A'-A0 byproduct is most likely initiated by hRRP6 and followed by the action of hDIS3.

## 718 Peptide nucleic acid oligomers targeted at bacterial rRNA

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Bacterial rRNA is a target for many antibiotics which block proper function of ribosomes leading to inhibition of bacterial growth. However, rRNA can be also targeted in a sequence-specific manner by complementary oligonucleotides (e.g., see review [1]). Here, we have targeted rRNA sites with peptide nucleic acid oligomers (PNA). PNA is a synthetic DNA analogue with a neutral peptide-mimicking backbone, so it is not degraded by proteases and nucleases.

Considering various descriptors of rRNA in the ribosome context such as accessibility, flexibility, energetics of rRNA strand opening, as well as similarity to human rRNA, we proposed a few rRNA target sites for sequence-specific inhibition by PNA [2]. To determine rRNA flexibility we performed and examined all-atom molecular dynamics simulations of ribosome subunits in explicit solvent [3].

A few PNA sequences were tested experimentally to verify if they inhibit the synthesis of a reporter protein in a cell-free *E. coli* extract. One example was a PNA sequence aimed to target Helix 69 of 23S rRNA and the other one - helix 26 of 16S rRNA. We also verified the binding of fluorescein-labeled PNA to either 23S or 16S rRNA in isolated total RNA and to ribosomes. Further, we conjugated PNA to a cell-penetrating peptide in order to deliver it to cells and we verified that these peptide-PNA conjugates inhibit the growth of *E. coli* and *S. Typhimurium* cultures in a sequence-specific manner.

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**719 Redox-regulation of the mammalian tRNA ligase complex**

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In mammals, the maturation of a portion of tRNAs involves the removal of an intron followed by the ligation of both remaining exon halves. This step is catalyzed by RTCB, a subunit of the pentameric tRNA ligase complex. Although the reaction mechanisms during tRNA splicing are well characterized, potential regulators of the tRNA ligase activity have so far not been studied. Using phylogenetic approaches, we identified a hitherto uncharacterized pyridine nucleotide-dependent oxidoreductase as a novel regulator of tRNA splicing in human cells. We have shown that this protein indeed harbors oxidoreductase activity and that its redox properties are essential for the function of the tRNA ligase complex. Interestingly, we also observed that the tRNA ligase complex loses activity upon treatment of cells with pro-oxidants like hydrogen peroxide or menadione, indicating that it is a redox-regulated enzyme. We are currently working on (A) the identification of the substrate(s) of this oxidoreductase, (B) the effects on cellular metabolism upon depletion of the oxidoreductase, (C) its three-dimensional structure, and (D) the identification of the redox-switch in the tRNA ligase complex and the molecular mechanism leading to its inactivation during redox stress or in the absence of the oxidoreductase. Altogether, we are revealing the first regulatory mechanism of tRNA splicing in mammals with implications on both fundamental biology and in diseases triggered by altered redox responses, such as cancer and neurodegeneration.

**720 Analysis of a tRNA's fate in Escherichia coli - will it be processing or degradation?**

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In all three domains of life, tRNAs play a crucial adaptor role converting genetic information into an amino acid sequence. An important prerequisite for aminoacylation is the CCA triplet at the 3' end of tRNAs which is usually added posttranscriptionally by CCA-adding enzyme. Prior to CCA-addition, a series of RNases must act on nascent tRNA precursors to process them into mature tRNAs in both pro- and eukaryotes. In *Escherichia coli* (*E. coli*), the CCA triplet is gene encoded for all tRNAs. Therefore, CCA-addition is not mandatory but enhances efficient growth by restoring damaged tRNA ends.

It has been shown that the quality of tRNAs and their corresponding precursors is tightly controlled. Monitoring the integrity of (precursor) tRNAs is vital to avoid fatal effects of tRNA misfolding and malfunctioning within a cellular environment. Upon either polyadenylation via poly(A) polymerase or CCACCA-addition via CCA-adding enzyme, defective tRNAs are marked for subsequent degradation. However, a tag like this closely resembles the 3' trailer of a precursor tRNA which is recognized by the 3' processing machinery. Here, we investigate whether *E. coli* exoribonucleases acting on tRNA can distinguish a precursor tRNA (native 3' trailer) from a tRNA targeted for degradation (CCACCA tag). We focused on the activities of RNase R and RNase T. While RNase R is the only *E. coli* RNase able to digest through complex secondary structure autonomously, RNase T plays the key role in final trimming of short tRNA 3' trailers. Our results confirm that the degrading enzyme RNase R breaks down a tRNA-CCACCA primed for degradation. However, a corresponding tRNA with native 3' trailer is degraded to the same extent, supporting that RNase R is a rather non-specific RNase. Furthermore, we examined RNase T activity against a tRNA-CCACCA. Based on kinetic analyses, we are able to show that its conversion into a mature tRNA is very difficult for RNase T, which is in agreement with the reported inhibitory C-effect. A profound substrate preference for a tRNA with a common trailer sequence over a tRNA-CCACCA is demonstrated and suggests an indirect involvement of RNase T in tRNA quality control.



## 721 Genetic bypass of RNA repair enzymes in *S. cerevisiae*

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The essential RNA ligase *TRL1* ligates intron-containing tRNA and participates in the non-canonical splicing of the messenger RNA *HAC1*. Trl1 is a multifunctional protein that joins 2',3'-cyclic phosphate and 5'-hydroxyl RNA fragments in successive steps, creating a new phosphodiester linkage with a 2'-PO<sub>4</sub> at the junction. After ligation, the 2'-phosphotransferase Tpt1 removes the 2'-PO<sub>4</sub> left behind by Trl1. We developed a genetic bypass of *trl1Δ* and *tpt1Δ* by expressing "intronless" versions of the ten intron-containing tRNAs in *S. cerevisiae*. We confirmed that the essential functions of these two enzymes are limited to tRNA splicing. In contrast, expression of intronless tRNAs failed to rescue deletion of any component of the SEN complex, consistent with previous studies indicating an additional essential function for the splicing endonuclease. Bypassed *trl1Δ* and *tpt1Δ* cells have growth defects at high temperature and in the presence of drugs that inhibit translation, suggesting that the intronless tRNAs are suboptimal for protein synthesis. RNA repair mutants show reduced fitness under oxidative stress conditions, suggesting a role for RNA repair in recovery from oxidative damage. We are using these cells to search for novel substrates of RNA repair and to understand the physiological roles for RNA repair.

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## 722 Modified tRNA adenines and their interaction with plant hormone cytokinins

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Cytokinins are a class of plant hormones involved in a wide range of biological processes affecting plant growth and development. They are adenine derivatives with aromatic or isoprene side chain. In the major biosynthetic pathway in plants, cytokinins are synthesized from precursor molecules by the enzyme isopentenyl transferase, which utilizes adenosine phosphates (either AMP, ADP or ATP) as a source of adenine. Some cytokinins (e.g., *N*6-(*cis*-hydroxyisopentenyl) adenosine, *cis*-zeatin) are present also in tRNAs (A37 position), and have been evidenced as an additional source of free cytokinins. Although this phenomenon has been known for decades, the deeper biological role of tRNA-mediated cytokinin synthesis remains poorly understood. Our extensive search revealed that there are also several other modifications present at A37 position of tRNA. We hypothesize that these molecules may have cytokinin activity as well.

To this end, cytokinin activity of A37 tRNA modification t6A was tested, and the candidate mutants with perturbed t6A formation in *A. thaliana* have been analyzed for cytokinin related defects. We found that mutations in different genes involved in t6A formation have different impacts on plant development. We reveal that, in addition to several defects possibly ascribed to altered cytokinin activity, several genes are essential for early morphogenesis steps, including development of the gametophyte.

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## 723 Role of microtubule-associated proteins in the control of nucleic-acid mediated type I IFN responses

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The innate immune system detects viral nucleic acids and induces type I interferon (IFN) responses. The retinoic acid-inducible gene-I (RIG-I) has been well known to play a major role in pathogen sensing of RNA virus infection to initiate antiviral immunity. Therefore, it is critical to regulate the expression of RIG-I for optimal induction of immune responses. Recently, it has been shown that microtubule-associated proteins (MAPs) are involved in nucleic acid mediated type I IFN responses. We have observed that MAPs can regulate the production of IFN- $\beta$  positively or negatively depending on the family members of MAPs. Depletion of MAP4 abolished RNA-mediated IFN responses. In contrast, inhibition of expression of MAP7 family members caused an upregulation of RIG-I proteins in cells, leading to increased phosphorylation of IRF3 and enhanced IFN- $\beta$  production in response to stimulation with poly I:C and SeV infection. Knockout of MAP7 family members did not affect poly dA:dT and DNA virus-mediated IFN responses. Furthermore, we showed that regulation of RIG-I by MAP7 proteins occurs at transcription level. Our data suggest that besides traditional roles for MAPs as cytoskeletal components, MAPs can act as a positive regulator in modulating RNA-mediated immune responses.

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## 724 Protein-assisted RNA folding mediates specific RNA-RNA genome segment interactions in segmented RNA viruses

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Segmented RNA viruses, including influenza viruses and rotaviruses, are ubiquitous human, animal and plant pathogens. A major challenge in understanding their assembly is the combinatorial problem of a non-random selection of a full genomic set of distinct single-stranded (ss)RNAs. This process involves multiple, complex RNA-RNA and protein-RNA interactions, which to date have been obscured by non-specific binding and aggregation at concentrations approaching in vivo assembly conditions. To interrogate specific inter-segment interactions in rotaviruses, we employ two-color fluorescence cross-correlation spectroscopy (FCCS) for detecting stable RNA-RNA interactions taking place in complex RNA and protein mixtures. We show that binding of the rotavirus non-structural protein NSP2 to ssRNAs results in RNA conformational rearrangements conducive to forming stable contacts between RNA segments. To identify the sites of inter-segment interactions, we developed an RNA-RNA SELEX approach for mapping the RNA sequences mediating stable inter-molecular base-pairing between the interacting ssRNAs. Our findings elucidate the molecular basis underlying inter-segment interactions in rotaviruses, paving the way for studying genome packaging of other segmented RNA viruses. The integrated approach expands the arsenal of techniques much needed for delineating dynamic RNA-RNA interactions involved in the assembly of large ribonucleoprotein complexes.

## 725 Biochemical, Biophysical, and Infection-Based Studies of the ciRNA-RNase L Complex

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Many viruses use structured non-coding RNA elements to manipulate cellular machinery for infection and replication. These elements are rarely found in coding regions, but one example is the competitive inhibitor RNA (ciRNA) within the C3 protease coding region of group C enteroviruses, including coxsackievirus (CV) and poliovirus (PV). This 303 nt element competitively inhibits a powerful antiviral enzyme, RNase L (RL), that once activated rapidly degrades RNA. The ciRNA inhibits this enzyme, suggesting pressure to retain the ciRNA sequence to reduce the antiviral response. Understanding the detailed molecular events during infection is key for the advance of new therapies against PV and CV infection. These molecular events could be important for a novel therapy with CV strain A21 in clinical trials as a therapy against melanoma and prostate cancers. The molecular, biophysical and in vivo features of the ciRNA that inhibits RL are unknown; understanding the interactions between RL and ciRNA is vital in understanding how an RNA is able to directly inhibit an enzyme made to ensure its destruction.

We hypothesize that the ciRNA envelopes the area around the RL active site and occludes single-stranded RNA, and during infection the ciRNA and RL co-localize in the cell to sequester and inhibit RL function. We aim to define the ciRNA structural elements needed for binding to RL and define the cellular localization of the RL-ciRNA complex during viral infection.

We have identified mutations in the ciRNA resulting in various levels of RL inhibition and have found key regions for RL inhibition. We have shown that CVA21 acts akin to PV cell infection. To address the cellular location for complex formation we have used RNA FISH with CVA21 infection of HeLa and melanoma cells that show punctate regions consistent with viral replication complexes and stress granules. To determine the biological role of this conserved viral RNA element we are testing its role in peripheral blood mononuclear cells and mature and immature dendritic cells.

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## 726 Detection and characterization of aberrant HIV RNAs expressed in latently infected cells

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HIV latency is a major hurdle to overcome in the efforts to cure AIDS. Latently infected cells do not express viral proteins and escape immune surveillance during the anti-retroviral treatment. These cells produce infectious viruses upon cessation of the treatment. A challenge to achieving an effective HIV anti-latency therapy (HALT) is that these latently infected cells are very difficult to detect. To this end, it is critical to develop a sensitive method to detect latently infected cells in HIV-infected individuals. Although latently infected cells do not express productive viral mRNAs, defective or non-productive viral RNA, such as short RNAs, antisense RNAs, host-viral hybrid RNAs and splice variants from cryptic splicing sites, are expressed and can be detected by RT-qPCR analysis. Many of these RNAs are products of host cellular mechanisms that strongly suppress viral transcription, suggesting that these RNA species play important roles in the viral latency. Thus, these aberrant HIV RNAs could serve as an excellent biomarker to detect latently infected cells. Here, we report our attempt to obtain comprehensive catalogues of viral RNA species expressed in latently infected cells using methods that can also detect unstable and short-lived RNA species. First, RNA-seq analysis using well-established Jurkat latent models indicate that these cells exclusively express host-viral hybrid RNAs in unstimulated states. Also, reduction of the RNA exosome components increases the level of these hybrid RNAs as well as antisense viral RNA. Patterns of viral RNA expression during the establishment of viral latency and its reactivation are revealed using *ex vivo* latency models of primary CD4<sup>+</sup> cells as well as HIV-infected cells derived from HAART-suppressed patients. These analyses determine the proportion of latently infected cells that express these aberrant RNAs, and whether there is a bias in the correlation between HIV RNA expression and provirus integration sites (orientation, genetic environment). These studies will provide important information for developing an efficient therapeutic approach to improve our current HALT regimen.

## 727 m6A Modification Plays a Central Role in Lytic Viral Gene Expression During Kaposi's Sarcoma-Associated Herpesvirus Infection

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Methylation at the N6 position of adenosine (m6A) is a highly prevalent reversible modification within eukaryotic mRNAs. This modification is thought to be similarly widespread in viral mRNAs, and recent reports from HIV and flavivirus infected cells demonstrate that the m6A machinery plays key roles during infection with these RNA viruses. However, it is unknown whether other viruses similarly engage proteins within the m6A pathway, and at present we have an incomplete understanding of how m6A readers impact viral replication. Here, we report that m6A levels are dramatically increased in cells infected with the oncogenic human DNA virus Kaposi's sarcoma-associated herpesvirus (KSHV), which is the leading etiologic agent of cancer in AIDS patients. Transcriptome-wide m6A-sequencing during KSHV lytic reactivation revealed the presence of m6A across multiple kinetic classes of viral transcripts, and a concomitant decrease in m6A levels across much of the host transcriptome. Silencing multiple components of the m6A machinery resulted in distinct impacts upon viral replication in Caki-1 and B-cell lymphoma derived cells. Collectively, our data show that m6A modification can function in a pro-viral or anti-viral fashion at multiple stages of the viral lifecycle. On going studies seek to examine the role of reader proteins in modulating the stability of m6A-modified KSHV transcripts, and determine the viral and cellular cofactors that interact with the m6A machinery.

## 728 Replication competence served as a driving force for experimental evolution of a satellite RNA associated with Plant RNA virus

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Genetic mutation is one of the driving forces for evolution. RNA viruses are highly mutable, thus can accumulate advantageous mutations when adapting to different environmental cues. Here, we showed how a simple RNA replicon evolved mutations experimentally. We used satellite RNA (satRNA), parasite of virus, as a working model, because satRNA with small genome totally depends on its cognate helper virus (HV) for replication and encapsidation. To mimic the natural infection in the field, we serially passaged a prototype satRNA associated with Bamboo mosaic virus (BaMV) on (1) wild-type plants co-infected with BaMV, designated as medium-stress (MS), and (2) transgenic tobacco plants expressing entire BaMV genome as high stress (HS) and assayed its evolutionary dynamics under different replication stresses. Deep sequencing revealed the patterns of genetic variations in satBaMV evolved populations were markedly different among different stresses and passages. However, mutation frequency and population diversity increased in a passage-dependent manner regardless which conditions satBaMV passages. Notably, single-nucleotide substitutions located in the 5' UTR of satBaMV were observed, which are the key determinants involved in the replication competence of satBaMV in BaMV-coinfected plants. By in vivo competition assays in tobacco protoplasts, we confirmed that evolved satBaMV winners always contain the key single-nucleotide substitution involved in replication competence. In addition, the preferential mutations for satBaMV to adapt replication and movement stresses were also selected in inoculation leaves and systemic leaves, respectively. Taken together, the satellite RNA provides a wonderful replicon for RNA evolution experimentally. Our findings provide insights into the dynamic evolution of a satellite RNA adapted to different replication stresses which revealed part of the complicate interactions between satRNA, virus and their hosts.



## 729 Evolutionary analysis of HIV-1 pol proteins reveals critical residues for virus subtype differentiation

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RNA viruses have been used as a model system to understand patterns and processes of molecular evolution, since they have high mutation rates and genetic diversity. Human Immunodeficiency Virus type 1 (HIV-1), an etiologic agent of Acquired Immune Deficiency Syndrome (AIDS), has high genetic diversity and is classified into several groups/subtypes. Meanwhile, it has been difficult to utilize those diverse sequences to see the overall phylogenetic relationships and tendencies of sequence conservation through calculating phylogenetic trees. In this study, we focused on pol protein sequence that is often utilized to classify HIV groups/subtypes. In order to identify regions on pol protein responsible for HIV-1 subtype differentiation and evolution, here we systematically characterized mutation sites on 2,052 pol proteins derived from HIV-1 Group M (subtype A: 144, B: 1,528, C: 380) using sequence similarity networks. In addition, we performed spectral clustering which divides sequences into clusters based on the network graph's structure. Stepwise analysis of the hierarchy of clusters enables to estimate a possible evolutionary process of pol proteins. The result showed that subtype A sequences were divided into further clusters based on when and where viruses were isolated, whereas each subtypes B and C sequences remain as one cluster. Since the pol protein has several functional domains, we further analyzed what regions were discriminative, by comparing the structure of each domain-based network. Consequently, it is suggested that sequence alterations on RNase H domain and reverse transcriptase connection domain are responsible for the subtype classification. We revealed that a few specific amino acid residues could represent the difference among subtypes by analyzing the difference in the amino acid composition at each site of both domain sequences. Mapping of the residues onto the structure of reverse transcriptase showed that these sites were localized in one side of the tertiary structure.

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## 730 Viral synergism and transcriptomic reprogramming in *Phalaenopsis* orchid co-infected with *Cymbidium mosaic virus* and *Odontoglossum ringspot virus*

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Synergistic viral interaction is commonly hallmarked with reinforced virus colonization and hindered host growth in mixed infected plants. In orchids, the taxonomically distinct *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV) are often dually detected in diseased plants and cause economic losses. Here we characterized asymmetrical enhancement of CymMV accumulation and movement under co-infection with ORSV in *Phalaenopsis amabilis*. Notably, chlorotic ringspots and lesions were observed as another sign of synergism at early as 10 days post inoculation. Based on symptom formation and progression of virus infection, we further designated the inoculated and adjacent non-inoculated tissues representing late and early stages of infection, respectively. To decipher defensive-offensive interactions and especially the effects of CymMV-ORSV synergism on *Phalaenopsis* gene networks, we compared *de novo* assembled and annotated transcriptomes from tissues of different infection stages. Large numbers of differentially expressed genes were first identified, indicating drastic reprogramming of *Phalaenopsis* transcriptome upon viral attack. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses showed terms related to cell wall and endoplasmic reticulum-associated degradation were enriched in initial stage of infection. Biosynthesis pathways of secondary metabolites were highly activated along the progression of viral pathogenesis. Arrays of calcium binding proteins, WRKY transcription factors, and pathogenesis-related proteins were up-regulated in infected tissues. This result suggests canonical roles of salicylic acid-dependent signaling in *Phalaenopsis* in response to viral infection. In addition, we noticed "sequence-specific DNA binding (GO:0043565)" term was commonly enriched in all the clusters, and further demonstrated multiphasic regulations of transcription factors via co-expression analysis. Through quantitative reverse transcription polymerase chain reaction validation and transiently virus-induced gene silencing assays, we identified potential key genes and processes involved in viral synergism and anti-viral resistance in *Phalaenopsis*. In conclusion, our results provided a broad view of regulatory network in aspects of orchid defense, viral pathogenesis and synergism.

**731 Development and extensive analysis of the Hepatitis C IRES Variation Database - HCVIVdb**Anas Khawaja<sup>1</sup>, Vaclav Vopalensky<sup>1</sup>, Evan Floden<sup>1</sup>, Ludek Roznovsky<sup>2</sup>, Jakub Mrazek<sup>3</sup>, Martin Pospisek<sup>1</sup><sup>1</sup>Charles University, Faculty of Science, Prague, Czech Republic; <sup>2</sup>Clinic of Infectious Medicine, University Hospital Ostrava, Ostrava, Czech Republic; <sup>3</sup>Institute of Health Ostrava, Ostrava, Czech Republic

Synthesis of the hepatitis C virus polyprotein is fully controlled by an internal ribosome entry site (IRES) located within the long and highly structured 5'UTR of the viral RNA. Analysis of the structural and functional impact of the nucleotide mutations within the HCV IRES is in the centre of interest of the hepatitis C virus biology. Prior to the development of the Hepatitis C Virus IRES Variation Database (<http://hcvivdb.org>), there was no central repository for the various HCV IRES mutations and their effect on the hepatitis C virus polyprotein translation efficiency. We have developed a syntax for collating this information and, to date, have generated a large dataset containing 1564 entries comprising 1967 HCV IRES sequence variations. The collected data have been characterized in multiple categories and are presented through the sophisticated but user-friendly web-based graphical interface that assists users in conducting comparative and functional analyses among various HCV IRES regions. We further complemented this research with data collected from analyses of HCV IRESs from our patients. All that together allowed us to describe and characterize >20 novel mutations in HCV IRES, to predict impact of single and multiple mutations on HCV IRES activity according to domains and subdomains where they appear and to discover rare examples of long-range intra-domain and inter-domain interactions which can lead either to sharp decline of the HCV IRES activity or to the compensation of the HCV IRES functional defects. Careful analysis of the collected data also led to discovery of new hyper-variable nucleotides and confirmation of the conserved regions in the HCV IRES, which we further supported by calculation of the frequency of occurrence of each nucleotide at each position of the HCV IRES in ~2000 independent full-length HCV genome sequences from the NIAID Virus Pathogen Database and Analysis Resource (ViPR).

**732 A single nucleotide change in a functionally undefined region of the RRE induces an extensive RRE conformational switch**Chringma Sherpa<sup>1</sup>, Stuart Legrice<sup>1</sup>, David Rekosh<sup>2,3</sup>, Marie-Louise Hammarksjold<sup>2,3</sup>, Patrick E. Jackson<sup>2</sup>, Gelare Ghajar-Rahimi<sup>2</sup><sup>1</sup>National Cancer Institute, National Institute of Health, Frederick., MD, USA; <sup>2</sup>Myles H. Thaler Center for AIDS Center, Department of Microbiology, Immunology, and Cancer Biology, University of Virginia School of Medicine, VA, USA; <sup>3</sup>Department of Microbiology, University of Venda, Thohoyandou, Limpopo, South Africa

The HIV-1 Rev Response Element (RRE) is a *cis*-acting RNA element with multiple stem-loops. Binding and subsequent multimerization of the Rev protein on the RRE are essential steps in HIV replication. Most of our understanding of the Rev-RRE regulatory axis comes from studies on a few lab-adapted HIV clones. However, from a therapeutic standpoint, in a rapidly evolving virus like HIV, mechanistic studies of naturally occurring Rev and RRE sequences are critical. A recent study on Rev-RRE variation in serum samples from HIV infected individuals reported that, in one patient, RRE sequences present within 1 ½ years of seroconversion (early SC3) and after AIDS onset 5 years later (late SC3), differed only by 4 isolated nucleotide changes in functionally undefined regions of the RRE. Additionally, the late SC3 RRE was more active than the early SC3 RRE in a Gag-Pol reporter assay. Interestingly, the Rev sequence which paired with each of these RREs remained unchanged between the two time-points. (Sloan et al. J Virol. 2013 87:11173-11186)

To understand the mechanism underlying the functional differences between these two RREs, these RRE RNAs were synthesized by in-vitro transcription and their secondary structure was determined using CE-SHAPE and SHAPE-MaP. The late RRE formed a canonical 5 stem-loop structure whereas the early RRE formed a structure with an alternative folding of stem-loops I, IV, and V. To investigate this further, four early SC3 RRE variants, each containing only one of the four signature late RRE nucleotides, were created and their RNA structure was determined by SHAPE-MaP. We found that only one nucleotide change (G to C) in a functionally undefined central loop region of the RRE was responsible for the structural switch between the early and late RRE. Thus, we have shown for the first time that a single nucleotide change in the functionally undefined central loop region of the RRE can affect global RRE structure. Furthermore, preliminary experiments also suggest that the same single nucleotide change can change the activity of the early RRE to the late RRE level. Studies investigating the effect of these mutations on Rev binding and multimerization are underway.

### 733 Elimination of carry-over contamination by the use of a thermostable restriction endonuclease for RT-qPCR for diagnosis of RNA virus

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A repeated real-time reverse transcription polymerase chain reaction (rRT-PCR) for the same target genome increases the risk of carry-over contamination which is used widely for the diagnosis of foot-and-mouth disease (FMD) virus for its rapidity and high accuracy. In this study, we adapted the technique for the detection of RNA virus and successfully choose a thermostable restriction endonuclease *Taq I* which selectively neutralizes the PCR product carried over from the previous assay. This method also show negligible effect on the viral RNA for detection and quantification.

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### 734 Functional Characterization of Hepatitis C Virus Non-Structural 5A in Viral Replication

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The genome of hepatitis C virus (HCV), same as other members in the flaviviridae family, is maintained as a positive-sense single-stranded [(+) ss] RNA in the virion. Once the HCV virions enter human hepatocytes, this (+) ssRNA immediately serves as a template for translation into a single open reading frame, which is subsequently processed into at least 10 proteins by viral and host proteases. For genome replication, the newly produced viral RNA-dependent RNA polymerase (RdRp), i.e. non-structural protein 5B (NS5B), recognizes the (+) ssRNA to synthesize the (-) strand RNA which later serves as the replication template for generating more (+) ssRNA by NS5B. Thus, many screening pipelines have focused on viral proteases and RdRp for the development of the direct-acting antivirals (DAA) to suppress viral replication in the host cells. One exception is the discovery of the HCV NS5A inhibitors, such as FDA-approved daclatasvir, ledipasvir, ombitasvir, and elbasvir. The NS5A inhibitors are effective in a very low concentration (pico-molar range) and relative safer. Inevitably, resistance mutations to NS5A inhibitors were observed in HCV genotype 1a patients, urging solutions to overcome the emerging genetic barriers. HCV NS5A is an RNA-binding protein without enzymatic activity and it is required for HCV genome replication and virion assembly. The precise function of NS5A at the molecular levels remains poorly understood. To gain more insight to the roles NS5A plays, we first seek to search for the functionally interacting host partners by a genetic approach. We took the advantage of the non-essential genes deletion collection and the conditional mutant library of the essential genes to screen for alterations which exhibited genetic phenotypes in budding yeast overexpressing NS5A. The corresponding human counterparts of identified candidates will be validated for their interaction to NS5A in Huh7.5 cells with HCV replicon. In addition, we performed modified Photoactivatable Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP) in Huh7.5 cells to examine the interacting RNA landscape of NS5A with and without the HCV replicon. We will present and discuss the results of these exploring studies on NS5A in the meeting.

### 735 Structural rearrangements of genomic RNA during HIV-1 maturation process

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To be infectious HIV-1 particles have to undergo a maturation step. This process occurs concomitantly and shortly after the budding from the infected cells and concerns at least two partners. In one hand, proteolytic cleavages of Pr55<sup>Gag</sup> precursors allow the production of the structural proteins and trigger morphological rearrangements to form the mature particle with a cone-shaped core containing the genomic RNA (gRNA) in a dimeric form. On the other hand, gRNA dimer also rearranges with a concomitant increase in its stability. It has been shown that the genomic maturation is essential to allow efficient reverse transcription during the next viral cycle. Moreover, the nucleocapsid protein (NCp7) stabilizes the genomic dimer, with intermolecular interactions probably taking place all along the genome, even if their location remains unknown.

Our goal is to better understand the link between proteolytic processing and genomic maturation and we are interested in determining the different steps leading to the formation of a stable and mature dimeric RNA. Since the RNA reactivity profile can be explained by a structural change or by direct protein binding (footprints), we analyzed the *in vitro* structure of the packaging signal in presence of Gag, mature nucleocapsid (NCp7) and maturation intermediates containing the NC domain, using chemical probing analyzed by capillary electrophoresis. Gag and cleavage products induce a protection at identical sites with a higher NCp7 ability to stabilize RNA. Comparing reactivity profiles of RNA only and RNA-protein complexes treated with proteinase K, we were able to show that proteins did not induce significant permanent conformational rearrangements of RNA.

In order to confirm our *in vitro* results, we studied gRNA structure inside immature and mature viral particles. These results, visualized by gel electrophoresis, also indicate a rearrangement of the nucleotides between the SL3 stem-loop and the AUG start codon of *gag*, from the immature to the mature virus. Since these technical approaches are not suited to analyze mutants mimicking the sequential processing of Gag, we developed an *in virio* high-throughput chemical probing technique (h-SHAPE-Seq).

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### 736 PAN RNA from Kaposi's sarcoma-associated herpesvirus promotes late lytic viral gene expression by sequestering cytoplasmic poly(A) binding protein

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an opportunistic pathogen of HIV patients and the etiological agent of several human cancers, including Kaposi sarcoma and primary effusion lymphoma. The KSHV life cycle includes a latent phase, when viral gene expression is largely absent, and a lytic phase, when viral gene expression increases dramatically and progeny virions are produced. During the lytic phase, the most abundant polyadenylated RNA is a 1-kb, nuclear, non-coding viral transcript called PAN RNA that accumulates to approximately 500,000 copies per cell. The work of Joan Steitz and colleagues has described a triple-helical RNA secondary structure that caps the 3'-end of this viral lncRNA, thereby contributing to the accumulation of PAN RNA. Previous work has demonstrated that loss of PAN RNA, either through genetic knockout from the viral genome or antisense depletion of the transcript, results in misregulation of late lytic genes and host immune response genes. This highlights the essential role of PAN RNA during the lytic phase, but the mechanism underlying these phenotypes associated with loss of PAN RNA is unclear. PAN RNA is exclusively nuclear, which prompted efforts to demonstrate that PAN RNA associates directly with the human and viral genomes. Using a technique known as CHART (Capture Hybridization Analysis of RNA Targets) we have sought to revisit this hypothesis. Briefly, CHART involves the use of biotinylated DNA oligonucleotides to bind and isolate RNA that is associated with chromatin. The associated chromatin is mapped back to the genome using high-throughput sequencing analysis, thereby allowing identification of specific DNA sites bound by the RNA of interest. This analysis of PAN RNA from two related viruses, KSHV and Rhesus macaque rhadinovirus (RRV), suggests that the primary function of herpesviral PAN RNA may not be regulation of gene expression through chromatin interactions. We will discuss an alternative model for PAN RNA function whereby sequestration of cytoplasmic poly(A) binding protein selectively promotes viral gene expression.



### 737 Widespread induction of antisense transcription from the human host cell genome in lytic Herpes simplex virus 1 infection

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Herpesviruses have evolved multiple ways to modify the functional program of infected cells to their need. Here, we show that Herpes simplex virus 1 (HSV-1) induces the expression of about 1500 antisense transcripts from the human host cell genome. A subset of these is also activated by the closely related Varicella zoster virus. Antisense transcripts either originate at gene promoters, or within the gene body. Their temporal expression patterns group into ten distinct clusters. Antisense transcripts show different susceptibility to the inhibition of early and immediate early viral gene expression, indicating that products of these genes are causal for our observation. Overexpression of viral ICP4 alone is sufficient to turn on a subset of antisense transcripts. Histone marks around transcription start sites of HSV-1 induced and constitutively transcribed antisense transcripts are very similar, indicating that the genetic loci are already poised to transcribe these novel RNAs. Furthermore, we provide evidence that an antisense transcript overlapping about half of the BBC3/PUMA gene transcriptionally silences this potent inducer of apoptosis *in cis*. Taken together, we describe a new way how a common pathogen manipulates the infected host. Beyond that, the HSV-1 induced antisense transcripts can serve as a search template to investigate this phenomenon in other biological contexts.

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### 738 Splicing factors Esrp1/2 enhance transcription factor-mediated somatic cell reprogramming

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Despite the great interest in induced pluripotent stem cells (iPSCs), the molecular aspects of the reprogramming process remain insufficiently understood. Alternative splicing, a process that enables the expression of multiple transcript isoforms from a given gene, was recently identified to play an important role in the reprogramming cascade [1-6]. By analyzing an extensive set of publicly available RNA-seq datasets obtained from human, chimpanzee and mouse reprogramming systems, we identify a conserved core set of regulatory splicing factors that undergo large expression changes (>10-fold) during the process, including the Epithelial Splicing Regulatory Proteins Esrp1 and Esrp2. We show that ectopic expression of either factor in a mouse embryonic fibroblast-based, inducible, secondary reprogramming system [7] accelerates the kinetics of reprogramming towards an intermediate stage and leads to an increase in overall reprogramming efficiency. We further show that Cd44, which has been identified as a cell surface marker for reprogramming [8] and whose alternative splicing is regulated by Esrp proteins during epithelial-to-mesenchymal transition (EMT) [9-10], exhibits splicing changes during the early stages of reprogramming that are diametrically opposed to those observed during EMT, and that overexpression of either Esrp1 or Esrp2 speeds up this transition. To identify other relevant splicing events driving the observed boost in reprogramming efficiency, we are currently studying transcriptome expression changes along a reprogramming time series, in the presence and absence of Esrp1/2. A better understanding of the molecular mechanisms underlying somatic cell reprogramming would pave the way towards a more precise, varied and potentially safer use of this technology.

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### 739 Structural characterization of the hnRNP C tetramer and the basic bZIP-like RNA binding domain

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The heterogeneous nuclear ribonucleoprotein hnRNP C1/C2 is an abundant nuclear protein associated with nascent transcripts. It is involved in alternative splicing (1), RNA packaging and sorting (2). The human protein exists as a (C1)<sub>3</sub>C2 heterotetramer, with C2 isoform harboring an additional 13 residue exon. It wraps ~230nt increments of elongating transcript, while three tetramers wrap ~700 nt of RNA into a triangular 19S complex, considered as the repeating ribonucleosome unit (3). The N-terminal RRM domain recognizes uridine (U) pentamers (4), however large fractions of natural RNA targets contain longer U-tracts consistent with binding of a dimer (5). Recombinant C1 and C2 are both capable to form homotetramer *in vitro*. We purified the *in vitro* reconstituted complex of the recombinant hnRNP C1 tetramer and its 185nt CD55 RNA target, containing an exon regulated by hnRNP C, situated between two RRM binding U-tracts separated by 165 nt RNA. In addition, we also purified the (C1)<sub>3</sub>C2 tetramer directly from human HeLa cells using affinity tags and ultracentrifugation. Electron microscopy analysis of the hnRNP C1/C2 tetramer is currently in progress and initial micrographs suggest that the tetramer has a different shape when bound to RNA. In addition to its RRM domain that confers specificity of RNA binding, a basic bZIP-like domain preceding the tetrameric leucine zipper domain confers high-affinity RNA interactions. We use NMR to study the basic bZIP domain and found that it undergoes a large structural re-arrangement upon RNA binding. Altogether, by combining NMR and EM, we aim to shed light on the RNA binding properties and the general arrangement of the RNA nucleosome.

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### 740 Post-transcriptional and translational regulation of mRNA-like long non-coding RNAs by microRNAs in early developmental stages of zebrafish embryos

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At the post-transcriptional and translational levels, microRNA (miRNA) represses protein-coding genes via seed pairing to the 3' untranslated regions (UTRs) of mRNA. Although working models of miRNA-mediated gene silencing are successfully established using miRNA transfections and knockouts, the regulatory interaction between miRNA and long non-coding RNA (lncRNA) remain unknown. In particular, how the mRNA-resembling lncRNAs with 5' cap, 3' poly(A)-tail, or coding features, are regulated by miRNA is yet to be examined. We therefore investigated the functional interaction between miRNAs and lncRNAs with/without those features, in miRNA-transfected early zebrafish embryos. We observed that the greatest determinants of the miRNA-mediated silencing of lncRNAs were the 5' cap and 3' poly(A)-tails in lncRNAs, at both the post-transcriptional and translational levels. The lncRNAs confirmed to contain 5' cap, 3' poly(A)-tail, and the canonical miRNA target sites, were observed to be repressed in the level of both RNA and ribosome-protected fragment, while those with the miRNA target sites and without 5' cap and 3' poly(A)-tail, were not robustly repressed by miRNA introduction, thus suggesting a role as a miRNA-decoy.

## **741 ELIXIR - a distributed infrastructure for life-science information. Training opportunities for RNA scientists!**

Marian Novotny

**Charles University, Prague, Czech Republic**

ELIXIR (<https://www.elixir-europe.org>) is a distributed European infrastructure for safeguarding an ever growing amount of life-science data. It integrates and supports bioinformatics resources across its member states and enables users in academia and industry to access services that are vital for their research, including databases and tools for RNA data.

An integral part of ELIXIR activities is a training dedicated to ELIXIR resources. Elixir has a training portal called TeSS (Training e-Support System - <https://tess.elixir-europe.org>). TeSS offers information on planned courses, training materials, workflows and information on training providers from ELIXIR nodes and 3<sup>rd</sup> party providers. It currently holds information about 7 RNA-related courses in 2017 and more than 100 training materials for RNA data.

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## NOTES

## NOTES

## NOTES



## NOTES

The floor plan illustrates the layout of the 1st floor, featuring several key areas:

- Meeting Hall I:** Divided into sections A and B.
- Meeting Hall II:** Located near the top left.
- Congress Hall:** A large central hall with a blue background.
- Forum Hall Foyer I:** Located near the top right.
- Congress Hall Foyer IA and IB:** Foyer areas adjacent to the Congress Hall.
- Clubs A through E:** A row of rooms on the right side.
- Club H:** Located at the bottom right.
- ZOOM Restaurant:** A yellow area on the right side.
- Buffet:** A yellow area near the Congress Hall.
- Cloakroom:** Located near the top center.
- Clubs Foyer:** A yellow area at the bottom right.
- Panorama Hall:** A large blue area on the right side.

Accessibility icons are placed throughout the plan to indicate accessible routes and facilities, including wheelchair access, stroller access, and other mobility aids.

Second Floor



Third Floor





# ABBREVIATED SCHEDULE

## TUESDAY 30 MAY

14:00 – 19:00	Registration	Congress Hall Foyer 2B (floor 2)
18:00 – 20:00	Welcome Dinner	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3)
20:00 – 21:15	Opening and Keynote 1 [Robert Singer]	Congress Hall
21:15 – 23:00	Social Time	Zoom Restaurant (floor 1)

## WEDNESDAY 31 MAY

08:00 – 09:00	Sponsored Seminar	Panorama Hall (floor 1)
08:00 – 19:00	Registration continues	Congress Hall Foyer 2B (floor 2)
09:00 – 12:30	Plenary Session 1: RNA Editing/Modification (1-12) [Michael Jantsch]	Congress Hall
10:30 – 11:00	Coffee Break	Congress Hall Foyer 2B (floor 2)
12:30 – 14:00	Lunch	Congress Hall Foyer 2A, 2C (floor 2)
12:30 – 14:00	Mentoring Lunch [Nancy Greenbaum]	North Hall, Terrace 1 & 2, Foyer 3A (floors 2 & 3)
14:00 – 16:15	Concurrent Session 1: RNA Processing (13-21) [Yongsheng Shi]	Congress Hall
	Concurrent Session 2: RNA Chemistry/Methods (22-31) [Roland K. O. Sigel]	Meeting Hall 1 (floor 1)
16:15 – 16:45	Coffee Break	Congress Hall Foyer 1B, 2B (floors 1 & 2)
16:45 – 17:45	Keynote 2 [Eric Sontheimer]	Congress Hall
17:45 – 19:15	Concurrent Session 3: Splicing (32-38) [David Stanek]	Congress Hall
	Concurrent Session 4: Retro Non-coding RNA (39-45) [Stepanka Vanacova]	Meeting Hall 1 (floor 1)
19:15 – 20:15	Junior Scientists Social	Zoom Restaurant (floor 1)
19:15 – 21:00	Dinner	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3), North Hall, Terrace 1, Terrace 2 (floor 2)
	RNA Society Meetings Committee Dinner Meeting	Club E (floor 1)
20:30 – 23:00	Poster Session 1 (even numbers)	Forum Hall (floor 2)
23:00 – 23:30	Social Time	Zoom Restaurant (floor 1)

## THURSDAY 1 JUNE

08:00 – 13:30	Registration continues	Congress Hall Foyer 2B (floor 2)
09:00 – 12:30	Plenary Session 2: RNA/RNP Structure (46-57) [Martin Jinek]	Congress Hall
10:30 – 11:00	Coffee Break	Congress Hall Foyer 2B (floor 2)
12:30 – 13:30	Keynote 3 [Marina Rodnina]	Congress Hall
13:30	Free afternoon and evening	

## FRIDAY 2 JUNE

08:00 – 19:00	Registration continues	Congress Hall Foyer 2B (floor 2)
09:00 – 12:30	Plenary Session 3: Splicing (58-70) [Kristen Lynch]	Congress Hall
10:30 – 11:00	Coffee Break	Congress Hall Foyer 2B (floor 2)
12:30 – 14:00	Lunch	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3), North Hall, Terrace 1, Terrace 2 (floor 2)
14:00 – 16:15	Concurrent Session 5: RNA Localization/Transport (71-78) [Maria Carmo-Fonseca]	Congress Hall
	Concurrent Session 6: Translation Regulation (79-87) [Daniel Wilson]	Meeting Hall 1 (floor 1)
	Career Development Workshop: Combating Imposter Syndrome [Fadi Marayati]	Panorama Hall (floor 1)
16:15 – 16:45	Coffee Break	Congress Hall Foyer 1B, 2B (floors 1 & 2)
16:45 – 17:45	Science and Society Lecture [Adrian Krainer]	Congress Hall
17:45 – 19:15	Concurrent Session 7: RNA Therapeutics (88-92,94) [Adrian Krainer]	Meeting Hall 1 (floor 1)
	Concurrent Session 8: Non-coding RNA (95-101) [Stefan Ameres]	Congress Hall
19:15 – 21:00	Dinner	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3), North Hall, Terrace 1, Terrace 2 (floor 2)
19:15 – 21:00	RNA Society Board of Directors Dinner Meeting	Club E (floor 1)
20:30 – 23:00	Poster Session 2 (odd numbers)	Forum Hall (floor 2)
23:00 – 23:30	Social Time	Zoom Restaurant (floor 1)

## SATURDAY 3 JUNE

08:00 – 18:00	Registration continues	Congress Hall Foyer 2B (floor 2)
09:00 – 12:30	Plenary Session 4: Non-coding RNA (102-113) [Ling-Ling Chen]	Congress Hall
10:30 – 11:00	Coffee Break	Congress Hall Foyer 2B (floor 2)
12:30 – 13:30	Lunch	Congress Hall Foyer 2A, 2C, 3A (floors 2 & 3)
13:30 – 14:30	Keynote 4 [Juan Valcarcel]	Congress Hall
14:30 – 17:15	Concurrent Session 9: RNA in Disease (114-121) [Javier Martinez]	Panorama Hall (floor 1)
	Concurrent Session 10: Regulation & Mechanisms of RNA Turnover (122-130) [Kristian Baker]	Congress Hall
	Concurrent Session 11: RNA Catalysis/Folding (131-139) [Jeffrey Kieft]	Meeting Hall 1 (floor 1)
15:30 – 16:00	Coffee Break	Congress Hall Foyer 1B, 2B (floors 1 & 2)
17:30 – 18:30	Awards Ceremony	Congress Hall
19:00 – 24:00	Reception, Gala Dinner, Dance	Zofin Palace

## SUNDAY 4 JUNE

Conference Concludes

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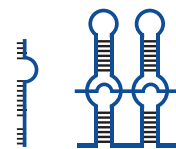
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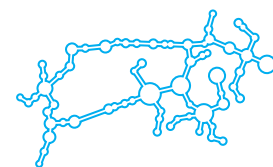
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# ADDENDUM to the PROGRAM – RNA 2017

May 30 – June 3, 2017  
Prague, Czech Republic

*Corrections, withdrawals and new abstracts received after the program book was printed.*

## SESSION CHANGES

**Friday, 2 June      09:00 – 12:30**  
**Plenary Session 3: Splicing**

**Congress Hall**

*Withdrawn*

- 62      Structural insight into the mechanism of splicing inhibition by modulators**  
*Vladimir Pena, Constantin Cretu*  
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

**Saturday, 3 June      14:30 – 17:15**  
**Concurrent Session 11: RNA Catalysis/Folding**

**Meeting Hall 1 (floor 1)**

*Change of Presenting Author*

- 133      Folding and splicing of group II intron ribozymes at the single molecule level**  
*Susann Zelger-Paulus, Roland K. O. Sigel*  
Department of Chemistry, University of Zurich, Zurich, Switzerland

## POSTER CHANGES

*Withdrawn*

- 211      Epigenetic switch of a macrosatellite repeat in colorectal cancer**

*Withdrawn*

- 216      Long non coding RNAs as New Paradigm for Lung Cancer Pathogenesis**

*Withdrawn*

- 220      Structural analyses of human and mouse NEAT1 lncRNAs suggest long-range RNA interactions contribute to paraspeckle architecture**

*Withdrawn*

- 293      Cytosine methylation by DNMT2 facilitates stability and survival of HIV RNA in the host cell during infection**

*Withdrawn*

- 305      RIsearch2-CRISPR: Predicting off-targets in CRISPR technology with energy models**

*Withdrawn*

- 357      A strategy for selectively altering genetic information at the level of RNA**

Correction to authors list

**389 Integrator complex and 3'-end snRNA processing**

*Eva Gomez-Orte, Beatriz Sáenz-Narciso, Begoña Ezcurra, Juan Cabello*  
CIBIR (Center for Biomedical Research of La Rioja), Logroño, La Rioja, Spain

Withdrawn

**497 RAIN: Inferring RNA-protein networks from associations and interactions**

Correction to authors list

**589 A novel type of intronic circRNA in nuclear genes of euglenids (Euglenida)**

*N. Gumińska, B. Zakryś, R. Milanowski*  
University of Warsaw, Warsaw, Poland

## ADDED POSTERS

Added in topic area *Emerging and High-throughput Techniques* (in the space vacated by withdrawn 167)

**167x Nanopores allow direct sequencing of full-length RNA strands and modified RNA nucleotides.**

*Elizabeth A. Snell, Daniel R. Garalde, Daniel G. Jachimowicz, Botond Sipos, Joseph H. Lloyd, Christopher Wright, Javier Blasco-Herrera, Anne-Marie Wadsworth, Clare Winter, Jemma Keenan, Anthony Warland, Jonah Ciccone, Nadia Pantic, David Alves, Andrew J. Heron, Lakmal Jayasinghe, Forrest Brennen, Jayne Wallace, James Clarke and Daniel J. Turner*

**Oxford Nanopore Technologies Ltd., 4 Robert Robinson Avenue, Oxford, OX4 4GP, United Kingdom**

Nanopores are the only sequencing technology which can sequence a strand of native RNA directly without the need to convert to DNA. The direct RNA library prep developed by Oxford Nanopore Technologies Ltd is simple, whereby the RNA-specific motor-protein is ligated directly to the RNA molecule and then the prep is loaded on the flow cell and the RNA strand translocates through the nanopore, blocking the current at specific levels allowing the individual bases to be read and identified. Without the need to convert to DNA, long RNA molecules are able to be sequenced; for example we sequenced the full Human Rhinovirus (HRV) genome (~7 kb) as one full-length transcript using a single-stranded RNA protocol. Furthermore, the lack of amplification should reduce quantitation bias. To test this, and to ensure the ability to determine spliceosomal isoforms is straightforward, we sequenced Lexogen's SIRV panel and were able to discern different isoforms, but we did observe some mismapping due to sequences with high degrees of similarity. However we experienced a high correlation of expected *versus* observed identified read counts when we sequenced the ERCC panel of 92 different transcripts (Spearman  $r = 0.97$ ;  $p = 5.9e-56$ ). We expect these both to improve with further enhancements to accuracy. The ability to sequence long read lengths allowed us to distinguish two highly abundant isozymes of GAPDH among *Saccharomyces cerevisiae* S228C direct mRNA transcriptome sequences. Perhaps most importantly, directly sequencing native RNA allows us to determine modified bases present based on distinct current-blocking signals. As such, we are able to discern both m6A and m5C bases from their canonical rAMP and rCMP counterparts in modified and unmodified fLuc transcripts which would normally be lost or converted by traditional sequencing methods. With Oxford Nanopore's Direct RNA sequencing kit, complete RNA strands can be sequenced on the MinION™, GridION™ and PromethION™ using a simple library prep, without the need to convert to double-stranded DNA.



Added in topic area *Long Non-coding RNAs* (in the space vacated by withdrawn 214)

**214x The Xist A-repeat fragment associates with chromatin through its conserved region independent of chromosomal accumulation.**

*Yuta Chigi<sup>1</sup>, Hiroyuki Sasaki<sup>2</sup> and Takashi Sado<sup>1</sup>*

<sup>1</sup>Department of Advanced Bioscience, Graduate School of Agriculture, Kindai University, Nara, Japan;

<sup>2</sup>Division of Epigenomics and Development, Medical Institute of Bioregulation, Kyushu-University, Fukuoka, Japan.

X inactive-specific transcript (Xist) is a long non-coding RNA that plays an essential role for X chromosome inactivation. Although Xist RNA like common protein coding mRNAs is transcribed by RNA polymerase II, spliced and polyadenylated, it is retained in the nucleus and associates with the X chromosome it originates from. A previous study showed that hnRNP U is required for the accumulation of Xist RNA on the inactive X chromosome (Xi). However, we found that Xist RNA dissociated from Xi upon knockdown of hnRNP U was still localized in the nucleus with apparent association with chromatin. This raises the possibility that a distinct mechanism might operate to promote chromatin association of Xist RNA, which is independent of the hnRNP U-mediated mechanism. Analysis of the previous CLIP-seq data set in human cultured cells revealed that hnRNP U was poorly localized in the first 950-nt region of XIST RNA. Assuming that the corresponding region in mouse Xist RNA was also free of hnRNP U, we overexpressed RNA coding for this mouse sequence and examined its behavior. The result demonstrated that this fragment associated with chromatin through its conserved repeat sequence that is important for Xist RNA-mediated silencing. Our results suggest that the A-repeat captures and relocates local chromatin nearby sites, at which Xist RNA is initially loaded upon the initiation of X inactivation, into the core of heterochromatin domain.

Added in topic area *Long Non-coding RNAs* (in the space vacated by withdrawn 220)

**220x Structure-functional studies on human long non-coding RNA MEG3**

*Tina Uroda<sup>1</sup>, Jean-Marie Teulon<sup>2</sup>, Jean-Luc Pellequer<sup>2</sup>, Marco Marcia<sup>1</sup>*

<sup>1</sup> EMBL Grenoble, Grenoble, France; <sup>2</sup> Institut de Biologie Structurale, Grenoble, France

Human maternally expressed gene 3 (MEG3) is an imprinted, alternatively-spliced long non-coding RNA (lncRNA) possessing a key role as a tumor suppressor. MEG3 activates p53 inhibiting cell proliferation and it interacts with *Polycomb* group proteins controlling cell differentiation. Evidence suggests that MEG3 is highly structured and that its structure regulates its cellular functions. For instance, MEG3 is well conserved in mammals and has an unusually high GC content. Moreover, MEG3 forms putative secondary structure motifs, whose disruption impairs p53 activation (Zhang et al., 2010). Such motifs may also affect splicing efficiency and thus the abundance of its 27 splicing variants, which exhibit different p53 activation capacity (Zhang et al., 2010). Thus, there may be a correlation between exonic organization, structural architecture and function. Finally, MEG3 structure may participate in protein recognition, particularly *Polycomb* group proteins, which curiously do not possess canonical RNA binding domains.

While the physiological and medical relevance of MEG3 is known, its molecular mechanism still remains largely unexplained, partly because little is known of its biochemical and structural properties. Therefore, in our project we set out to determine MEG3 secondary and tertiary structure with an unconventional biochemical and biophysical approach.

We purified highly homogeneous MEG3 samples in non-denaturing conditions (Chillon et al., 2015), which allowed us to obtain the first experimentally-determined MEG3 secondary structure map by chemical probing (SHAPE). Such secondary structure map reveals that MEG3 is organized in highly-structured domains, which we are going to visualize in 3D by small-angle X-ray scattering and atomic force microscopy. By *in vivo* and *in vitro* functional assays we will now also establish precise correlations between these structural domains and the functional roles of MEG3 in chromatin remodelling and cell cycle regulation.

References:

Zhang X, et al. Maternally expressed gene 3 (MEG3) noncoding ribonucleic acid: isoform structure, expression, and functions. *Endocrinology* 2010; 151: 939-47.

Chillon I, et al. Native Purification and Analysis of Long RNA. *Methods in Enzymology* 2015; 558, 3-37.

Added in topic area *RNA Bioinformatics* (in the space vacated by withdrawn 305)

**305x Identification of internal priming events in Lexogen's QuantSeq library preparation leads to accurate gene quantification and detection of 3' ends**

Patrick Schagerl, Michael Ante, Lukas Paul and Andreas Tuerk  
Lexogen GmbH, Campus Vienna Biocenter 5, Vienna, Austria

Accurate gene quantification and identification of 3'UTRs is important in many areas of biological research. Lexogen's QuantSeq library preparation provides an easy and fast protocol for generating highly strand-specific NGS libraries close to the 3' end of polyadenylated RNAs. The use of oligo(dT) primers in QuantSeq, however, also generates reads at long internal poly(A) stretches. Such reads can negatively affect the accuracy of end site detection and gene quantification. We, therefore, developed an internal priming filter (IPF) to remove reads associated with internal priming events. For this purpose, we investigate properties of the genome sequence and annotation around the read ends. In a window immediately downstream of the read end we count the number of A's, upstream we search for 3' motifs. We find the optimal cut-off for the number of A's by fitting a logistic regression model to sets of high-confidence internal priming and end sites having the same size ratio as internal priming and end sites in one chromosome. This ratio is determined by fitting a Gaussian mixture to the bimodal distribution of A-count frequencies. On the MAQC dataset the IPF retained 85.32% of the original reads in 3'UTRs, whereas only 5% and 3.68% were retained in 5'UTRs and introns, respectively. An increased concentration of reads at the gene ends can also be observed in coverage plots. Base content around priming sites after IPF showed the typical end site signature with an increased frequency of CA at the read end site, A upstream and GU/U downstream. Correlation between log fold changes of read counts and qPCR values increased after IPF from  $R^2=0.7234$  to  $R^2=0.8175$  suggesting superior accuracy in detecting differentially expressed genes. The described method is part of the QuantSeq pipeline including gene quantification and differential expression analysis which can be downloaded from the Lexogen website.

Added in topic area *RNA Processing* (in the space vacated by withdrawn 386)

**386x Long read sequencing of nascent RNA in *S. pombe* reveals coupling among RNA processing events**

Lydia Herzel, Korinna Straube, and Karla M. Neugebauer

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven USA

Pre-mRNA splicing is mediated by the spliceosome, a megadalton complex that assembles anew on each intron during transcription by RNA polymerase II (Pol II). We recently used single molecule RNA-Seq methods to determine the position of Pol II within budding yeast genes when the second step of splicing was completed<sup>1</sup>. Our data indicate that the spliceosome can act on the 3' splice site as soon as it emerges from Pol II. This leads to the prediction that splicing of multi-intron transcripts occurs in the order of their transcription, unless regulated by alternative splicing. We evaluated this in the fission yeast, *S. pombe*, which harbors >1000 genes with more than one constitutively spliced intron, by long-read sequencing of nascent transcripts. Similar proportions of 'in order' and 'not in order' splicing were observed, suggesting that splicing in order is not enforced and/or can be regulated. However, partially spliced transcripts were surprisingly rare when compared to a simulation based on individual intron splicing efficiencies determined from 75bp reads: 2.5-fold fewer partially spliced transcripts were observed (18%) than expected (45%). The largest fraction was fully spliced or unspliced, indicating that splicing of any given intron may depend on the splicing status of the other introns in the transcript. We show that fully unspliced transcripts failed to cleave at the polyA site, underwent transcriptional read-through at gene 3'ends, and were degraded by the exosome. Specific cases of retained individual introns were transported to the cytoplasm. We conclude that intron splicing and transcriptional read-through are dependent on the splicing status of neighboring introns, suggesting crosstalk among the spliceosomes and the 3'end processing machinery as they assemble during transcription.

<sup>1</sup> Carrillo Oesterreich, F., L. Herzel, K. Straube, K. Hujer, J. Howard, and K.M. Neugebauer. 2016. Splicing of nascent RNA coincides with intron exit from RNA polymerase II. *Cell* 165: 372-381.

Added in topic area *RNA Structure and Folding* (position between 455 and 456)

**ADD1 Combining asymmetric <sup>13</sup>C-labeling and isotopic filter/edit NOESY: rapid and logical RNA resonance assignment for identification of binding sites in RNA drug discovery**

*Regan M. LeBlanc*<sup>1,2</sup>, *Andrew P. Longhini*<sup>1</sup>, *Fardokht A. Abulwerdi*<sup>2</sup>, *Stuart F.J. Le Grice*<sup>2</sup>, *Theodore K. Dayie*<sup>1</sup>

<sup>1</sup> Center for Biomolecular Structure and Organization, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD, United States; <sup>2</sup> Basic Research Laboratory, National Cancer Institute, Frederick, MD, United States

RNA is an underutilized target for drug discovery. In fact, less than 1% of all approved drugs do not bind protein targets and more than 80% of drugs target only two classes of proteins: enzymes and receptors. RNA plays a critical role in essentially all aspects of biology including signaling, gene regulation, catalysis, and retroviral infection suggesting RNA is a promising target for drug discovery. However, less than 2% of the protein data bank structures comprise RNA yielding sparse structural information for rational drug design. The combined difficulties of crystallizing RNA for X-ray crystallography along with the rapid signal decay and spectral crowding associated with NMR have led to a stagnation in RNA structure publication. While half of the deposited RNA structures in the PDB were solved by NMR methods, the usefulness of NMR is still limited by the high cost of sample preparation and difficulties in resonance assignment. Here we propose a novel strategy for resonance assignment that combines new strategic <sup>13</sup>C labelling technologies with filter/edit type NOESY experiments to greatly reduce spectral complexity and crowding. This new strategy allowed us to assign important non-exchangeable resonances of proton and carbon (1', 2', 2,5,6, and 8) using only one sample and less than 24 hours of NMR instrument time for a 27 nt model RNA. The method was further extended to assigning a 6 nt bulge from a 61 nt viral RNA element justifying its use for wide range RNA chemical shift resonance assignment. Combining developments in fast pulsing techniques and non-uniform sampling methods, these improvements in resonance assignment methods have allowed us to implement chemical shift perturbation mapping as a preferred screening method for RNA drug discovery at greatly reduced costs.

Added in topic area *RNA Structure and Folding* (position between 455 and 456)

**ADD2 A case of polymorphism in a tetraloop due to a crystal packing interaction**

*Mélanie Meyer*<sup>1</sup>, *Vincent Olieric*<sup>2</sup>, *Jiro Kondo*<sup>3</sup>, *Henrik Nielsen*<sup>4</sup>, *Steinar Johansen*<sup>5</sup>, *Benoît Masquida*<sup>6</sup>

<sup>1</sup> Former address: Département de Biologie et de Génomique Structurales, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR7104, INSERM U964, Université de Strasbourg, Illkirch, France; <sup>2</sup> Swiss Light Source at Paul Scherrer Institute, Villigen, Switzerland; <sup>3</sup> Department of Materials and Life Sciences, Sophia University, Yotsuya Campus, Tokyo, Japan; <sup>4</sup> Department of Cellular and Molecular Medicine, The Panum Institute, University of Copenhagen, Copenhagen, Denmark; <sup>5</sup> Department of Medical Biology, University of Tromsø, Tromsø, Norway; <sup>6</sup> UMR 7156 GMGM - Centre National de la Recherche Scientifique, Université de Strasbourg, Strasbourg, France

To generate a single RNA conformer of the Lariat-Capping (LC) ribozyme, we engineered a circular permutation leading to the opening of the scissile bond and to the closure of the natural 5' and 3' extremities using a UUCG tetraloop [1,2]. The loop was supposed to have no effect on the whole structure. When we solved the structure, we noticed that the loop actually opened up in order to weave intimate interactions with a symmetry-related ribozyme molecule.

In order to check whether the conformation of the loop was due to the interaction, or if it opened up due to the design of our construct, we shortened the stem bearing the UUCG loop by one base-pair and solved the structure of this new construct. The crystal structure shows that in this slightly different context, the loop actually adopts the UUCG closed conformation that is observed in general.

We can conclude from these crystal structures that UUCG are indeed polymorphic and dynamic, and can serve interaction purposes, a function usually devoted predominantly to the GNRA tetraloops. This finding changes somehow the status of this kind of loops, which can be thought as a sensor as well as their cousins, the GNRA tetraloops. This finding may also orient future research to better characterize these loops to use them as crystallization modules among other applications.

1. Meyer, M.; Nielsen, H.; Olieric, V.; Roblin, P.; Johansen, S.D.; Westhof, E.; Masquida, B. (2014) Speciation of a group I intron into a lariat capping ribozyme. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 7659-7664.

2. Meyer, M.; Masquida, B. (2014) Cis-acting 5' hammerhead ribozyme optimization for in vitro transcription of highly structured RNAs. *Methods in molecular biology*, 1086, 21-40.

Added in topic area RNA Turnover (position between 496 and 497x)

**ADD3 NAD<sup>+</sup>-capped RNA and deNADding Enzymes in Human Cells**

Xinfu Jiao<sup>1</sup>, Selom K. Doamekpor<sup>2</sup>, Jeremy G. Bird<sup>3</sup>, Bryce E. Nickels<sup>3</sup>, Liang Tong<sup>2</sup>, Ronald P. Hart<sup>1</sup> and Megerditch Kiledjian<sup>1</sup>

<sup>1</sup>Dept. Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ, USA; <sup>2</sup>Dept. Biological Sciences, Columbia University, New York, NY, USA; <sup>3</sup>Department of Genetics and Waksman Institute, Rutgers University, Piscataway, NJ, USA

Eukaryotic mRNAs are post-transcriptionally modified at the 5' end by the addition of a m<sup>7</sup>G-cap to promote mRNA processing, translation and stability. The 5' end could be further modified by additional methylations, including generation of a trimethylated, m<sup>2</sup>,2'<sup>7</sup>G cap or the production of Cap1 by methylation at the 2'O-ribose of the first nucleotide. More recently prokaryotic mRNAs were found to carry an unusual 5' end modification consisting of a nicotinamide adenine dinucleotide (NAD<sup>+</sup>) moiety. This modification is proposed to protect the RNA from 5' end decay by the bacterial RppH and RNaseE nucleases in vitro unless the NAD<sup>+</sup> cap is hydrolyzed by the bacteria Nudix motif protein, NudC. We now demonstrate that mammalian mRNAs can also carry a 5'-end NAD<sup>+</sup> cap and in contrast to the m<sup>7</sup>G cap, does not support translation but instead promotes mRNA decay. RNAs introduced into mammalian cells harboring a 5'-end NAD<sup>+</sup> cap was less stable than RNAs with a m<sup>7</sup>G-cap or uncapped RNAs and failed to support translation any better than uncapped RNA. We further identify the DXO noncanonical decapping enzyme family of proteins as potent "deNADding" enzymes that efficiently remove NAD<sup>+</sup> caps. Cocystal structures of mammalian DXO and fungal Rai1 with 3'-NADP<sup>+</sup> illuminate the molecular mechanism for how the deNADding reaction produces NAD<sup>+</sup> and 5'-phosphate RNA. Removal of DXO from cells increases NAD<sup>+</sup>-capped mRNA levels and enables detection of NAD<sup>+</sup>-capped intronic snoRNAs, suggesting NAD<sup>+</sup> caps can be added to 5'-processed termini by an unknown NAD-capping mechanism. Our findings establish NAD<sup>+</sup> as an alternative mammalian RNA cap and DXO as a deNADding enzyme modulating cellular levels of NAD<sup>+</sup>-capped RNAs. Collectively, these data reveal mammalian RNAs can harbor a 5'-end modification distinct from the classical m<sup>7</sup>G cap that promotes, rather than inhibits, RNA decay.

Added in topic area RNA-protein Interactions (in the space vacated by withdrawn 497)

**497x New Insights into RNA Structure and Dynamics of *Trypanosoma brucei* Telomerase**

Abhishek Dey<sup>1,2</sup>, Rania Elbarki<sup>1,2</sup>, Ranjodh Sindhu<sup>3</sup>, Samantha Sanford<sup>4</sup>, Niyati Jain<sup>5</sup>, Blanton Tolbert<sup>5</sup>, Bibo Li<sup>3</sup> and Kausik Chakrabarti<sup>1,2</sup>

<sup>1</sup>Dept. of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA; <sup>2</sup>Center for Nucleic Acid Science and Technology (CNAST), Carnegie Mellon University, Pittsburgh, PA, USA; <sup>3</sup>Department of Biological, Geo and Environmental Sciences, Cleveland State University, Cleveland, OH, USA; <sup>4</sup>University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA; <sup>5</sup>Dept. of Chemistry, Case Western Reserve University, Cleveland, OH, USA

The control of cell proliferation is of central importance to the proper development of most organisms. Increased cell proliferation is essential to cancer and many lymphoproliferative diseases. Also, rapid and continuous cell proliferation is required by unicellular parasitic organisms to sustain long-term infection and cause disease pathogenesis. One of the major regulators of cellular proliferation in eukaryotes is a large RNA-protein enzyme complex, called Telomerase (1). Although much is known about telomerase regulation and function in higher eukaryotes, the intricate details of telomerase catalysis for chromosome end replication is poorly understood in pathogenic protists such as *Trypanosoma sp.*, which represent the early branches eukaryotic phylogeny and cause devastating consequences on world health and economy with neurodegenerative and heart diseases. This is mainly because the RNA component of this enzyme, which provides the critical active site function by binding to the telomeric DNA substrate at the chromosome end, was unknown (2). Recently, we have identified the telomerase RNA in *T. brucei* (3) and provided genetic and biochemical evidence for its activity. Remarkably, this RNA has several unique features that suggest a mechanistically different process of telomerase-mediated telomere maintenance at the chromosome ends in *T. brucei* compared to yeast, mammalian, and Tetrahymena models. First, the RNA 'template' domain contains unique sequence permutations, suggesting an RNA-dependent, rather than a protein-dependent, anchoring interaction of the enzyme to its cognate substrate. Second, *T. brucei* and humans make identical telomeric DNA repeats (TTAGGG) using template domains that are vastly different in sequence composition, suggesting discrete

pathways of telomere replication. Third, many Telomerase RNA domains that interact with its integral *Reverse Transcriptase* protein component, are a composite of features from Telomerase RNAs of various organisms, however their functional significance remains unknown. Using RNA-SHAPE, NMR and functional assays, we have determined the structure and folding of the ‘catalytic core’ of this RNA molecule in *T.brucei* which provide in-depth understandings of the mechanistic aspects underlying RNA structure formation. These data should further our knowledge on understanding novel aspects of telomerase regulation in deep-branching eukaryotes.

#### References:

1. Blackburn EH, Greider CW, Szostak JW. 2006. Telomeres and telomerase: the path from maize, Tetrahymena and yeast to human cancer and aging. *Nat Med.* 12(10):1133-8.
2. Muñoz DP, Collins K. 2004. Biochemical properties of *Trypanosoma cruzi* telomerase. *Nucleic Acids Res.* 32(17): 5214-22.
3. Sandhu R, Sanford S, Basu S, Park M, Pandya UM, Li B, Chakrabarti K. 2013. A trans-spliced telomerase RNA dictates telomere synthesis in *Trypanosoma brucei*. *Cell Research* 23:537-551.

*Added in topic area RNAs in Disease (position between 567 and 568)*

### **ADD4 The serotonin receptor 2C in pituitary is deregulated in Prader-Willi syndrome**

*Justin Welden<sup>1</sup>, Zhaiyi Zhang<sup>1</sup>, Tim Wells<sup>2</sup> and Stefan Stamm<sup>1</sup>*

<sup>1</sup>University of Kentucky, Lexington, KY, USA; <sup>2</sup>Cardiff University, Cardiff, Wales, UK

The serotonin receptor 2C is a seven transmembrane receptor regulating mood and appetite in the central nervous system. Its pre-mRNA undergoes alternative splicing of exonVb creating two 5HT2C isoforms: RNA1 that encodes a truncated receptor and RNA2 that encodes a full-length receptor. Due to additional editing, the 5HT2C generates a total of 33 mRNAs encoding 25 proteins. ExonVb inclusion is promoted by a snoRNA, SNORD115, likely by changing the pre-mRNA structure around exonVb. SNORD115 is missing in Prader-Willi syndrome, a genetic cause for obesity and intellectual disability. The truncated receptor encoded by RNA1 heterodimerizes with the full-length receptor (RNA2), leading to an internalization of the full-length receptor stopping 5HT2C signaling. Thus, the isoform ratio between RNA1 and RNA2 controlled by alternative splicing regulates the activity of the 5HT2C, and possibly other receptors, such as the ghrelin receptor (GHSR1) due to heterodimerization.

Short stature caused by low growth hormone levels is a characteristic feature of PWS. Growth hormone is secreted after ghrelin receptor activation from the pituitary. Since growth hormone deficiency is central to PWS, we tested expression of the 5HT2C in pituitary and detected expression of RNA1 and RNA2 in mice, human and rat pituitaries. In addition, SNORD115 that regulates alternative splicing of 5HT2C is expressed as well, suggesting a regulation of the 5HT2C isoforms through SNORD115 in pituitary. We therefore analyzed mice that lack SNORD115 and found a 1.6 fold increase of the RNA1/RNA2 ratio, concomitant with the loss of SNORD115.

We next tested the effect of food withdrawal on the 5HT2C isoforms and found an almost complete loss of RNA1 after 48 hrs of fasting, suggesting that 5HT2C isoform ratios are under control of physiological stimuli. We previously developed an oligonucleotide <sup>1</sup> that promotes exonVb inclusion, similar to SNORD115 and found that after tail vein injection, this oligo strongly promotes exonVb inclusion in pituitary, further demonstrating a regulation of the RNA1/RNA2 ratio.

Our data suggest that the 5HT2C plays, so far, an unknown role in pituitary, possibly regulating growth hormone release through heterodimerization with other receptors, such as the GHSR1a. It is likely deregulated in PWS, contributing to the hormonal distortions characteristic for the disease.

1.Zhang Z, Shen M, Gresch P, Ghamari-Langroudi M, Rabchevsky AG, Emeson RB, Stamm S. Oligonucleotide-induced alternative splicing of serotonin 2C receptor reduces food intake. *EMBO molecular medicine.* 2016;8(8):878-94.PubMed PMID: 27406820.



Added in topic area *RNAs in Disease* (position between 567 and 568)

**ADD5 Modeling retinitis pigmentosa - investigation of changes in pre-mRNA binding and alternative splicing to understand the onset and cause of photoreceptor degeneration**

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Background and aims: Germline mutations in genes encoding components of the pre-mRNA splicing machinery underlie development of retinitis pigmentosa (RP), a degenerative disease characterized by progressive loss of photoreceptor cells. Given the eye-exclusive manifestation, the phenotype suggests enhanced sensitivity of ocular cell populations for proper spliceosome assembly and/or its outcomes. Using a blend of *in vivo* and *in vitro* models, we aim to specifically explore the molecular principles by which aberrant splicing events contribute to retinal malfunction.

Methods and Results: We used TALEN and CRISPR/Cas9 genome editing tools to establish novel murine strains harboring the RP-causative substitution mutations Prpf3 Thr494Met, Prpf8 Tyr2334Asn, and moreover Brr2 Ser1087Leu. Secondary to correct SNPs, we also gained *Prpf3* and *Prpf8* null alleles by small Indel events; the latter strains serve comparison with presumptive loss-of-function RP mutations. In aging mice, the retinal condition is being repeatedly assessed using noninvasive optical coherence tomography and electroretinography, with histopathological analysis performed in parallel cohorts. In addition, physiological as well as diseased retinal transcriptome and the related splicing patterns will be examined by deep sequencing of the RP-mutant and wild-type counterpart retinæ.

In parallel, we used corresponding nuclease editing systems to introduce tagged variants of RP-relevant splicing factors to cultured, human retina-derived cells RPE-1. Here, we will employ the iCLIP technique to explore, whether altered magnitude of binding and/or specificity in site recognition may lead to aberrant splicing events.

Conclusions: We have established an innovative array of *in vivo* and *in vitro* tools to scrutinize the splicing execution in RP-compromised retinal cells. Using transcriptome-wide data in combination with protein-RNA interaction assays, we hope to dissect how inappropriate splicing corrupts the fitness of RP-stricken retinal cell populations.

Added in topic area *Splicing Mechanisms* (position between 605 and 606)

**ADD6 An Atomic Structure of the Human Spliceosome**

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Mechanistic understanding of pre-mRNA splicing requires detailed structural information on various states of the spliceosome. Here we report the cryo-EM structure of the human spliceosome just prior to exon ligation (the C\* complex) at an average resolution of 3.76 Å. The local resolution for about 20 components in the core of the spliceosome reaches 3.0-3.5 Å, allowing identification of amino acid side chains and assignment of RNA nucleotides. Compared to the yeast C\* complex, a notable difference is formation of a considerably longer duplex between U6 snRNA and the 5'SS and ensuing sequences of the intron in the human C\* complex. The splicing factor Prp17 stabilizes the active site conformation by interacting with multiple proteins. The step II factor Slu7 adopts an extended conformation, binds Prp8 and Cwc22, and is poised for selection of the 3'-splice site. Remarkably, the intron lariat traverses through a positively charged central channel of RBM22; this unusual organization suggests mechanisms of intron recruitment, confinement, and release. The protein PRKRIP1 forms a 100-Å α-helix linking the distant U2 snRNP to the catalytic center, suggesting an important role in splicing reaction. A 35-residue fragment of the ATPase/helicase Prp22 latches onto Prp8. The distance between the 3'-end of the intron lariat and the RNA binding site of Prp22 spans approximately 100 Å, which requires a minimal of 15 nucleotides in their fully extended conformation. The quaternary exon junction complex (EJC) recognizes upstream 5'-exon sequences and associates with Cwc22 and the GTPase Snu114. These structural features reveal important mechanistic insights into exon ligation.

**676x RNA-binding proteins influence stem cells' fate decisions**

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Post-transcriptional regulation of gene expression plays an important role during fundamental physiological processes in eukaryotes. The decision whether stem cells will keep their self-renewal potency or switch into differentiation pathway occurs at each stem cell division and has to be under strict control by numerous stem-cell maintenance and differentiation factors. Fruit fly early germline development has been used as a powerful model for studying translational control. The precise translational regulation employs diverse RNA-binding proteins (RBPs) and chemical modifications which have a crucial effect on adjusting the translation and protein folding dynamics. By forming ribonucleoproteins (RNPs) upon binding to specific motifs of mRNA transcripts, RBPs act as regulatory elements and provide additional level of translational control through RNPs' dynamic and constant remodeling events. Any defects in this regulation may lead to continuous proliferation of stem cells resulting in carcinogenesis and infertility.

To further our understanding of how mRNA transcripts are efficiently "fine-tuned" by RNPs assembly and control protein translation, we have chosen a novel macromolecular complex involved in translational repression during stem cells switch to differentiation pathway. Translational repression of particular transcripts relies on protein-RNA and protein-protein interactions. Upon binding of RBPs to U-rich RNA elements present in the 3' untranslated region (3' UTR) of mRNA, the synthesis of stem cells maintenance factors is inhibited and differentiation events occur. Our knowledge on how RBPs orchestrate mRNA binding and subsequently control translation remains limited.

To study regulatory mechanisms of protein synthesis we combine protein biochemistry and biophysics with structural biology. By determining structural characteristic of RBPs interactions with molecular partners (RNA and other proteins) we plan to identify exact protein domains and RNA motifs important for translational regulation during oogenesis. To address the hierarchy of RNPs assembly events within the repressive complex we perform protein interaction analyses and competition assays of reconstituted sub-complexes. This approach allows to determine if RNA-binding proteins bind cooperatively or stepwise to the regulatory motifs on mRNA transcripts. Through gaining details about specific inhibitory protein-RNA interactions and RNPs formation we aim to deepen our knowledge of important regulatory processes that determine cell fate.

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