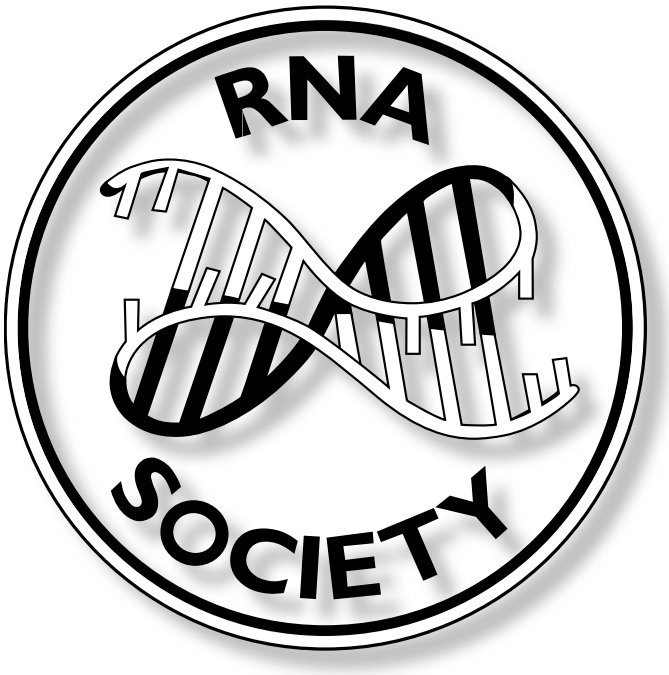
RNA 2013

The 18th Annual Meeting of the RNA Society

PROGRAM & ABSTRACTS



June 11–16, 2013

Davos, Switzerland

Frédéric Allain, *ETH-Zürich*
Witold Filipowicz, *Friedrich Miescher Institute*
Adrian Krainer, *Cold Spring Harbor Laboratory*
Osamu Nureki, *University of Tokyo*
Sarah Woodson, *Johns Hopkins University*

ACKNOWLEDGEMENTS

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

The organizers wish to thank Dr. Marc Ruepp and Ms Isabelle Allen for their tremendous effort in securing sponsorship support

Throughout the program listing, the numbers next to the titles refer to corresponding Oral or Poster numbers in the Abstract section of this book. The letters next to each poster abstract designates the poster session within which that poster will be presented. These abstracts should not be cited in bibliographies. Material contained herein should be treated as personal communication, and should be cited only with the consent of the author.

To encourage sharing of unpublished data at the RNA Society Meeting, taking of photographs and/or videos during scientific sessions (oral or posters), or of posters outside of session hours, is strictly prohibited. Violators of this policy may have their equipment confiscated (cameras, cell phones, etc.) and/or they may be asked to leave the conference and have their registration privileges revoked without reimbursement.

Front Cover

View of the village of Sertig Dörfli in the Sertig Valley, approx. 8 km south of Davos. The massifs of the Mittaghorn are in the background [photo by Raphael Koch, reprinted with permission from the Davos Congress Centre]. Floating above the village is the crystal structure of yeast Prp8, a component of the U5 small nuclear ribonucleoprotein particle, in complex with Aar2, a U5 assembly factor [Galeij et al. (2013) Nature 493: 638]. The structure reveals the active site cavity of the spliceosome. Nestled in the valley is a cocrystal structure of the Stem I domain of the bacterial T-box riboswitch recognizing its cognate tRNA. [Zhang & Ferré-D'Amaré, manuscript in preparation].

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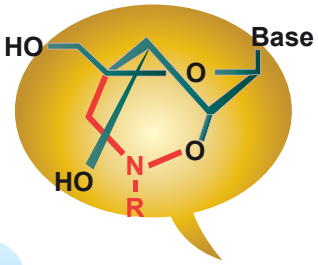

Biotechnologies

- Antisense
- RNA Interference
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- Antigene

- Chromosomal FISH Probes
- LightUp Probes
- DNA Chip
- Other tools for molecular biology and functional genomics

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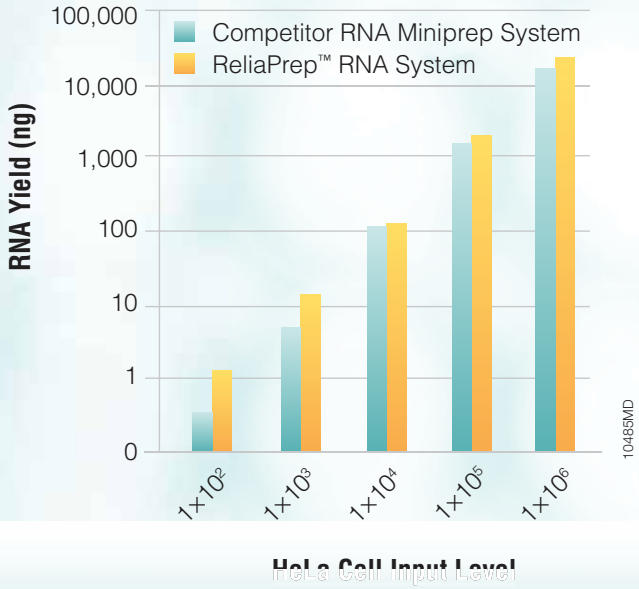

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RNA 2014

The 19th Annual Meeting of the RNA Society will be held in
Quebec City, Canada from June 3-8, 2014, at the Centre des Congrès de Québec

2014 Organizers

Benoît Chabot, *Université de Sherbrooke*
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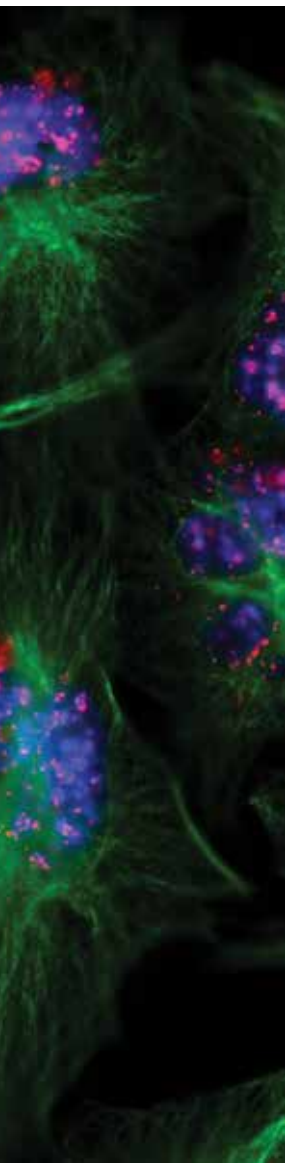

Frontiers in Basic Cancer Research
Chairperson: Scott W. Lowe
Co-Chairpersons: Joan S. Brugge, Hans Clevers, Carol L. Prives, and Davide Ruggero
September 18-22, 2013 • National Harbor, MD

Advances in Ovarian Cancer Research: From Concept to Clinic
Co-Chairpersons: David G. Huntsman, Douglas A. Levine, and Sandra Orsulic
September 18-21, 2013 • Miami, FL

Advances in Breast Cancer Research
Co-Chairpersons: Carlos L. Arteaga, Jeffrey M. Rosen, Jane E. Visvader, and Douglas Yee
October 3-6, 2013 • San Diego, CA

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics
Co-Chairpersons: Jeffrey A. Engelman, Lee J. Helman, and Sabine Tejpar
October 19-23, 2013 • Boston, MA

Twelfth Annual International Conference on Frontiers in Cancer Prevention Research
Chairperson: Paul J. Limburg
October 27-30, 2013 • National Harbor, MD

Pediatric Cancer at the Crossroads: Translating Discovery into Improved Outcomes
Co-Chairpersons: John M. Maris, Stella M. Davies, James R. Downing, Lee J. Helman, and Michael B. Kastan
November 3-6, 2013 • San Diego, CA

The Translational Impact of Model Organisms in Cancer
Co-Chairpersons: Cory Abate-Shen, A. Thomas Look, and Terry A. Van Dyke
November 5-8, 2013 • San Diego, CA

CTRC-AACR San Antonio Breast Cancer Symposium
Co-Directors: Carlos L. Arteaga, C. Kent Osborne, and Peter M. Ravdin
December 10-14, 2013 • San Antonio, TX

Sixth AACR Conference on The Science of Cancer Health Disparitites in Racial/Ethnic Minorities and the Medically Underserved
December 6-9, 2013 • Atlanta, GA

AACR-IASLC Conference on Molecular Origins of Lung Cancer
January 6-9, 2014 • San Diego, CA

AACR-Prostate Cancer Foundation Conference on Advances in Prostate Cancer Research
Co-Chairpersons: Arul M. Chinnaiyan, William G. Nelson, June M. Chan, and Jonathan W. Simons
January 18-21, 2014 • San Diego, CA

Cancer Susceptibility and Cancer Susceptibility Syndromes
Co-Chairpersons: Alan D. D'Andrea, Phillip A. Dennis and Pier Paolo Pandolfi
January 29-February 1, 2014 • San Diego, CA

AACR Annual Meeting 2014
April 5-9, 2014 • San Diego, CA

AACR EDUCATIONAL WORKSHOPS

Accelerating Anticancer Agent Development and Validation
Co-Chairpersons: H. Kim Lyerly and Richard Pazdur
May 8-10, 2013
Bethesda, MD

NEW! Integrative Molecular Epidemiology
Director: Thomas A. Sellers; Co-Directors: Peter L. Kraft and Margaret R. Spitz
July 15-20, 2013
Boston, MA

Molecular Biology in Clinical Oncology
Co-Directors: William G. Kaelin Jr., Mark Geraci, and Suzanne Topalian
July 21-28, 2013
Snowmass, CO

ASCO/AACR Methods in Clinical Cancer Research
Co-Directors: Jamie H. von Roenn, Neal J. Meropol, and Mithat Gönen
July 27-August 2, 2013
Vail, CO

Translational Cancer Research for Basic Scientists
Co-Directors: Tom Curran, George D. Demetri, and Pasi A. Jänne
November 10-15, 2013
Boston, MA

Invitation to Membership

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

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

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

Our members receive:

- Subscription to the Society journal, *RNA* (IF 6.051) with
  - 50% discount on page charges
  - 50% discount on first color figure charge (a savings of $225)
  - For those members who wish to have their articles completely open access immediately upon publication can do so at a reduced cost of $1500 (a $500 savings from non-member fee)
- Reduced registration fees for the annual meeting of the Society (more than $100 saving)
- The RNA Society Newsletter, a forum for disseminating information to members and discussing issues affecting the Society and RNA Science
- Numerous opportunities for junior scientists to become involved in the Society
- The Directory of Members, available online
- Free job postings on the Society website
- Opportunities to request Travel Fellowships and Meeting Support for RNA-related meetings you are organizing

These member savings more than offset the cost of a one-year membership in the Society. Two and three year memberships, as well as lifetime memberships, are now available through our online registration system with the added benefit of a discounted annual rate!

Take a moment to start or renew your membership using our online system at
http://rnasociety.org/become-a-member

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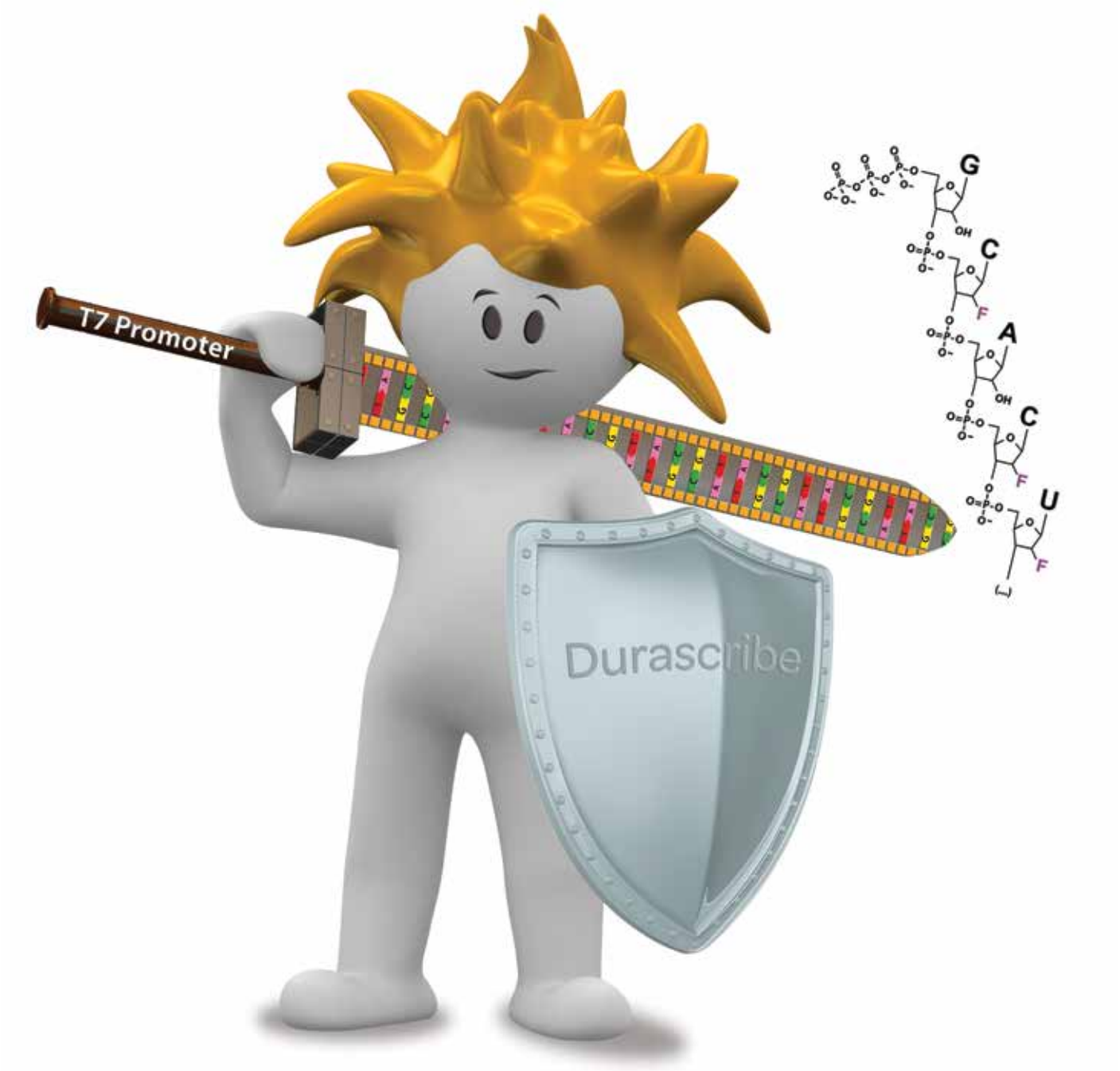

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PROGRAM – RNA 2013

The 18th Annual Meeting of the RNA Society
Davos, Switzerland
June 11 - June 16, 2013

Tuesday, June 11

11:00 – 19:00	**Registration**	Talstrasse Foyer
15:00 – 15:10	**Welcome, Opening Remarks**	Davos Ballroom
15:10 – 18:30	**Plenary Session 1: Ribosome biogenesis and translation (1 - 9, 397 B)** *Keynote:* **Venki Ramakrishnan** *(MRC-LMB, Cambridge)* *Chair:* **Nenad Ban** *(ETH Zürich)*	
18:30 – 23:00	**Welcome Grill Dinner and Beer Garden**	Kurpark, *adjacent to the Davos Congress Center*

Wednesday, June 12

07:30 – 18:30	**Registration**	Talstrasse Foyer
08:30 – 10:15	**Plenary Session 2A: Regulation by long non-coding RNAs (10 - 16)** *Chair:* **V. Narry Kim** *(Seoul National University)*	Davos Ballroom
10:15 – 10:45	Coffee Break	Foyer/Davos Ballroom
10:45 – 12:30	**Plenary Session 2B: RNA Modification (17 - 24)** *Chair:* **Juan Alfonzo** *(Ohio State University)*	Davos Ballroom
12:30 – 14:00	**Lunch**	Foyer/Davos Ballroom
14:00 – 16:30	**Concurrent Sessions**	
	Concurrent Session 1: Splicing mechanisms (25 - 33) *Chair:* **Soo-chen Cheng** *(Academia Sinica)*	Davos Ballroom
	Concurrent Session 2: RNA localization (34 - 42) *Chair:* **Ralf Jansen** *(Univ Tübingen)*	Aspen
	Concurrent Session 3: Viral RNAs (43 - 51) *Chair:* **Karen Beemon** *(Johns Hopkins University)*	Sanada
16:30 – 17:00	**Refreshment Break**	Foyer/Davos Ballroom

17:00 – 18:30 **Workshops**

Workshop 1: The Non-coding transcriptome (52 - 58) — Davos Ballroom
Chairs: **Constance Ciaudo** *(ETH Zürich) and* ***Claus Azzalin*** *(ETH Zürich)*
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Workshop 2: RNA chemistry (59 - 65) — Sanada
Chairs: **Ronald Micura** *(Leopold Franzens University) and* ***Mark Helm*** *(Johannes Gutenberg University)*

Workshop 3: Career Development Skills in Science — Aspen
Chair: **Michael Meers** *(University of North Carolina at Chapel Hill)*

18:30 – 20:10 **Buffet Dinner** — Foyer/Davos Ballroom

18:30 – 20:30 **Meetings Committee Meeting & Dinner** — Wisshorn

19:30 – 20:30 **Junior Scientists Social** — Chamonix

20:00 – 22:30 **Poster Session A** — Main Hallway and Sanada Foyer
Poster abstracts labeled with an "A" present

Sponsored by: Cell Press

	Abstracts
Non-coding and Regulatory RNAs	156 - 204, 717, 718
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RNA and Epigentics	223 - 231
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RNA Catalysis and Riboswitches	258 - 271
RNA structure and folding	272 - 297
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tRNA, snRNA, snoRNA, rRNA	316 - 334, 722
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RNP Structure, Function and Biosynthesis	580 - 598
Riboregulation in Development	599 - 600, 719
RNA Transport and Localization	601 - 609
RNAs in Diseases	610- 643
Viral RNAs	644 - 654
Interconnections Between Gene Expression Processes	655 - 671
RNA system biology	672 - 679
Bioinformatics	680 - 697
Emerging & High-throughput Techniques for RNA	698 - 716

21:30 – 23:30 **Beer Garden** — Kurpark

Thursday, June 13

08:00 – 12:30	**Registration**	Talstrasse Foyer
08:30 – 10:15	**Plenary Session 3A: RNA and disease (66 - 72)** *Chair:* **Tom Cooper** *(Baylor College of Medicine)*	Davos Ballroom
10:15 – 10:45	**Coffee Break**	Foyer/Davos Ballroom
10:45 – 12:30	**Plenary Session 3B: RNA systems biology (73 - 80)** *Chair:* **Brent Graveley** *(University of Connecticut)*	Davos Ballroom
12:30 – 13:00	**Takeaway Lunch**	Foyer/Davos Ballroom
12:30 – 20:00	**Free Afternoon**	
16:00 – 20:00	**Happy Hour in the Beer Garden** *Dinner on your own - snacks available for purchase in Beer Garden*	Kurpark
20:00 – 22:30	**Plenary Session 4: Architecture of RNPs (81 - 88)** *Keynote:* **Thomas R. Cech** *(HHMI, University of Colorado Boulder)* *Chair:* **Eric Westhof** *(University Strasbourg)*	Davos Ballroom

Friday, June 14

08:00 – 18:30	**Registration**	Talstrasse Foyer
08:30 – 10:15	**Plenary Session 5A: RNA Processing (89 - 95)** *Chair:* **Joan Steitz** *(HHMI, Yale University)*	Davos Ballroom
10:45 – 12:30	**Coffee Break**	Foyer/Davos Ballroom
	Plenary Session 5B: RNA decay (96 - 102) *Chair:* **Elena Conti** *(MPI Biochemistry)*	Davos Ballroom
12:30 – 14:00	**Lunch**	Foyer/Davos Ballroom
14:00 – 16:30	**Concurrent Sessions**	
	Concurrent Session 4: Regulation by small non-coding RNAs (103 - 111) *Chair:* **Jennifer Doudna** *(HHMI, Univ. of California - Berkeley)*	Davos Ballroom
	Concurrent Session 5: Structure, dyanamics, and catalysis (112 - 120) *Chair:* **David Rueda** *(Imperial College, London)*	Aspen
	Concurrent Session 6: High-throughput approaches to RNA biology (121 - 128) *Chair:* **Jernej Ule** *(University College London)*	Sanada

16:30 – 17:00	**Refreshment Break**	Foyer/Davos Ballroom
17:00 – 18:30	**Workshops**	
	Workshop 4: RNA in pharmaceutical research (129 - 134) *Chairs:* **Nicole Meisner** *(Novartis Institutes for Biomedical Research) and* ***David Morrissey*** *(Novartis Institutes for Biomedical Research)*	Davos Ballroom
	Workshop 5: The evolving landscape of scientific publishing and how best to evaluate scientific output ***This workshop will discuss how scientific articles and journals continue to evolve and will also raise questions about how best to evaluate the full spectrum of the scientific process.* *Chairs:* **Boyana Konforti** *(Cell Press)* and **Arianne Heinrichs** *(Nature Structural & Molecular Biology)*	Aspen
	Workshop 6: Tutorial on prediction of RNA secondary structure (135 - 141) *Chair:* **Alain Laederach** *(University of North Carolina at Chapel Hill)*	Sanada
18:30 – 20:00	**Buffet Dinner**	Foyer/Davos Ballroom
18:30 – 20:30	**Board of Directors Meeting and Dinner**	Wisshorn
20:00 – 22:30	**Poster Session B** ***Poster abstracts labeled with a "B" present*** *Sponsored by: Novartis* See Poster Session A for topic listing	Main Hallway and Sanada Foyer
21:30 – 23:30	**Beer Garden**	Kurpark

Saturday, June 15

08:00 – 18:30	**Registration**	Talstrasse Foyer
08:30 – 10:15	**Plenary Session 6A: Interconnections in RNA regulation (142 - 148)** *Chair:* **Ulrike Kutay** *(ETH Zürich)*	Davos Ballroom
10:15 – 10:45	**Coffee Break**	Foyer/Davos Ballroom
10:45 – 12:30	**Plenary Session 6B: Alternative splicing (149 - 155)** *Chair:* **Javier Cáceres** *(MRC Edinburgh)*	Davos Ballroom
12:30 – 14:00	**Lunch**	Foyer/Davos Ballroom
12:30 – 14:00	**Mentor - Mentee Lunch**	Aspen, Sanada, Chamonix

14:00 – 17:00	**Poster Session C** ***Poster abstracts labeled with a "C" present*** *Sponsored by: University of Zürich* See Poster Session A for topic listing	Main Hallway and Sanada Foyer
18:00 – 24:00	**Apero** **Banquet** **Awards Ceremony & Dance**	

Sunday, June 16

Conference Concludes

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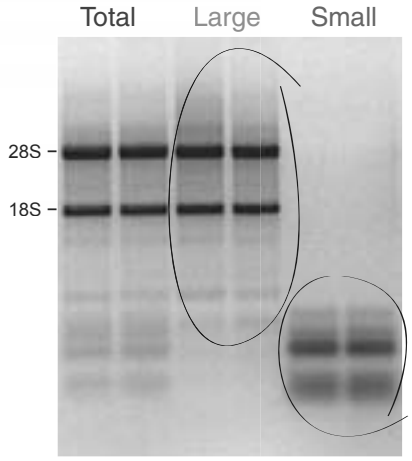
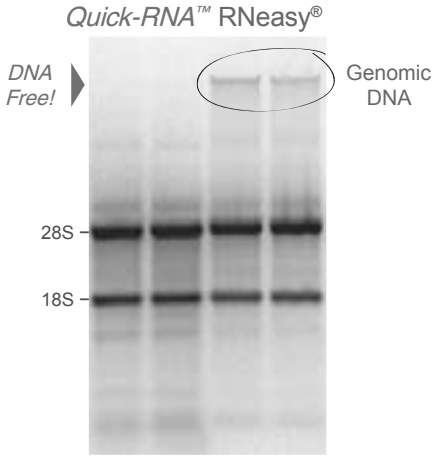


Quality



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ADDITIONAL SCHEDULED EVENTS AT RNA 2013

Junior Scientists Pre-Conference Hike and Lunch - Tuesday, June 11

09:00, See Facebook page for location

- Open to all graduate students and post docs
- No additional charge (bring money for lunch), no registration required

This is an informal gathering for graduate students and post docs to meet and socialize. It will be a great way to discover Davos and its surroundings and catch up with colleagues before the meeting starts. The tour will begin in the morning by taking the gondola lift to Rhinerhorn station, followed by a scenic hike around the mountain. We will then take a slowly descending path to Sertig Dörfli for lunch at a local restaurant. The planned hike is easy and does not change much in elevation, so it should be feasible for hikers of any skill level. In case of bad weather an alternative program will be prepared. A meeting point and maps will be available over the Facebook page and by email.

Meetings Committee Meeting - Wednesday, June 12

18:30-20:30, Wisshorn

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

This meeting is where the venues for future RNA Society meetings are reviewed and selected. Any member of the RNA Society is welcome to attend, but due to space constraints one should request participation in advance by sending an email to the Meetings Committee Chair, David Lilley (d.m.j.lilley@dundee.ac.uk).

Junior Scientists Career Development Workshop - Wednesday, June 12

17:00-18:30, Aspen

- Open to all attendees, but tailored for junior scientists

This is an opportunity for junior scientists to hear and discuss the issues of skill development for careers in science, with a focus on skills for career advancement regardless of career track. The session will touch on a wide range of common concerns related to initiating and advancing a career in science such as choosing the right career, career planning, time management, networking, resume building, work/life balance and other related skills that are recognized by hiring companies and institutions. The workshop will consist of a short talk from a career consultant followed by a panel discussion. Panel members include Elena Conti, Nicole Meisner-Kober, Brett Robb, and Françoise Stutz. This workshop should be particularly relevant to young members who are planning the next steps in their careers.

Junior Scientists Social - Wednesday, June 12

19:30-20:30, Chamonix

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

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

Beer Garden – Wednesday-Friday, June 12-14

June 12, 21:30-23:30, Kurpark
June 13, 16:00-20:00, Kurpark
June 14, 21:30-23:30, Kurpark

- Open to all attendees

Step outside for fresh air and a beer.

Free Afternoon – Thursday, June 13

12:30-20:00 Various options

Make your own decision about how to spend your free time enjoying all that Davos has to offer.

- **Hiking** Information about hiking options, including maps, will be provided at the end of the morning sessions.
- **Optional Excursions** Enjoy one of many excursions offered by Davos Services. If you booked an excursion through Davos Services during registration, you should have received a confirmation with details about your participation. If you have any questions, please contact Davos Services:

  Tourismus- und Sportzentrum
  Talstrasse 41
  Tel +41 81 415 23 23
  www.davos-services.ch
  ds@davos.ch
- **Lunch** is provided to all attendees, and you'll be able to take it with you – whether on a hike or just to enjoy outside in Kurpark.
- **Beer Garden** However you spend your afternoon, the beer garden will be open from 16:00 - 20:00 for happy hour, complete with snacks for purchase. Since dinner is "on your own" on Thursday, this might be a fun option!

Board of Directors Meeting – Friday, June 14

18:30-20:30 Wisshorn

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

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

Mentor/Mentee Lunch - Saturday, June 15

12:30-14:00, Aspen, Sanada, Chamonix

- Open to all attendees
- No additional charge, but registration is required before May 1

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.

Conference Closing Events - Saturday, June 15

Open to all attendees who pre-registered by May 15

- **Reception** 18:00-19:00, Davos Foyer
- **Conference Banquet** 19:00-19:45, Davos Ballroom
- **Awards Ceremony** 19:45-21:00, Davos Ballroom

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

  - Phillip Allen Sharp; RNA Society Lifetime Achievement Award
  - RNA Society/Scaringe Award winners
    - Wenwen Fang
    - Je-Hyun Yoon
  - Poster prize winners
- **Dessert and RNA Society Dance** 21:00-24:00, Davos Ballroom

  Celebrate a week of great science with live music!

RNA 2013 AWARDS

The RNA Society Lifetime Achievement Award

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

*Congratulations to **Phillip Sharp** who is the winner of the 2013 RNA Society Lifetime Achievement Award.*

The RNA Society Service Award

No award recipient was chosen for 2013

The RNA Society/Scaringe Award

The RNA Society/Scaringe Young Scientist Award was established to recognize the achievement of young scientists engaged in RNA research and to encourage them to pursue a career in the field of RNA. In 2004 and 2005, the RNA Society/Scaringe Award was made to the student author(s) of the best paper, as selected by the editors, published during the previous year in RNA. The winners of the 2004 and 2005 awards were Stefano Marzi and Ramesh Pillai, respectively. In 2006, this award was revamped and opened to all junior scientists (graduate students or postdoctoral fellows) from all regions of the world who have made a significant contribution to the broad area of RNA. The award is no longer restricted to authors who have published in the RNA journal. The award includes a cash prize and support for travel and registration costs for the awardee(s) to attend the annual RNA Society meeting. Previous graduate student winners include: Jeff Barrick (2006), Malte Beringer (2007), Qi Zhang (2008), Jeremey Wilusz (2009), John Calarco (2010), Jasmine Perez (2011), Chenguang Gong (2012) and Tatjana Trcek Pulisic (2012). 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) and Dipali Sashital (2012).

SCARINGE
Supporting the Future

*Congratulations to graduate student **Wenwen Fang**, and postdoctoral fellow **Je-Hyun Yoon**, who are the winners of the 2013 RNA Society/Scaringe Award.*

The ACS Chemical Biology Poster Prize

The journal *ACS Chemical Biology* is pleased to recognize junior scientists with a poster prize to be awarded at RNA 2013. The prize is for 'innovative use of chemical biology applied to the study of RNA', and consists of a $250 cash prize. All graduate students and postdoctoral fellows presenting posters at RNA 2013 are eligible.

The Biochemistry Poster Prize

The journal *Biochemistry* is pleased to recognize junior scientists with a poster prize to be awarded at RNA 2013. The prize is for 'innovation in the study of RNA biochemistry', and consists of a $250 cash prize. All graduate students and postdoctoral fellows presenting posters at RNA 2013 are eligible.

The NRMCB Poster Prizes

Nature Reviews Molecular Cell Biology (NSMB) is pleased to sponsor a poster prize to be awarded at the 2013 RNA Society Meeting. The prize is for 'innovation and interdisciplinary research,' and consists of a free one-year print and online subscription to NRMCB and a $200 cash award. All graduate students and postdoctoral fellows presenting posters at the meeting are eligible.

The NSMB Poster Prize

Nature Structural & Molecular Biology (NRMCB) is pleased to sponsor 3 poster prizes to be awarded at the 2013 RNA Society Meeting. The prizes, one in the area of molecular biology and biochemistry, one in genetics and development, and one in biophysics and structural biology, consist of a free one-year print and online subscription to NSMB and a $200 cash award. All graduate students and postdoctoral fellows presenting posters at the meeting are eligible.

ABSTRACT LISTING

Tuesday, June 11: 15:00 —18:30

Session P1: Ribosome biogenesis and translation—Davos Ballroom

Keynote: Venki Ramakrishnan (MRC-LMB, Cambridge)
Chair: Nenad Ban (ETH Zürich)
Abstracts 1–9, 397 B

1 **Keynote: Exceptions to Canonical Decoding by the Ribosome**
Venki Ramakrishnan

2 **Structure of the eukaryotic 40S ribosomal subunit in complex with initiation factors eIF1 and eIF1A**
Melanie Weisser, Marc Leibundgut, Nenad Ban

3 **Versatile binding of eukaryotic initiation factor 3 on the small ribosomal 40S subunit and the CSFV IRES**
Yaser Hashem, Robert A. Grassucci, Amedee Des Georges, Robert Langlois, Vidya Dhote, Tatyana V. Pestova, Christopher U.T. Hellen, Joachim Frank

4 **A novel strategy for protein synthesis initiation: 40S ribosomes bind to the 3' UTR of barley yellow dwarf virus (BYDV) mRNA**
Sohani Das Sharma, Bidisha Banerjee, Jelena Kraft, W. Allen Miller, Dixie Goss

5 **Probing the dynamics of Ribosome biogenesis in yeast**
Ralph D Hector, Elena Burlacu, Stuart Aitken, Atlanta Cook, Sander Granneman

6 **The casein kinase 1d homolog Hrr25 promotes dissociation of the ribosome assembly factor Ltv1 from nascent small ribosomal subunits to allow joining of large subunits.**
Homa Ghalei, Katrin Karbstein

7 **Exonucleolytic processing of the 18S rRNA precursors during nuclear export in human cells**
Marie-Francoise O'Donohue, Nathalie Montel-Lehry, Marie-Line Bortolin-Cavaille, Milena Preti, Hanna Gazda, Pierre-Emmanuel Gleizes

8 **An mRNA-derived ncRNA targets and regulates the ribosome**
Andreas Pircher, Kamilla Bakowska-Zywicka, Marek Zywicki, Norbert Polacek

9 **Structural basis of translational regulation of msl2 mRNA by SXL and UNR during dosage compensation in Drosophila**
Janosch Hennig, Iren Wang, Miriam Sonntag, Arie Geerlof, Cristina Militti, Fatima Gebauer, Grzegorz Popowicz, Michael Sattler

397 B **Dom34-mediated dissociation of non-translating ribosomes allows efficient restart of translation after stress**
Antonia van den Elzen, Bertrand Séraphin

Wednesday, June 12: 08:30 —10:15

Session P2A: Regulation by long non-coding RNAs—Davos Ballroom

Chair: V. Narry Kim (Seoul National University)
Abstracts 10–16

10 **Circular RNAs function as efficient microRNA sponges**
Jorgen Kjems, Thomas B. Hansen, Christian K Damgaard, Trine I. Jensen, Jesper B. Bramsen, Bettina H. Clausen, Bente Finsen

11 **The regulatory circuits mediated by RNAs in Staphylococcus aureus and implication of the endoribonuclease III**
Efthimia Lioliou, Cédric Romilly, Thomas Geissmann, François Vandenesch, Isabelle Caldelari, Cynthia Sharma, Joerg Vogel, Pascale Romby

12 **Messenger and long non-coding RNAs: dressed for the occasion?**
Alex Tuck, David Tollervey

13 **Non-coding RNAs prevent spreading of a repressive histone mark**
Marc Bühler, Claudia Keller, Raghavendran Kulasegaran-Shylini, Yukiko Shimada, Hans-Rudolf Hotz

14 **Single cell and genome-wide analysis to dissect antisense RNA-mediated gene silencing and pervasive transcription in S. cerevisiae**
Manuele Castelnuovo, Elisa Guffanti, Jurgi Camblong, Judith Zaugg, Nick Luscombe, Zhenyu Xu, Lars Steinmetz, Samir Rahman, Daniel Zenklusen, Françoise Stutz

15 **Telomeric non-coding RNA acts as a scaffold for telomerase high-order organization at short telomeres**
Emilio Cusanelli, Carmina Angelica Perez Romero, Pascal Chartrand

16 **Human α satellite derived transcripts interact with the active site of RNApolII**
Katarzyna Matylla-Kulinska, Renee Schroeder

Wednesday, June 12: 10:45 —12:30

Session P2B: RNA modification—Davos Ballroom

Chair: Juan Alfonzo (Ohio State University)

Abstracts 17–24

17 **Methylated mRNA recognition by the YTH domain**
Dominik Theler, Cyril Dominguez, Frederic Allain

18 **Determination of N6-methyladenosine RNA modification status at single nucleotide resolution and the application to a long non-coding RNA-protein interaction**
Nian Liu, Qing Dai, Guanqun Zheng, Chuan He, Marc Parisien, Xiaoyun Wang, Tao Pan

19 **Inosine-mediated modulation of RNA sensing by innate immune sensors**
Michael Gantier, Soroush Sarvestani, Bryan Williams

20 **Impact of ADARs on abundance and sequence of miRNAs and other non-coding RNAs.**
Cornelia Vesely, Michael F. Jantsch, Fritz J. Sedlazeck, Arndt von Haeseler, Stefanie Tauber

21 **ADAR proteins suppress activation of antiviral signaling by cellular RNA.**
Sam Greenwood, Niamh Mannion, Xianghua Li, Liam Keegan, Robert Young, Simona Paro, Sarah Cox, Leeanne McGurk, Marion Hogg, James Brindle, David Read, Rui Zhang, Christoffer Nellåker, Chris Ponting, Jin-Billy Li, Matthew Ronshaugen, Julia Dorin, Ian Adams, Mary O'Connell

22 **Mechanism of gRNA Biogenesis in Trypanosome Mitochondria**
Ruslan Afasizhev, Takuma Suematsu, Inna Afasizheva

23 **Biogenesis and function of cyclic N6-threonylcarbamoyladenosine (ct6A) as a widely distributed tRNA hypermodification**
Tsutomu Suzuki, Kenjyo Miyauchi, Takuya Sakashita, Satoshi Kimura, Tomoyuki Numata

24 **Unusual non-canonical editing important for tRNA processing in Trypanosomes as revealed by shallow sequencing.**
Mary Anne T. Rubio, Christopher R. Trotta, Juan D. Alfonzo

Wednesday, June 12: 14:00 —16:30

Concurrent Session C1: Splicing mechanisms—Davos Ballroom

Chair: Soo-chen Cheng (Academia Sinica)

Abstracts 25–33

25 **Structural insights into the assembly of spliceosomal U snRNPs**
Clemens Grimm, Jann Pelz, Utz Fischer

26 **Crystal structure of human spliceosomal U1 snRNP at 3.3 Å resolution.**
Yasushi Kondo, Chris Oubridge, Marike van Roon, Kiyoshi Nagai

27 **Crystal structure of Prp5p reveals intra-molecular interactions that impact splicing fidelity**
Fei Yang, Zhi-Min Zhang, Jiahai Zhou, Yong-Zhen Xu

28 **Sequential contacts of DExD/H-box protein Prp28p with Prp8p, Brr2p, and Snu114p during splicing as captured by a chemical cross-linking approach**
Fu-lung Yeh, Luh Tung, Hsien-Yeh Chou, Che-Ming Lin, Tien-Hsien Chang

29 **Functional spliceosome assembly without stable U4/U6 snRNA pairing**
Jordan Burke, Samuel Butcher, David Brow

30 **A group II intron-like catalytic triplex in the U6 snRNA forms during spliceosome activation**
Sebastian Fica, Melissa Mefford, Joseph Piccirilli, Jonathan Staley

31 **3D Cryo-EM structure of the yeast activated spliceosome (Bact) and localisation of functionally important regions**
Holger Stark, Norbert Rigo, Chengfu Sun, Prakash Dube, Kum-Loong Boon, Berthold Kastner, Reinhard Rauhut, Patrizia Fabrizio, Reinhard Lührmann

32 **A conformational switch in PRP8 mediates metal ion coordination that promotes pre-mRNA exon ligation**
Matthew Schellenberg, Tao Wu, Dustin Ritchie, Sebastian Fica, Jonathan Staley, Karim Atta, Paul Lapointe, Andrew MacMillan

33 **Versatile reaction catalyzed by the Spliceosome in a competitive manner**
Chi-Kang Tseng, Hui-Fang Wang, Che-Sheng Chung, Soo-Chen Cheng

Wednesday, June 12: 14:00 — 16:30

Concurrent Session C2: RNA localization—Aspen

Chair: Ralf Jansen (University Tübingen)

Abstracts 34–42

34 **An unexpected role of the nuclear periphery for mRNA export in yeast**
Mark-Albert Saroufim, Daniel Zenklusen

35 **NMD3 regulates mRNA nuclear export via an XpoI-linked mechanism**
Pegine Walrad, Melanie Bühlmann, Pegine Walrad, Paul Capewell, Arunasalam Naguleswaran, Isabel Roditi, Elisabetta Ullu, Keith R. Matthews

36 **RNA recognition and architectural activity of Zipcode Binding Protein 1**
Giuseppe Nicastro, David Hollingworth, Alain Oregioni, Adela Candel, Andres Ramos

37 **Identification and analysis of Staufen2 target RNAs from rat brain**
Jacki Heraud-Farlow, Michael Doyle, Martin Bilban, Stefanie Tauber, Michael Kiebler

38 **The order of assembly and disassembly of nuclear ASH1-mRNPs**
Annika Niedner, Marisa Müller, Dierk Niessing

39 **Novel players and novel mRNAs transported by the Bic-D / Egl / Dynein RNA localization machinery**
Paula Vazquez, Bogdan Schaller, Rémy Bruggmann, Samuel Neuenschwander, Henning Urlaub, Beat Suter

40 **An RNA biosensor for imaging translation of single mRNAs in living cells.**
Jeffrey Chao, Timothée Lionnet, Robert Singer

41 **Single molecule systems biology of RNA silencing**
Nils Walter, Sethuramasundaram Pitchiaya, Márcio Mourão, Corey Custer, Laurie Heinicke, Katelyn Doxtader, Vishalakshi Krishnan, Santiago Schnell

42 **GRSF1 regulates RNA processing in mitochondrial RNA granules**
Alexis A. Jourdain, Jean-Claude Martinou

Wednesday, June 12: 14:00 —16:30

Concurrent Session C3: Viral RNAs—Sanada

Chair: Karen Beemon (Johns Hopkins University)

Abstracts 43–51

43 **Virus-Induced Dysregulation of Cellular mRNA Decay and Alternative Polyadenylaton – Implications for Pathogenesis**
Stephanie L. Moon, Michael D. Barnhart, Carol J. Wilusz, Liang Liu, Bin Tian, Jeffrey Wilusz

44 **A dengue virus 2 non-coding RNA downregulates translation of antiviral interferon-stimulated mRNAs through interaction with host RNA binding proteins.**
Katell Bidet, Sharon Jamison, Mariano Garcia-Blanco

45 **RNA/RNA interactions govern selective packaging of influenza A genomic segments**
Cyrille Gavazzi, Matthieu Yver, Emilie Fournier, Boris Essere, Jean-Christophe Paillart, Jean-Daniel Sirbat, Annie Cavalier, Jean-Paul Rolland, Daniel Thomas, Manuel Rosa-Calatrava, Bruno Lina, Catherine Isel, Vincent Moules, Roland Marquet

46 **Interplay between retroviral genomic RNA packaging and mRNA translation**
Katarzyna J. Purzycka, Mastooreh Chamanian, Katarzyna J. Purzycka, Paul Wille, Janice S. Ha, David McDonald, Yong Gao, Stuart F.J. Le Grice, Eric J. Arts, Ryszard W. Adamiak

47 **HIV1, Antisense RNA and ADAR editing**
Siripong Tongjai, Keanan McGonigle, Yeou-Cherng Bor, David Rekosh, Marie-Louise Hammarskjöld

48 **The Identification of a novel posttranscriptional regulatory element in gamma retroviruses**
Guy Pilkington, Jenifer Bear, Katarzyna Purzycka, Stuart Le Grice, Barbara Felber

49 **Shunting revisited.**
Thomas Hohn

50 **Inquiry into the variability of HCV IRES and its impact on function by developing and evaluation of a large-scale mutation database that also unfolds potential of some new nucleotides.**
Anas Khawaja, Vaclav Vopalensky, Ludek Roznovsky, Jakub Mrazek, Ondrej Horvath, Martin Pospisek

51 **Novel Insights from Structural Analysis of Lentiviral and Gammaretroviral Reverse Transcriptases in Complex with RNA/DNA Hybrids.**
Stuart Le Grice, Mikalai Lapkouski, Lan Tian, Jennifer Miller, Enzbieta Nowak, Wojciech Potrzebowski, Peter Konarev, Jason Rausch, Marion Bona, Dmitri Svergun, Janusz Bujnick, Marcin Nowotny, Wei Yang

Wednesday, June 12: 17:00 —18:30

Workshop Session W1: Function of Non coding Transcriptome—Davos Ballroom

Chairs: Constance Ciaudo (ETH Zürich) and Claus Azzalin(ETH Zürich)

Abstracts 52–58

52 **RNAi dependent and independent control of LINE1 mobility and accumulation in mouse ES cells**
Constance Ciaudo, Florence Jay, Ikuhiro Okamoto, Chong-Jian Chen, Nicolas Servant, Emmanuel Barillot, Edith Heard, Olivier Voinnet, Alexis Sarazin

53 **Role of Telomeric Repeat-containing RNA in Alternative Lengthening of Telomeres**
Rajika Arora, Claus Azzalin

54 **RNA and DNA Targeting CRISPR-Cas Immune Systems of Pyrococcus furiosus**
Joshua Elmore, Caryn Hale, Sonali Majumdar, Jason Carte, Hong Li, Sara Olson, Brenton Graveley, Lance Wells, Claiborne Glover, Rebecca Terns, Michael Terns

55 **Involvement of TERT-RdRP in heterochromatin maintenance**
Kenkichi Masutomi, Mami Yasukawa, Yoshiko Maida

56 **The role of the Arabidopsis exosome complex in siRNA-independent silencing of heterochromatic loci**
Junhye Shin, Hsiao-Lin Wang, Brandon Dinwiddie, Julia Chekanova

57 **Involvement of the novel complex consisting of the splicing factor Prp14p/DHX38 RNA helicase and centromeric non-coding RNAs in the regulation of chromosome segregation**
Masatoshi Mutazono, Takashi Ideue, Kanako Nishimura, Yukiko Cho, Chihiro Tsukahara, Misato Morita, Madoka Chinen, Jun-ichi Nakayama, Kojiro Ishii, Tokio Tani

58 **A genome-wide RNAi screen draws a genetic framework for transposon control and primary piRNA biogenesis in Drosophila**
Paloma M Guzzardo, Felix Muerdter5, Jesse Gillis, Yicheng Luo, Yang Yu, Caifu Chen, Richard Fekete, Gregory J Hannon

Wednesday, June 12: 17:00 — 18:30

Workshop Session W2: RNA chemistry—Sanada

Chair: Ronald Micura (Leopold Franzens University) and Mark Helm (Johannes Gutenberg University)
Abstracts 59–65

59 **New Approaches in RNA Chemical Biology**
Jonathan Hall, Andreas Brunschweiger, Luca Gebert, Jochen Imig, Mario Rebhan, Ugo Pradere, Boris Guennewig

60 **Resolving functional RNA dynamics by NMR**
Christoph Wunderlich, Romana Spitzer, Thomas Moschen, Martin Tollinger, Christoph Kreutz

61 **RNA-based fluorescent biosensors for live cell imaging of second messengers cyclic di-GMP and cyclic AMP-GMP.**
Colleen Kellenberger, Stephen Wilson, Jade Sales-Lee, Ming Hammond

62 **Sequence Specific Modulation of G-Quadruplex Folding**
Samuel Rouleau, Jean-Denis Beaudoin, Jean-Pierre Perreault

63 **Structural stabilization of toxic CUG repeats reverses mis-splicing associated with myotonic dystrophy**
Elaine deLorimier, Jeremy Copperman, Alex Taber, Leslie Coonrod, Emily Reister, Feras Ackall, Kush Sharma, Peter Todd, Marina Guenza and J. Andrew Berglund

64 **A chemo-enzymatic approach for selective modification of the RNA cap**
Daniela Schulz, Josephin Holstein, Andrea Rentmeister

65 **Bromomethylcoumarins as selective reagents for RNA labeling**
Mark Helm, Stefanie Kellner

Thursday, June 13: 08:30 — 10:15

Session P3A: RNA and disease—Davos Ballroom

Chair: Tom Cooper (Baylor College of Medicine)
Abstracts 66–72

66 **Somatic spliceosomal factor mutations in bone marrow neoplasms lead to alterations in alternative splicing patterns that relate to the splicing mechanism**
Richard Padgett, Bartloniej Przychodzen, Amina Kozaric, Hideki Makishima, Magda Konarska, Jaroslaw Maciejewski

67 **Mnk2 alternative splicing inactivates its tumor suppressor activity as a modulator of the p38-MAPK stress pathway**
Avi Maimon, Maxim Mogilevsky, Asaf Shilo, Ben Davidson, Rikiro Fukunaga, Rotem Karni

68 **Multiple myeloma-associated hDIS3 mutations cause perturbations in cellular RNA metabolism and suggest potential drug targets**
Rafal Tomecki, Karolina Drazkowska, Iwo Kucinski, Krystian Stodus, Roman Szczesny, Jakub Gruchota, Andrzei Dziembowski

69 **Defective RNP Assembly in Prostate and Other Cancers**
Rosario Machado-Pinilla, Phillip J. Iaquinta, Charles L. Sawyers, U. Thomas Meier

70 **Loss of MBNL1 function impairs neuronal morphology in myotonic dystrophy type 1**
Ting-Yu Kuo, Pei-Ying Wang, Hsing-Jung Chen, Mi-Hua Tao, Guey-Shin Wang

71 **Mutations in the gene encoding U11/U12-65K protein leads to pituitary hypoplasia and isolated growth hormone deficiency type I**
Bhupendra Verma, Ali Oghabian, Ivon Cuscó, Gabriel Á. Martos-Moreno, Armand Gutiérrez, Julie A. Chowen, Jesús Argente, Luis A. Pérez-Jurado, Mikko J. Frilander

72 **miR-34c-5p is a novel regulator of naive T-cell activation that impacts HIV replication**
Andreia Amaral, Jorge Andrade, Ana Matos, Russel Foxall, Paula Matoso, Mariana Santa-Marta, Rita Tendeiro, Ana Serra-Caetano, Rui Soares, João Gonçalves, Ana Sousa, Margarida Gama-Carvalho

Thursday, June 13: 10:45 —12:30

Session P3B: RNA systems biology—Davos Ballroom

Chair: Brent Graveley (University of Connecticut)

Abstracts 73–80

73 **Genome-wide mapping of RBM10 binding sites reveals its role in splicing regulation: Implications for cleft palate and TARP syndrome**
Julie Rodor, David Fitzpatrick, Javier Caceres, Margarida Gama-Carvalho

74 **Muscleblind-like proteins negatively regulate embryonic stem cell-specific alternative splicing and reprogramming**
Hong Han, Manuel Irimia, Joel Ross, Hoon-Ki Sung, Babak Alipanahi, Laurent David, Azadeh Golipour, Mathieu Gabut, Iacovos Michael, Emil Nachman, Eric Wang, Dan Trcka, Tadeo Thompson, Christopher Burge, Jason Moffat, Brendan Frey, Andras Nagy, James Ellis, Jeffrey Wrana, Benjamin Blencowe

75 **A pair of RNA binding proteins regulate neuron-subtype specific alternative splicing in C. elegans**
Adam Norris, John Calarco

76 **CFlm25 Links Global change in APA to Cell Growth Control and Glioblastoma Survival**
Chioniso Masamha, Zheng Xia, Wei Li, Ann-Bin Shyu, Todd Albrecht, Eric Wagner

77 **Subsets of introns are abundant in poly(A)+ RNA**
Paul Boutz, Arjun Bhutkar, Phillip Sharp

78 **Genome-wide analysis of pre-mRNA splicing in budding yeast from the perspective of the intron**
Daoming Qin, Lei Huang, Jonathan Staley

79 **Lariat Sequencing in a Unicellular Yeast Identifies Regulated Alternative Splicing of Exons that are Evolutionarily Conserved with Humans**
Ali Awan, Amanda Manfredo, Jeffrey Pleiss

80 **Global Analysis of Phosphorylation by SR Protein Kinases and Their Effects on Genome-wide Splicing in Schizosaccharomyces pombe**
Michael Marvin, Jesse Lipp, Kevan Shokat, Christine Guthrie

Thursday, June 13: 20:00 —22:30

Plenary Session P4: Architecture of RNPs—Davos Ballroom

Keynote: Thomas R. Cech (HHMI, University of Colorado Boulder)

Chair: Eric Westhof (University Strasbourg)

Abstracts 81–88

81 **Keynote: The Future of RiboScience**
Thomas R. Cech

82 **The architecture of Tetrahymena telomerase holoenzyme**
Jiansen Jiang, Edward J. Miracco, Kyungah Hong, Barbara Eckert, Henry Chan, Darian D. Cash, Bosun Min, Z. Hong Zhou, Kathleen Collins, Juli Feigon

83 **Deciphering the assembly of box C/D snoRNP complexes**
Jonathan Bizarro, Bérengère Pradet-Balade, Marc Quinternet, Xavier Manival, Bruno Charpentier, Christiane Branlant, Céline Verheggen, Edouard Bertrand

84 **Crystal Structure of the Bacterial Pnkp1/Rnl/Hen1 Heterohexamer: A New RNA Repair Complex**
Pei Wang, Kiruthika Selvadurai, Raven Huang

85 **Molecular basis of translation activation by the non-coding RNA RsmZ**
Olivier Duss, Maxim Yulikov, Erich Michel, Mario Schubert, Gunnar Jeschke, Frédéric Allain

86 **Single-molecule analysis of L7Ae protein binding to a k-turn : induced fit or conformational selection ?**
Jia Wang, Tomáš Fessl, Kersten T. Schroeder, David M. J. Lilley

87 **The structural basis of SRP receptor recruitment and GTPase activation by SRP RNA**
Nikolaus Schmitz, Felix Voigts-Hoffmann, Kuang Shen, Shu-ou Shan, Sandro F. Ataide, Nenad Ban

88 **Crystal structure of Prp8 and its implications for the spliceosomal active site**
Wojciech Galej, Chris Oubridge, Andy Newman, Kiyoshi Nagai

Friday, June 14: 08:30 — 10:15

Session P5A: RNA processing—Davos Ballroom

Chair: Joan Steitz (HHMI, Yale University)

Abstracts 89–95

89 **Structural and functional studies of pre-mRNA 5' and 3'-end processing**
Liang Tong

90 **Analysis of eukaryotic orthologous groups reveals Archease as a crucial factor in human tRNA splicing.**
Johannes Popow, Alexander Schleiffer, Javier Martinez

91 **Control of myogenesis by rodent SINE-containing lncRNAs**
Jiashi Wang, Chenguang Gong, Lynne Maquat

92 **DNA Damage induces targeted, genome-wide variation of poly(A) sites in budding yeast**
Joel Graber, Fathima Nazeer, Pei-chun Yeh4 Jason Kuehner, Sneha Borikar, Derick Hoskinson, Claire Moore

93 **Polyadenylated histone mRNAs accumulate upon PARN knock-down**
Claudia Weißbach, Christiane Harnisch, Heike Berndt, Lars Anders, Elmar Wahle

94 **Non-coding Y1/3 RNAs promote the 3'-processing of canonical histone pre-mRNAs**
Marcel Köhn, Stefan Hüttelmaier

95 **Dicer-2 is involved in mRNA activation through cytoplasmic polyadenylation**
Ana Villalba, Olga Coll, Tanit Guitart, Catherine Papin, Martine Simonelig, Fátima Gebauer

Friday, June 14: 10:45 — 12:30

Session P5B: RNA decay—Davos Ballroom

Chair: Elena Conti (MPI Biochemistry)

Abstracts 96–102

96 **Assembly and function of the NOT module of the CCR4-NOT complex**
Ying Chen, Andreas Boland, Tobias Raisch, Stefanie Jonas, Duygu Kuzuoglu-Öztürk, Lara Wohlbold, Oliver Weichenrieder, Elisa Izaurralde

97 **Structural insights into the Not module of the Ccr4-Not complex**
Varun Bhaskar, Jerome Basquin, Vladimir Rudko, Bertrand Séraphin, Elena Conti

98 **Roquin Promotes Constitutive mRNA Decay via a Conserved Class of Stem-Loop Recognition Motifs**
Kathrin Leppek, Johanna Schott, Sonja Reitter, Fabian Poetz, Ming C. Hammond, Georg Stoecklin

99 **The crystal structure of the nucleolar exosome engaged with RNA**
Elizabeth Wasmuth, Christopher Lima

100 **Nonsense mediated mRNA decay is inefficient on long ORF transcripts**
Laurence Decourty, Antonia Doyen, Christophe Malabat, Emmanuel Frachon, Delphine Rispal, Bertrand Séraphin, Alain Jacquier, Cosmin Saveanu, Alain Jacquier

101 **eIF4E-bound mRNPs are substrates for nonsense-mediated mRNA decay in mammalian cells**
Simone C. Rufener, Oliver Mühlemann

102 **PAXT-1 binds XRN-2 and promotes its activity**
Takashi Miki, Stefan Rueegger, Hannes Richter, Helge Grosshans

Friday, June 14: 14:00 — 16:30

Concurrent Session C4: Regulation by small non-coding RNAs—Davos Ballroom

Chair: Jennifer Doudna (HHMI, Univ. of California - Berkeley)

Abstracts 103–111

103 **Molecular Clues to Tissue-Specific Control of MiRNA Biogenesis**
Nila Roy Choudhury, Flavia de Lima Alves, Luisa de Andrés-Aguayo, Thomas Graf, Javier F. Cáceres, Juri Rappsilber, Gracjan Michlewski

104 **Mouse Tudor domain containing 12 (Tdrd12) is essential for biogenesis of piRNAs associating with the nuclear Piwi protein Miwi2**
Radha Raman Pandey, Zhaolin Yang, Ramesh S Pillai

105 **Regulation of miRNAs and endo-siRNAs during oocyte-to-zygote transition in the mouse**
Matyas Flemr, Radek Malik, Vedran Franke, Jana Nejepinska, Kristian Vlahovicek, Petr Svoboda

106 **Kinetic and biophysical models improve identification of miRNA targets**
Mihaela Zavolan, Jean Hausser, Mohsen Khorshid, Erik van Nimwegen, Erik Sontheimer

107 **Insights into the recruitment of the PAN2-PAN3 deadenylase complex to miRNA targets by the GW182/TNRC6 proteins**
Mary Christie, Andreas Boland, Eric Huntzinger, Oliver Weichenrieder, Elisa Izaurralde

108 **Translational repression and eIF4A2 activity are critical for microRNA-mediated gene regulation.**
Anna Wilczynska, Hedda Meijer, Wei-Ting Lu, Yi-Wen Kong, Ruth Spriggs, Jack Godfrey, Sue Robinson, Anne Willis, Martin Bushell

109 **The conserved concave surface of the MIF4G domain of CNOT1 is involved in miRNA-mediated translational repression**
Hansruedi Mathys, Witold Filipowicz

110 **Single-molecule observation of DNA targeting and cleavage by the RNA-guided Cas9 endonuclease**
Samuel Sternberg, Eric Greene, Sy Redding, Martin Jinek, Jennifer Doudna

111 **Processing-Independent CRISPR RNAs Limit Natural Transformation in Neisseria meningitidis**
Yan Zhang, Nadja Heidrich, Biju Joseph Ampattu, Carl Gunderson, Hank Seifert, Christoph Schoen, Jörg Vogel, Erik Sontheimer

Friday, June 14: 14:00 — 16:30

Concurrent Session C5: Structure, dynamics, and catalysis—Aspen

Chair: David Rueda (Imperial College, London)

Abstracts 112–120

112 **T box riboswitch decodes both the information content and geometry of tRNA to affect gene expression**
Ailong Ke, Jason Grigg, Yujie Chen, Frank Grundy, Tina Henkin, Lois Pollack, Ailong Ke

113 **Structural basis of specific tRNA recognition by the T-box riboswitch**
Jinwei Zhang, Adrian Ferre-D'Amare

114 **Crystal Structure and Biophysical Analysis of a Class 2 PreQ1 Riboswitch**
Joseph Liberman, Mohammad Salim, Jolanta Krucinska, Joseph Wedekind

115 **A novel class of self-cleaving ribozymes is prevalent in many species of bacteria and eukarya**
Adam Roth, Zasha Weinberg, Andy Chen, Peter Kim, Tyler Ames, Ronald Breaker

116 **Spliceosomal Prp24 unwinds a minimal U2/U6 complex from yeast**
Chandani Warnasooriya, Zhuojun Guo, Samuel Butcher, David Brow, David Rueda

117 **Conformational Heterogeneity of the Protein-Free Human Spliceosomal U2-U6 snRNA Complex**
Caijie Zhao, Ravichandra Bachu, Nancy Greenbaum

118 **A new class of minimal Hammerhead ribozymes conserved in the eukaryotic family of Penelope-like retroelements**
Amelia Cervera, Marcos de la Peña

119 **Probing EF-G Power Stoke During Ribosome Translocation**
Yuhong Wang, Li Yao, Yue Li, Shoujun Xu

120 **Understanding RNA Interference One Molecule at a Time**
William Salomon, Victor Serebrov, Mellissa Moore, Phillip Zamore

Friday, June 14: 14:00 — 16:30

Concurrent Session C6: High-throughput approaches to RNA biology—Sanada

Chair: Jernej Ule (MRC-LMB, University College London)

Abstracts 121–128

121 **Promoter directionality is controlled by U1 splicing and polyadenylation signals**
Albert Almada, Xuebing Wu, Andrea Kriz, Christopher Burge, Phillip Sharp

122 **Suppression of promoter upstream transcripts (PROMPTs) by polyadenylation site-induced RNA decay provides directionality to transcription of human promoters**
Evgenia Ntini, Aino Järvelin, Jette Bornholdt-Lange, Yun Chen, Mette Jørgensen, Robin Andersson, Aleks Schein, Peter Refsing Andersen, Pia Kjølhede Andersen, Vicente Pelechano, Lars Steinmetz, Albin Sandelin, Torben Heick Jensen

123 **Pathway of histone mRNA decay determined by high-throughput sequencing (HTS)**
Mike Slevin, Rebecca Bigler, Staci Meaux, William Marzluff

124 **Global poly(A)-tail length measurements reveal that the relationship between tail length and translational efficiency varies between biological contexts**
Alexander Subtelny, Stephen Eichhorn, Grace Chen, Hazel Sive, David Bartel

125 **Capture of a microRNA targetome using chemically modified miRNA mimics ("miR-CLIP")**
Jochen Imig, Andreas Brunschweiger, Anneke Brümmer, Nitish Mittal, Boris Guennewig, Mihaela Zavolan, André P. Gerber, Jonathan Hall

126 **Identification of multiple regulatory microRNAs by miTRAP**
Juliane Braun, Knut Krohn, Stefan Hüttelmaier

127 **Neurodegenerative diseases: Quantitative predictions of protein-RNA interactions**
Davide Cirillo, Gian Tartaglia

128 **New in vivo RNA-binding architectures discovered by RBDmap**
Alfredo Castello, Bernd Fischer, Sophia Foehr, Anne-Marie Alleaume, Tomaz Curk, Jeroen Krijgsveld Krijgsveld, Matthias W Hentze

Friday, June 14: 17:00 — 18:30

Workshop Session W4: RNA in pharmaceutical research—Davos Ballroom

Chairs: Nicole Meisner (Novartis Institutes for Biomedical Research) and David Morrissey (Novartis Institutes for Biomedical Research)

Abstracts 129–134

129 **Development of Dynamic Polyconjugates for tissue-targeted delivery of siRNA**
Christine Wooddell, So Wong, Jason Klein, Andrei Blokhin, Magdolna Sebestyén, Weijun Cheng, Julia Hegge, Qili Chu, Vladimir Trubetskoy, Collin Hagen, Anthony Perillo-Nicholas, Jacob Griffin, Jonathan Benson, Jeffrey Carlson, Darren Wakefield, Holly Hamilton, Stephanie Bertin, Jessica Montez, Alan McLachlan, David Rozema, David Lewis

130 **Specific Gene Activation by Disruption of PRC2-lncRNA Interactions**
James McSwiggen

131 **RNA-based immunotherapeutics against cancer**
Andreas Kuhn, Janina Buck, Florian Eberle, Britta Vallazza, Joanna Kowalska, Jacek Jemiliety, Edward Darzynkiewicz, Ugur Sahin

132 **Antisense Oligonucleotide Therapies for the Treatment of Neurodegenerative and Muscular Diseases**
Frank Rigo, C. Frank Bennett

133 **Extracellular RNAs are markers of muscle myogenesis following splice switching oligonucleotide therapy in a mouse model of Duchenne muscular dystrophy**
Matthew Wood

134 **Silencing gene expression by recruiting RISC**
Jennifer Broderick, Neil Aronin, Phillip Zamore

Friday, June 14: 17:00 —18:30

Workshop Session W6: Tutorial on prediction of RNA secondary structure—Sanada

Chair: Alain Laederach (University of North Carolina at Chapel Hill)

Abstracts 135–141

135 **What can you know about noncoding RNAs without doing any experiments?**
Zasha Weinberg, Ronald Breaker

136 **Methods of Predicting RNA Structure Change Due to Mutation**
Matt Halvorsen, Joshua Martin, Justin Ritz, Alain Laederach

137 **RNAsnp: Predicting SNP Effects on Local RNA Secondary Structure**
Radhakrishnan Sabarinathan, Hakim Tafer, Stefan E Seemann, Ivo L Hofacker, Peter F Stadler, Jan Gorodkin

138 **Navigating through the MC-Flashfold 2D suboptimal solution maze using simple structural transformation rules**
Paul Dallaire, Stefanie Schirmer, Francois Major

139 **How to determine binding affinities and binding motifs for RNA-bindingproteins from CLIP-seq data**
Rolf Backofen, Daniel Maticzka, Fabrizio Costa

140 **The HIV-2 Rev-Response Element: Determining Secondary Structure and Defining Folding Intermediates**
Joanna Sztuba-Solinska, Sabrina Lusvarghi, Katarzyna Purzycka, Gary Pauly, Jason Rausch, Stuart Le Grice

141 **Structural spectrum of long non-coding RNAs revealed by experiment**
Irina Novikova, Scott Hennelly, Bin Zhang, David Spector, Karissa Sanbonmatsu

Saturday, June 15: 08:30 —10:15

Session P6A: Interconnections in RNA regulation—Davos Ballroom

Chair: Ulrike Kutay (ETH Zürich)

Abstracts 142–148

142 **The transcription factors ILF2 and ILF3 are trans-acting factors for 60S ribosomal biogenesis**
Franziska Wandrey, Christian Montellese, Lukas Badertscher, Lukas Bammert, Ulrike Kutay

143 **Novel function for human Argonaute 2 in gene regulation at the tRNA genes**
Jessica Woolnough, Keith E. Giles

144 **The human cap-binding complex is functionally connected to the nuclear RNA exosome**
Peter Refsing Andersen, Michal Domanski, Maiken S. Kristiansen1, Evgenia Ntini1, Celine Verheggen, Jakob Bunkenborg, Ina Poser, Marie Hallais, Anthony Hyman, John LaCava, Michael P. Rout, Jens . Andersen, Edouard Bertrand, Torben Heick Jensen

145 **A splicing-dependent transcriptional checkpoint**
Keerthi Chathoth, Shaun Webb, David Barrass, Jean Beggs

146 **The Exon Junction Complex core component MLN51 interacts with eIF3 and activates translation**
Pierre-Etienne Chazal, Elisabeth Daguenet, Catherine Tomasetto, Bruno Sargueil, Hervé Le Hir

147 **SRSF1-mediated translational regulation and its role in cellular transformation**
Magdalena Maslon, Sara Heras, Nicolas Bellora, Eneritz Aguirre, Eduardo Eyras, Javier Caceres

148 **The RNA kinase CLP1 is required for efficient tRNA splicing and regulates p53 activation in response to oxidative stress**
Stefan Weitzer, Toshikatsu Hanada, Barbara Mair, Josef Penninger, Javier Martinez

Saturday, June 15: 10:45 —12:30

Session P6B: Alternative splicing—Davos Ballroom

Chair: Javier Cáceres (MRC Edinburgh)

Abstracts 149–155

149 **Genome-Wide Identification of Recursive Splicing in Drosophila**
Sara Olson, Xintao Wei, Ahmad Osman, Michael Duff, Susan Celniker, Brenton Graveley

150 **Isolated pseudo-RRMs of SR proteins can regulate splicing using a non-canonical mode of RNA recognition**
Antoine Cléry, Rahul Sinha, Olga Anczukow, Anna Corrionero, Ahmed Moursy, Gerrit Daubner, Juan Valcarcel, Frédéric Allain, Adrian Krainer

151 **RBM5 OCRE domain modulates alternative splicing regulation by recognition of proline-rich motifs in spliceosomal SmN/B/B'**
Lisa Warner, André Mourão, Sophie Bonnal, Juan Valcárcel, Michael Sattler

152 **GSK3-Induced Regulation of the Protein- and RNA-Binding Activity of PSF Modulates Signal-Induced Alternative Splicing.**
Christopher A Yarosh, Nicole Martinez, Jinsong Qiu, Xiang-dong Fu, Kristen Lynch

153 **Rhythmic U2AF26 alternative splicing regulates the circadian clock in mice**
Marco Preussner, Florian Heyd

154 **RNA Binding Protein Sfpq is required for the expression of neuron-specific long pre-mRNAs essential for brain development.**
Akihide Takeuchi, Kei Iida, Kensuke Ninomiya, Mikako Ito, Kinji Ohno, Masatoshi Hagiwara

155 **The SWI/SNF subunit Brahma modulates the choice of alternative terminal exons by recruiting the BRCA1/BARD1 ubiquitin ligase**
Gabriele Fontana, Aurora Rigamonti, Reinaldo Alvarez, Silvia Lenzken, Marco Bianchi, Silvia Barabino

Poster Sessions

Main Hallway and Sanada Foyer

Non-coding and Regulatory RNAs

Abstracts 156–204

156 A **Natural antisense transcripts in Neurospora crassa**
Yamini Arthanari, Sam Griffiths-Jones, Christian Heintzen, Susan Crosthwaite

157 B **Overexpression of small noncoding RNAs and its effects on biofilm-related phenotypes in Escherichia coli**
Geunu Bak, Kwang-sun Kim, Younghoon Lee

158 C **Defense against viral attack: single-molecule view on a bacterial adaptive immune system**
Timothy Blosser, Edze R. Westra, Cees Dekker, Stan J. J. Brouns, Chirlmin Joo

159 A **TDP-43 regulates cancer-associated microRNAs**
Xiaowei Chen, Zhen Fan, Mengmeng Chen, Ruirui Kong, Pushuai Wen, Tengfei Xiao, Warren McGee, Xiaomin Chen, Jianghong Liu, Li Zhu, Runsheng Chen, Jane Wu

160 B **An RNA Degradation Machine Sculpted by Ro Autoantigen and Noncoding RNA**
Xinguo Chen, David Taylor, Casey Fowler, Soyeong Sim, Jorge Galan, Hong-Wei Wang, Sandra Wolin

161 C **The role of RNA degradation in moderating RNAi**
Cristina Cruz, Jon Houseley

162 A **The role of non coding RNA at sites of DNA damage in the control of genome integrity**
Fabrizio d'Adda di Fagagna

163 B **The microRNA pathway mediates expression of yolk lipoproteins in the Caenorhabditis elegans intestine**
Robert Dowen, Gary Ruvkun

164 C **The exosome subunit Rrp6 regulates the expression of retrotransposons and non-coding heterochromatic sequences in Drosophila melanogaster**
Andrea Brigitte Eberle, Viktoria Hessle, Antoni Ganez Zapater, Gilad Silberberg, Anne von Euler, Neus Visa

165 A **Identification and Analysis of New Genes Targeted for sRNA Regulation in Bacteria**
Martha Faner, Andrew Feig

166 B **Novel RNA Structures Controlling RIbosomal Protein Biosynthesis in E. coli and Beyond**
Yang Fu, Michelle Meyer

167 C **The RNA subunit of RNase MRP: extra nucleotides at the 3' end**
Katherine Goldfarb, Elaine Podell, James Goodrich, Thomas Cech

168 A **Alu RNAs as possible modulators of microRNA function**
Daniele Hasler, Gunter Meister

169 B **RNA annealing activity of Hfq is sensitively modulated by various physical parameters**
Wonseok Hwang, Véronique Arluison, Sungchul Hohng

170 C **Identification of stage specific microRNAs during the developmental stages in Triops cancriformis (tadpole shrimp)**
Kahori Ikeda, Yuka Hirose, Kiriko Hiraoka, Emiko Noro, Kosuke Fujishima, Masaru Tomita, Akio Kanai

171 A **A tandem-stem RNA motif mediates X-chromosome dosage compensation in Drosophila**
Ibrahim Avsar Ilik, Jeffrey J. Quinn, Filipe Tavares-Cadete, Plamen Georgiev, Yue Wan, Robert C. Spitale, Nicholas Luscombe, Howard Y. Chang, Asifa Akhtar

Posters labeled with an "A" present Wednesday, June 12, 20:00 – 22:30
Posters labeled with an "B" present Friday, June 14, 20:00 – 22:30
Posters labeled with an "C" present Saturday, June 15, 14:00 – 17:00

172 B **Long ncRNA NEAT1-dependent SFPQ relocation between nuclear body paraspeckle and gene promoter region mediates the transcription of IL8 gene in immune response**
Katsutoshi Imamura, Gen Akizuki, Naoto Imamachi, Michiko Kumakura, Atsushi Kawaguchi, Kyosuke Nagata, Akihisa Kato, Yasushi Kawaguchi, Hiroki Sato, Misako Yoneda, Chieko Kai, Tetsushi Yada, Shinichi Nakagawa, Kiyomi Kaneki, Kenji Inoue, Tatsuhiko Kodama, Youichiro Wada, Kazuhisa Sekimizu, Nobuyoshi Akimitsu

173 C **A Natural Antisense Transcript is Involved in the Destabilization of Cyclooxygenase 2 mRNA by Acetaminophen**
Hiroyuki Inaba, Emi Yoshigai, Keikichi Sugiyama, Hoyoku Nishino, Mikio Nishizawa

174 A **Circular RNAs are Abundant, Conserved and Linked to ALU Repeats**
William Jeck, Jessica Sorrentino, Michael Slevin, Zefeng Wang, William Marzluff, Norman Sharpless

175 B **Recognition of brain cytoplasmic 200 RNA by a human anti-RNA antibody**
Euihan Jung, Jungmin Lee, Hyo Jeong Hong, Insoo Park, Younghoon Lee

176 C **Evf2 (Dlx6AS) long non-coding RNA regulation of interneuron gene expression and behavior**
Jhumku Kohtz, Hao Luo, Sean Chen, Shari Birnbaum

177 A **The double stranded RNA transcriptome of E. coli reveals novel antisense RNAs**
Meghan Lybecker, Bob Zimmermann, Ivana Bilusic, Nadia Tukhtubaeva, Renee Schroeder

178 B **Roles for roX RNA and the RNA helicase MLE in the assembly of the dosage compensation complex in Drosophila**
Sylvain Maenner, Marisa Müller, Jonathan Fröhlich, Diana Langer, Peter B. Becker

179 C **Molecular Mechanism Involved in Antisense-Mediated Transcriptional PHO84 Gene Silencing**
Andrea Maffioletti, Nissrine Beyrouthy, Francoise Stutz

180 A **MiR-19 and miR-155 role in oncogene-induced senescence bypass**
Lian Mignacca, Emmanuelle Saint-Germain, Gerardo Ferbeyre

181 B **Fission yeast Cactin silences chromosome ends and retrotransposons and links heterochromatin establishment to telomere length regulation**
Martin Moravec, Luca E Lorenzi, Amadou Bah, Harry Wischnewski, Marco Santagostino, Claus M Azzalin

182 C **Revealing the elusive molecular biology of the vault RNA**
Birgit Nachbauer, Melanie Amort, Aloys Schepers, Arnd Kieser, Norbert Polacek

183 A **Structure and function of Zucchini endoribonuclease in piRNA biogenesis**
Osamu Nureki, Hiroshi Nishimasu, Hirotsugu Ishizu, Mikiko Siomi

184 B **Malignant Transformation Changes Packaging and Targeting of Extracellular MicroRNAs**
Jaime Palma, William Pearse, Mallory Havens, Michelle Hastings, Dominik Duelli

185 C **Identification and functional characterization of the long non-coding RNA in myogenesis**
Jinyoung Park, Jiwon Lee, Hanyoung Lee, Chanhee Jo, Ahreum Choi, Jae-Hyun Yang, Eun-Jung Cho

186 A **Degradation of ribosomal RNA and ribosomal proteins constitute separate pathways of ribophagy**
Anna Pastucha, Joanna Kufel

187 B **Discovery of Hfq-binding nanoRNAs in Escherichia coli**
Jennifer Patterson, Shugeng Cao, Jon Clardy, Cameron Mura

188 C **Mutations in the 5'UTR of SERPINA1 transcripts are involved in the disease associated mechanisms**
Gabriela Phillips, Chetna Gopinath, Matt Halvorsen, Justin Ritz, Amanda Solem, Alain Laederach

189 A **RNA Structure and Ligand Interactions Probed by Strategically Positioned 15N-Labels**
Tobias Santner, Jasmin Levic, Christoph Kreutz, Ronald Micura

190 B **Integrated genome-wide in silico and capture array approach discovers a large spectrum of novel structured RNAs associated to regulatory elements**
Stefan E Seemann, Claus Hansen, Claus H Bang-Berthelsen, Aashiq H Mirza, Mikkel Christensen-Dalsgaard, Hui Xiao, Zizhen Yao, Elfar Torarinsson, Flemming Pociot, Henrik Nielsen, Niels Tommerup, Walter L Ruzzo, Jan Gorodkin

191 C **Identification and characterization of rice non-coding RNAs involved in nitrogen-starvation stress response**
Sang-yoon Shin, Chanseok Shin

192 A **Divergent transcription of long noncoding RNA/mRNA gene pairs in embryonic stem cells**
Alla Sigova, Alan Mullen, Benoit Molinie, Sumeet Gupta, David Orlando, Matthew Guenther, Albert Almada, Charles Lin, Phillip Sharp, Cosmas Giallourakis, Richard Young

193 B **Uncovering novel microRNAs involved in homeostatic plasticity**
Mariline M. Silva, Joana Fernandes, Sandra D. Santos, Ana Luísa Carvalho

194 C **Transcriptional regulation and non-coding RNA: The Steroid Receptor RNA activator**
Stéphane Thore, Fabiana Arieti, Caroline Gabus-Darlix, Sandrine Coquille

195 A **RNA polymerase-binding elements attenuating transcription in E. coli**
Nadia Tukhtubaeva, Vitaliy Epshtein, Bob Zimmermann, Meghan Lybecker, Ivana Bilusic, Evgeny Nudler, Renee Schroeder

196 B **A link between long intervening noncoding RNAs and microRNA regulation**
Igor Ulitsky, Alena Shkumatava, Hazel Sive, David Bartel

197 C **Evolutionary relationships between PB1 mRNA of Influenza A virus and host microRNAs among vertebrates**
Yuki Usui, Kahori Ikeda, Motomu Matsui, Masaru Tomita, Akio Kanai

198 A **Rat mir-155 generated from the lncRNA Bic is 'hidden' in the alternate genomic assembly and reveals the existence of novel mammalian miRNAs and clusters**
Paolo Uva, Letizia Da Sacco, Manuela Del Cornò, Paola Sestili, Sandra Gessani, Antonella Baldassarre, Massimiliano Orsini, Andrea Masotti, Alessia Palma, Mattia Locatelli

199 B **HEN1-directed labeling of small non-coding RNAs**
Giedrius Vilkaitis, Alexandra Plotnikova, Aleksandr Osipenko, Viktoras Masevicius, Saulius Klimašauskas

200 C **Noncoding RNA-mediated chromosomal fusions**
Xing Wang, John Bracht, Keerthi Shetty, Xiao Chen, Mariusz Nowacki, Laura Landweber

201 A **Regulation of transcription by Pol II-binding RNA aptamers**
Adam Weiss, Maximilian Radtke, Frederike von Pelchrzim, Jennifer Boots, Bob Zimmermann, Marek Zywicki, Katarzyna Matylla-Kulinska, Renée Schroeder

202 B **A short guide to human long non-coding RNA gene nomenclature**
Matt Wright, Ruth Seal, Elspeth Bruford

203 C **RNA functional profiling by gene deletion in S. cerevisiae**
Jian Wu, Steven Parker, Sara Shamsah, Daniela Delneri, Raymond O'Keefe

204 A **Scaffold function of long noncoding RNA HOTAIR in protein ubiquitination**
Je-Hyun Yoon, Kotb Abdelmohsen, Xiaoling Yang, Kumiko Tominaga-Yamanaka, Elizabeth J. White, Arturo V. Ojalo, John L. Rinn, Stefan G. Kreft, Gerald M. Wilson, Myriam Gorospe

Mechanisms of RNA interference

Abstracts 205–222

205 B **Towards improved shRNA inhibitors with a Dicer-independent processing route**
Ben Berkhout

206 C **AGO1 requires interaction with GW182 to repress translation of miRNA targets**
Andreas Boland, Eric Huntzinger, Duygu Kuzuoglu-Öztürk, Maria Fauser, Elisa Izaurralde

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Posters labeled with an "B" present Friday, June 14, 20:00 – 22:30
Posters labeled with an "C" present Saturday, June 15, 14:00 – 17:00

207 A **Argonaute-associated factors required for translational repression in plant RNA silencing**
Clément Chevalier, Jacinthe Azevedo-Favory, Grégory Schott, Christophe Himber, Mohamed-Ali Hakimi, Olivier Voinnet

208 B **Endogenous RNA interference is driven by copy number**
Cristina Cruz, Jon Houseley

209 C **Identification of anhydrobiosis-related genes using RNA interference with 27-bp RNA duplexes**
Cláudia Evangelista, Gustavo Borges, Alan Tunnacliffe, Tiago Pereira

210 A **Human Dicer caught in the act via single-molecule fluorescence spectroscopy**
Mohamed Fareh, Kyu-Hyeon Yeom, Anna S. Haagsma, V. Narry Kim, Chirlmin Joo

211 B **Single-molecule view on the action of Drosophila Dicer-2**
Anna C. Haagsma, Mohamed Fareh, Chirlmin Joo

212 C **Analysis on viral suppressors of RNA silencing in plant cell-free RISC assembly system**
Taichiro Iki, Olivier Voinnet

213 A **Real-time observation of target binding and dissociation of Argonaute-guide complex**
Seung-Ryoung Jung, Eunji Kim, Soochul Shin, Wonseok Hwang, Ji-Joon Song, Sungchul Hohng

214 B **Investigating the potential role of an archaeal Argonaute in the subtype III-B CRISPR system of M. piezophila**
Emine Kaya, Jennifer A. Doudna

215 C **NMR structural study of the two N-terminal dsRBDs of TRBP in complex with siRNA.**
Gregoire Masliah, Christophe Maris, Juerg Hunziker, Nicole Meisner, Frederic Allain

216 A **crRNA-guided R-loop formation and the architecture of the Type I-C Cascade**
Ki Hyun Nam, Ailong Ke

217 B **P body-associated RNA silencing complex PRSC of Cryptococcus effects transposon suppression**
Prashanthi Natarajan, Joshua Dunn, Jonathan Weissman, Phillip Dumesic, Hiten Madhani

218 C **The rough Endoplasmatic Reticulum is the central nucleation site of siRNA-mediated RNA silencing**
Lukas Stalder, Wolf Heusermann, Lena Sokol, Dominic Trojer, Anja Fritzsche, Jan Weiler, Martin Hintersteiner, Florian Aeschimann, David Morrissey, Nicole Meisner Kober

219 A **Functional characterization of DCL1 and DCL2 proteins from Medicago truncatula**
Aleksander Tworak, Anna Urbanowicz, Jan Podkowinski, Marek Figlerowicz

220 B **Distinct activities of the Dictyostelium discoideum RNA-dependent RNA polymerases in post-transcriptional gene regulation**
Stephan Wiegand, Carsten Seehafer, Marek Malicki, Patrick Hofmann, Annika Schmith, Thomas Winckler, Balint Földesi, Benjamin Boesler, Wolfgang Nellen, Johan Reimegård, Lotta Avesson, Fredrik Söderbom, Christian Hammann

221 C **Computational Analyses and Experiments Indicate Role of Guide RNA Structure in MicroRNA::Target Interaction**
Yilong Wu, Jun Zhong, Christian Köberle, Stefan Kaufmann, Volker Patzel

222 A **The CCR4-NOT complex releases PABP from silenced miRNA targets in the absence of deadenylation**
Latifa Zekri, Elisa Izaurralde

RNA and Epigenetics

Abstracts 223–231

223 B **Characterization of small RNAs that contribute to ribosomal RNA gene silencing through the siRNA machinery**
Blake Atwood, Keith Giles, Karen Beeman

224 C **Structural Characterization of the RNA, Cyp33, MLL, Histone H3 Interaction Network**
Markus Blatter, Charlotte Meylan, Frédéric H.-T. Allain

Posters labeled with an "A" present Wednesday, June 12, 20:00 – 22:30
Posters labeled with an "B" present Friday, June 14, 20:00 – 22:30
Posters labeled with an "C" present Saturday, June 15, 14:00 – 17:00

225 A **The Igf2as transcript is exported into the cytoplasm and is associated with polysomes**
Carolina Duart-Garcia, Martin Braunschweig, Tosso Leeb

226 B **Human Argonaute Suppresses Cryptic RNA Polymerase II Transcription and Chromatin Structure of the Silent rRNA Genes.**
Keith Giles, Gaelle Lefevre, Blake Atwood, Mariana Saint Just Ribeiro, Gary Felsenfeld

227 C **Histone replacement reveals distinct essential functions for H3K36 methylation**
Michael Meers, A. Gregory Matera

228 A **Transcriptome-wide Analysis of the m6A Landscape Reveals Pervasive Adenosine Methylation in 3' UTRs and near Stop Codons**
Kate Meyer, Yogesh Saletore, Paul Zumbo, Olivier Elemento, Christopher Mason, Samie Jaffrey

229 B **Single cell analysis reveals aspects of antisense RNA regulation and mode of action in PHO84 transcription repression**
Samir Rahman, Manuele Castelnuovo, Elisa Guffanti, Francoise Stutz, Daniel Zenklusen

230 C **Regulation of piRNA production**
Prashanth Rangan

231 A **Mmi1, an RNA-binding protein, mediates heterochromatin gene silencing by recruiting the nuclear exosome to long non-coding RNAs in fission yeast**
Andre Verdel, Edwige Hiriart, Michael Reuter, Benoit Gilquin, Ravi Sachidanandam, Ramesh Pillai, Leila Touat-Todeschini

Small RNAs

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232 B **Introns of plant pri-miRNAs are required for proper biogenesis and function of miRNAs**
Dawid Bielewicz, Malgorzata Kalak, Maria Kalyna, David Windels, Andrea Barta, Franck Vazquez, Zofia Szweykowska-Kulinska, Artur Jarmolowski

233 C **Common regulation of micro-RNAs by oncogenic transcription factors in B-cell lymphomas**
Mohan Bolisetty, Karen Beemon

234 A **RNA-binding proteins vs. microRNAs: how IGF2BP1 modulates tumor-suppressive microRNA-action in tumor cells**
Bianca Busch, Juliane Braun, Stefan Hüütelmaier

235 B **miRNA profiles characterise distinct states of cellular pluripotency**
Jennifer Clancy, Hardip Patel, Nicole Cloonan, Andrew Corso, Mira Puri, Pete Tonge, Andras Nagy, Thomas Preiss

236 C **Quantitative analysis reveals extensive target specificity of individual let-7 miRNA family members in vivo**
Matyas Ecsedi, Helge Grosshans

237 A **Piwi-Interacting RNAs Protect DNA Against Loss During Oxytricha Genome Rearrangement**
Wenwen Fang, Xing Wang, John Bracht, Mariusz Nowacki, Laura Landweber

238 B **Positive regulation of inflammation by miR-19**
Michael Gantier, H. James Stunden, Bryan Williams

239 C **Characterization of microRNAs derived from the HIV-1 TAR RNA hairpin**
Alex Harwig, Ben Berkhout, Atze Das

240 A **miRNA degradation during C. elegans development**
Gert-Jan Hendriks, Dimosthenis Gaidatzis, Helge Grosshans

241 B **Temporal expression of tRNA fragments in development of *Triops cancriformis* (Tadpole shrimp)**
Yuka Hirose, Kahori Ikeda, Emiko Noro, Kiriko Hiraoka, Masaru Tomita, Akio Kanai

242 C **Highly potent and specific siRNAs isolated from E. coli with endogenous p19 expression**
Linfeng Huang, Jingmin Jin, Larry McReynolds, Judy Lieberman

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243 A **Novel NFL mRNA targeting microRNAs in Amyotrophic Lateral Sclerosis**
Muhammad Ishtiaq, Danae Campos-Melo, Kathryn Volkening, Michael Strong

244 B **A Specialized Mechanism of Translation Regulation in Quiescence**
Sooncheol Lee, Samuel Truesdell, Syed Irfan Bukhari, Ju Huck Lee, Olivier Le Tonqueze, Shobha Vasudevan

245 C **X-ray crystal structure of Maelstrom**
Naoki Matsumoto, Hiroshi Nishimasu, Kaoru Sato, Ryuichiro Ishitani, Mikiko Siomi, Osamu Nureki, Haruhiko Siomi

246 A **DmGTSF1 is essential for effective retrotransposon silencing by Piwi in Drosophila ovarian somatic cells**
Hitoshi Ohtani, Haruhiko Siomi

247 B **Competition between spliceosome and microprocessor complex regulates processing of Splice site Overlapping (SO) 34b pri-miRNA**
Franco Pagani, Chiara Mattioli, Giulia Pianigiani

248 C **Molecular mechanisms of the piRNA biogenesis machinery**
Ramesh Pillai

249 A **Abstract Withdrawn**

250 B **Poly(A) and histone mRNA processing factor Symplekin is involved in endo-siRNA biogenesis**
Mindy Steiniger, William Marzluff

251 C **Sensitized Backgrounds Reveal Critical Roles for microRNA Families**
Elizabeth Jeanne Thatcher, Victor Ambros

252 A **A proteomic screen identifies novel regulators of micro-RNA biogenesis**
Thomas Treiber, Nora Treiber, Simone Harlander, Henning Urlaub, Gunter Meister

253 B **The role of the RNA chaperone protein Hfq in the translation regulation by small noncoding RNAs targeting ompD mRNA from Salmonella typhimurium.**
Zuzanna Urbaniak, Aleksandra Kaszynska, Joanna Strózecka, Daria Grygiel, Mikolaj Olejniczak

254 C **Novel stress-induced smRNAs from Brachypodium distachyon**
Hsiao-Lin V. Wang, Brandon L. Dinwiddie, Herman Lee, Julia Chekanova

255 A **The novel Tetrahymena gene COI12 is crucial for siRNA loading into the Argonaute protein Twi1p**
Sophie Wöhrer, Kazufumi Mochizuki

256 B **An Exchange-Induced Remnant Magnetization Technique for MicroRNA Detection**
Shoujun Xu, Li Yao, Yuhong Wang

257 C **Nonstop decay in C. elegans: examination of a possible role for 22G RNAs**
Elaine Youngman, WeifengGu , Craig Mello

RNA Catalysis and Riboswitches

Abstracts 258–271

258 A **Spliceozymes: Ribozymes that act like Spliceosomes**
Zhaleh Amini, Ulrich Müller

259 B **Molecular Mechanism of preQ1 Riboswitch Action: a Molecular Dynamics Study**
Pavel Banáš, Petr Sklenovský, Joseph E. Wedekind, Jirí Šponer, Michal Otyepka

260 C **Characterization of Metal Ion Binding Sites in the P4 Helix of Bacillus subtilis RNase P**
Yu Chen, Xin Liu, Carol A. Fierke

261 A **Ligand-induced stabilization of the aptamer terminal helix in the adenine riboswitch**
Francesco Di Palma, Francesco Colizzi, Giovanni Bussi

262 B **Studying Conformational Changes in the Glycine Riboswitch using Electron Paramagnetic Resonance Spectroscopy**
Jackie Esquiaqui, Gail Fanucci

263 C **In vitro evolution of a calcium ion-sensing ribozyme from the natural glmS riboswitch-ribozyme**
Matthew Lau, Adrian Ferré-D'Amaré

264 A **Architectural Diversity of PreQ1 Riboswitches**
Phillip J. McCown, Jonathan J. Liang, Ronald R. Breaker

265 B **Theoretical Methods Suggest That Small Ribozymes May Use Multichannel Mechanism During Catalysis**
Vojtech Mlynsky, Pavel Banas, Nils G. Walter, Jiri Sponer, Michal Otyepka

266 C **Tuning the btuB riboswitch fold by chemically modifying its ligand coenzyme B12**
Anastasia Musiari, Sofia Gallo, Kai Zhou, Felix Zelder, Roland Sigel

267 A **A Riboswitch Class for the Bacterial Second Messenger c-di-AMP**
James Nelson, Narasimhan Sudarsan, Kazuhiro Furukawa, Zasha Weinberg, Joy Wang, Ronald Breaker

268 B **Metal Ion Requirement and Substrate Specificity of Kinase Ribozyme K28(1-77)C**
Raghav Poudyal, Elisa Biondi, Josh Forgy, Phuong Nguyen, Donald Burke

269 C **Formation of a catalytic supramolecular RNA 1D-array through self-assembly of an engineered group I intron RNA enzyme**
Narumi Uehara, Hiroyuki Furuta, Yoshiya Ikawa

270 A **A pinch model for activation of HDV-like ribozymes**
Chiu-Ho Webb, Andrej Luptak

271 B **The catalytic mechanism of a novel, widely disseminated small nucleolytic ribozyme**
Timothy Wilson, Tomas Fessl, David Lilley

RNA structure and folding

Abstracts 272–297

272 C **Anion-π or cation-π interactions in RNA?**
Pascal Auffinger, Luigi D'Ascenzo, Eric Westhof

273 A **The solution structure of Tetrahymena telomerase p65-RNA(TER)-TERT RBD determined by SAXS reveals contacts critical for particle stability**
Andrea Berman, Anne Gooding, Robert Rambo, John Tainer, Thomas Cech

274 B **Sizing large RNA molecules using single molecule fluorescence correlation spectroscopy**
Alexander Borodavka, Roman Tuma, Peter Stockley

275 C **CompaRNA: a server for continuous benchmarking of automated methods for RNA structure prediction**
Janusz Bujnicki, Tomasz Puton, Lukasz Kozlowski, Sam Mondal, Marcin Magnus, Kristian Rother

276 A **Symmetry and asymmetry in the unwinding of nucleic acids**
Francesco Colizzi, Yaakov Levy, Giovanni Bussi

277 B **A revised folding pathway of group II introns: Assigning specific structures to the individual FRET states**
Erica Fiorini, Cardo Lucia, Danny Kowerko, David Rueda, Roland K.O. Sigel

278 C **Elucidation of viroids structure by SHAPE**
Tamara Giguère, Charith Raj Adkar-Purushothama, François Bolduc, Jean-Pierre Perreault

279 A **Non-Canonical Base Pair Formation and Ion Binding in Small Bulged RNA**
Neena Grover

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280 B **The plasticity of a structural motif in RNA: structural polymorphism of a k-turn in response to its environment**
Lin Huang, Peter Daldrop, David Lilley

281 C **Ligand selectivity of the neomycin RNA aptamer is highly influenced by its ionic surroundings**
Muslum Ilgu, Bruce D Fulton, Ragothaman Yennamalli, Megan M Kleckler, Monica H Lamm, Taner Z Sen, Marit Nilsen-Hamilton

282 A **A predictive value optimizing the evaluation of biological RNA G-quadruplexes formation**
Rachel Jodoin, Jean-Denis Beaudoin, Jean-Pierre Perreault, Martin Bisaillon

283 B **Metal ions determine the kinetics and thermodynamics of single RNA-RNA associations according to the Irving-Williams series**
Sebastian L.B. König, Danny Kowerko, Mokrane Khier, Mélodie Hadzic, Roland Sigel

284 C **Crowded Environments Compensate Destabilizing Mutations in the Azoarcus Ribozyme**
Hui-Ting Lee, Duncan Kilburn, Sarah Woodson

285 A **A Universal RNA Structural Motif Docking the Elbow of tRNA in the Ribosome, RNAse P and T-box Leaders**
Jean Lehmann, Fabrice Jossinet, Daniel Gautheret

286 B **One-bead coarse grained models for RNA dynamics and folding**
Filip Leonarski, Fabio Trovato, Valentina Tozzini, Andrzej Les, Joanna Trylska

287 C **Crystallization of the active form of the Lariat-Capping ribozyme.**
Mélanie Meyer, Henrik Nielsen, Eric Westhof, Steinar Johansen, Benoît Masquida

288 A **Structural characterization of the yeast telomerase RNA core by SHAPE**
Rachel O. Niederer, David C. Zappulla

289 B **Molecular crowding accelerates ribozyme docking and catalysis**
Bishnu P. Paudel, David Rueda

290 C **Imp3p unfolds conserved and stable stem structures in both U3 snoRNA and pre-rRNA to promote annealing**
Binal Shah, Xin Liu, Carl Correll

291 A **The influence of metal ions on the structure of the CPEB3 ribozyme's P4 region**
Miriam Skilandat, Magdalena Rowinska-Zyrek, Roland K. O. Sigel

292 B **Structural complexity of Dengue untranslated regions: the role of cis-acting RNA motifs and pseudoknot interaction in modulating functionality of the viral genome.**
Joanna Sztuba-Solinska, Tadahisa Teramoto, Jason Rausch, Bruce Shapiro, Radhakrishnan Pademanabhan, Stuart Le Grice

293 C **Self-dimerizing group I ribozymes as a new class of modular units for RNA synthetic biology**
Takahiro Tanaka, Hiroyuki Furuta, Yoshiya Ikawa

294 A **Automated identification of RNA 3D modules with discriminative power in RNA structural alignments.**
Corinna Theis, Christian Hoener zu Siederdissen, Ivo L. Hofacker, Jan Gorodkin

295 B **The regulatory significance of RNA secondary structure in Arabidopsis**
Lee Vandivier, Fan Li, Qi Zheng, Matthew Willmann, Ying Chen, Brian Gregory

296 C **Crystallographic Studies of the Complex between G-quadruplex RNA and the RGG Domain of Fragile X Mental Retardation Protein**
Nikita Vasilyev, Anna Polonskaia, Dinshaw J. Patel, Alexander Serganov

297 A **How do platinum drugs interact with RNA?**
Marianthi Zampakou, Daniela Donghi

RNA chemistry

Abstracts 298–306

298 B **Synthesis of fluorophore- and spin-labeled RNA using deoxyribozymes**
Lea Büttner, Fatemeh Javadi-Zarnaghi, Claudia Höbartner

299 C **Spin-labeled cytidine nucleotides in long synthetic RNAs: solid-phase synthesis, post-synthetic labeling, ligation and EPR-spectroscopic characterization**
Claudia Höbartner, Jan Seikowski, Lea Büttner, Katarzyna Wawrzyniak, Anne Ochmann, Falk Wachowius, Giuseppe Sicoli

300 A **Inhibition of Bcl-2 using photo-crosslinking antisense oligonucleotides**
Akio Kobori, Yuko Nagae, Asako Yamayoshi, Akira Murakami

301 B **Translational regulation by ligand-inducible formation of RNA pseudoknot**
Saki Matsumoto, Changfeng Hong, Asako Murata, Kazuhiko Nakatani

302 C **Targeting secondary structures of pre-miRNA by small molecules: Development of potential inhibitors of pre-miRNA processing**
Asako Murata, Ayako Sugai, Takeo Fukuzumi, Shiori Umemoto, Chikara Dohno, Kazuhiko Nakatani

303 A **Suppression of miR-29a maturation by ligand binding**
Takahiro Otabe, Asako Murata, Fumie Takei, Kazuhiko Nakatani

304 B **Chemical Synthesis of 3'-Aminoacyl-tRNA Mimics to Investigate Antibiotic Induced Ribosome Stalling**
Lukas Rigger, Shanmugapriya Sothiselvam, Nora Vázquez-Laslop, Alexander Mankin, Ronald Micura

305 C **Coumarin fluorochrome binds to Rev responsible element RNA with extremely large absorption shift**
Tetsuya Tsuda, Takeo Fukuzumi, Kazuhiko Nakatani

306 A **Versatile phosphoramidation reactions for effective labeling and conjugation of nucleic acids**
Tzu-Pin Wang, Yu-Chih Su

Therapeutic RNAs

Abstracts 307–310

307 B **CACNA2D4: a novel paradigm for the application of antisense-mediated gene therapy to the cure of retinal dystrophies**
Niccolo Bacchi, Gian Carlo Demontis, Simona Casarosa, Michela Alessandra Denti

308 C **Antisense RNA-induced EXON-skipping for the Gene Therapy of Frontotemporal Dementia and Parkinsonism Associated with Chromosome 17 (FTDP-17).**
Giuseppina Covello, Kavitha Siva, Lara Mari, Elena Marchesi Marchesi, Perrone Daniela Perrone, Michela Alessandra Denti

309 A **Messenger RNA as a novel therapeutic approach**
Antonin de Fougerolles

310 B **Identification of novel anti-obesity genes in primary human adipocytes using RNAi screening**
Akanksha Gangar, Didier Grillot, Sandrine Martin, Valerie Linhart, Alizon Riou-Eymard, Raphaelle Guillard, Kim Carrein, Maarten Van Balen, Brigitta Witte, Nick Vandeghinste, Marlijn Steger, David Fischer, Steve De Vos, Dominique Langin, Vladimir Stich, Stephane Huet

Workshop: RNA in pharmaceutical research

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311 C **Inhibition of the HIV -1 virus RRE-Rev interaction by small molecule Rev mimics. A new synthetic scaffold for specific recognition of RNA structure.**
Luis Gonzalez-Bulnes, Ignacio Ibañez, Luis Miguel Bedoya, Silvia Catalan, Jose Alcami, Santos Fustero, Jose Gallego

Therapeutic RNAs

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312 A **RNA aptamer C3 is a non-ATP site inhibitor of the MAP kinase ERK2**
Sabine Lennarz, Günter Mayer

313 B **mRNA transfection - a transient transgene expression in human mesenchymal stem cells as an attractive tool for regenerative medicine**
Adam Nowakowski, Anna Andrzejewska, Hanna Kozlowska, Piotr Walczak, Barbara Lukomska, Miroslaw Janowski

314 C **Combined systemic and local morpholino treatment rescues the phenotype of the SMA Delta 7 mouse model**
Marc-David Ruepp, Monica Nizzardo, Chiara Simone, Sabrina Salani, Federica Rizzo, Margherita Ruggieri, Simona Brajkovic, Hong Moulton, Oliver Mühlemann, Nereo Bresolin, Giacomo P. Comi, Stefania Corti

315 A **RNA-i Therapy for Fronto Temporal Dementia and Parkinsonism Linked to Chromosome 17**
Kavitha Siva, Giuseppina Covello, Michela Alessandra Denti

tRNA, snRNA, snoRNA, rRNA

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316 B **Studies on structure-function relationships of the snoRNP assembly machinery**
Christiane Branlant, Régis Back, Benjamin Rothé, Jonathan Bizarro, Magali Blaud, Marc Quinternet, Solange Morera, Philippe Meyer, Cyril Dominguez, Frédéric Alain, Edouard Bertrand, Xavier Manival, Bruno Charpentier

317 C **Lateral tRNA gene transfer in Methanobrevibacter ruminantium**
Patricia Chan, Todd Lowe

318 A **Mitochondrial poly(A) polymerase is involved in tRNA editing**
Mario Fiedler, Elmar Wahle, Christiane Rammelt

319 B **High throughput quantification of tRNA function reveals unexpected interactions between tRNA residues**
Michael Guy, David Young, Yoshiko Kon, Xiaoju Zhang, Matthew Payea, Kimberly Dean, David Mathews, Elizabeth Grayhack, Stanley Fields, Eric Phizicky

320 C **Gene expression analysis of nematode-specific tRNAs (nev-tRNAs) that can decode an alternative genetic code**
Kiyofumi Hamashima, Yoshiki Andachi, Masaru Tomita, Yuji Kohara, Akio Kanai

321 A **Structure and Kinetic Mechanism of Protein-only RNase P from A. thaliana**
Michael Howard, Markos Koutmos, Carol Fierke

322 B **Biochemical Characterization of Archaeal RNase E-like Protein, FAU-1 in *Pyrococcus furiosus***
Yoshiki Ikeda, Asako Sato, Masaru Tomita, Akio Kanai

323 C **Strong anion exchange fast performance liquid chromatography as a versatile tool for preparation and purification of RNA produced by in vitro transcription**
Jiri Koubek, Ku Feng Lin, Yet Ran Chen, Richard Ping Cheng, Joseph Jen Tse Huang

324 A **Identification of proteins co-purifying with the yeast RNA exosome and their effect on the complex stabilization**
Rogerio Lourenco, Fernando Gonzales, Carla Oliveira

325 B **tRNAscan-SE and GtRNAdb: Improving Detection and Functional Prediction Based on Genomic Context, Structure, and Expression of Transfer RNAs**
Todd Lowe, Patricia Chan, Aaron Cozen, Andrew Holmes, Jay Kim, Eva Robinson

326 C **RIP-seq analysis of eukaryotic Sm proteins reveals interactions between snRNPs and mature mRNAs**
Zhipeng Lu, Xiaojun Guan, Greg Matera

327 A **Absence of a Universal Element for tRNAHis Identity in Eucarya**
Bhalchandra Rao, Jane Jackman

328 B **Thiolation of specific tRNAs by URM1 is required for efficient translation a subset of proteins by promoting ribosomal A-site binding**
Namit Ranjan, Vanessa Rezgui, Patrick Pedrioli, Kshitiz Tyagi, Marina Rodnina, Andrey Konevega, Joerg Mittelstaet, Matthias Peter

329 C **A comprehensive analysis of the natural variation of tRNA modification in two Saccharomyces species**
Peter Sarin, Sebastian Leidel

330 A **A Protease that Cleaves the C-terminal Domain of the RtcB-type RNA Ligase in the Hyperthermophilic Archaeon Pyrococcus furiosus**
Asako Sato, Takeshi Masuda, Masaru Tomita, Takashi Itoh, Akio Kanai

331 B **Human mitochondrial RNase P and its multiple faces**
Elisa Vilardo, Nadia Brillante, Christa Nachbagauer, Walter Rossmanith

332 C **Playing RNase P evolution: replacing a complex ribonucleoprotein enzyme with a single protein**
Christoph Weber, Roland K. Hartmann, Andreas Hartig, Walter Rossmanith

333 A **tRNA maturation abnormalities connected to stress conditions and transcription deregulation**
Dominika Wichtowska, Anita K. Hopper, Magdalena Boguta

334 B **A Genome-wide Analysis to Identify Novel Genes Involved in tRNA Metabolism and Subcellular Trafficking**
Jingyan Wu, Anita K. Hopper

Ribosomes and Translation

Abstracts 335–361

335 C **Expanded Function of Trans-Editing Domains To Edit Non-canonical Amino Acids Prevents Errors In Translation**
Jo Marie Bacusmo, Birgit Alber, Karin Musier-Forsyth

336 A **Characterization of Rbtf1 and its role in ribosome synthesis**
Lukas Bammert, Ulrike Kutay

337 B **When p53 senses faulty ribosomes: Induction of Tp53 correlates with enhanced expression of c-Myc target nucleolar proteins in Rpl11-deficient zebrafish**
Anirban Chakraborty, Tamayo Uechi, Pierre-Emmanuel Gleizes, Naoya Kenmochi

338 C **Exploring translation in S. pombe using ribosomal profiling**
Caia Duncan, Juan Mata

339 A **Escherichia coli ribosomal protein S1 unfolds structured mRNAs onto the ribosome for active translation initiation**
Mélodie Duval, Alexey Korepanov, Olivier Fuchsbauer, Pierre Fechter, Ronald Micura, Bruno Klaholz, Stefano Marzi, Pascale Romby

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340 B **Antibiotic-mediated frameshift during translation of the leader ORF reveals a new principle of gene regulation**
Pulkit Gupta, Nora Vazquez-Laslop, Alexander Mankin

341 C **High-resolution cryo-electron microscopy structure of the Trypanosoma brucei ribosome**
Yaser Hashem, Amedee Des Georges, Sarah Buss, Fabrice Jossinet, Amy Jobe, Qin Zhang, Robert Grassucci, Chandrajit Bajaj, Eric Westhof, Susan Madison-Antenucci, Joachim Frank

342 A **Cryo-EM structure of the mammalian ribosomal 43S preinitiation complex**
Yaser Hashem, Amedee Des Georges, Vidya Dhote, Robert Langlois, Robert Grassucci, Christopher U.T. Hellen, Tatyana V. Pestova, Joachim Frank

343 B **Alu RNPs target 40S ribosomal subunits to repress translation initiation**
Elena Ivanova, Audrey Berger, Elena Alkalaeva2 Anne Scherrer, Katharina Strub

344 C **Interactions of modified oligonucleotides with RNA of the prokaryotic and eukaryotic decoding site**
Maciej Jasinski, Sapna Thoduka, Ryszard Stolarski, Joanna Trylska

345 A **The intimate connection of RNA granules with human 4Es changes with use of different protein variants**
Klára Kazdová, Katerina Mocová, Tomáš Mašek, Silvia Mrvová, Martin Pospíšek

346 B **Crystal structures of the protozoal cytoplasmic A site in complex with aminoglycosides**
Jiro Kondo, Mai Koganei

347 C **Rrp5 binds the pre-rRNA at multiple sites and is required for ribosomal processing and assembly**
Simon Lebaron, Åsa Segerstolpe, Sarah French, Sander Granneman, Ann Beyer, Lars Wieslander, David Tollervey

348 A **Function of Fap7 in the maturation of the ribosome small subunit**
Jérôme Loc'h, Magali Blaud, Stéphane Réty, Simon Lebaron, Sander Granneman, David Tollervey, Patrick Deschamps, Julie Jombart, Joseph Bareille, Nicolas Leulliot

349 B **Molecular characterization of ribosomal E-site function**
Nicola Rusca, Nina Clementi, Matthias Erlacher, Norbert Polacek

350 C **Evolutionary decline for a nuclear-encoded human mitochondrial aminoacyl-tRNA synthetase**
Hagen Schwenzer, Gert Scheper, Nathalie Zorn, Luc Moulinier, Agnès Gaudry, Emmanuelle Leize, Franck Martin, Catherine Florentz, Olivier Poch, Marie Sissler

351 A **The communication between ribosome biogenesis and cell cycle machinery**
Md Shamsuzzaman, Mamata Thapa, Lasse Lindahl

352 B **Effect of tRNA core modifications and a D-loop sequence element on ribosomal decoding**
Irina Shepotinovskaya, Olke Uhlenbeck

353 C **Non-ribosomal Interaction Partners of Ribosomal Protein S3**
Tamsyn Stanborough, Barbara Koch, Valentin Mitterer, Johannes Niederhauser, Brigitte Pertschy

354 A **Sequence-specific targeting of bacterial ribosomal RNA as a way to look for inhibition pockets**
Sapna G. Thoduka, Zofia Dabrowska, Tomasz Witula, Maciej Jasinski, Anna Górska, Joanna Trylska

355 B **Molecular pathogenesis of ribosomopaties in zebrafish model for Diamond-Blackfan anemia**
Tamayo Uechi, Yukari Nakajima, Hiroko Sonoda, Masahiro keda, Yutaka Suzuki, Sumio Sugano, Naoya Kenmochi

356 C **Investigating the function of the RNA helicase Prp43 and its cofactor Pfa1 in 40S ribosomal subunit synthesis**
Stefan Unterweger, Brigitte Pertschy

357 A **A New Role for NOT5 of the CCR4-NOT Complex in Connecting Transcription with Translation**
Zoltan Villanyi, Sujatha Subbana, Olesya Panasenko, Zoltan Pahi, Imre Boros, Martine Collart

358 B **Ribosome biogenesis in plants and its impact on developmental processes**
Benjamin Weis, Sandra Missbach, Enrico Schleiff

359 C **Going through the motions: Network analysis reveals conserved intramolecular communication pathways within EF-Tu responsible for ribosome dependent GTPase activation and nucleotide exchange**
Hans-Joachim Wieden, Evan Mercier, Fan Mo, Dylan Girodat

360 A **Transcription regulation by cAMP-CRP of the rmf gene for 100S ribosome formation**
Hideji Yoshida, Tomohiro Shimada, Yasushi Maki, Shou Furuike, Masami Ueta, Chieko Wada, Akira Wada, Akira Ishihama

361 B **Towards understanding the inhibitory effects of codon-anticodon wobble base pairing on the ribosome**
Hani Zaher, Rachel Green

Translational Regulation

Abstracts 362–399

362 C **One messenger RNA and three initiation codons govern the synthesis of two human Glycyl-tRNA synthetases isoforms**
Jana Alexandrova, Caroline Paulus, Joëlle Rudinger-Thirion, Magali Frugier

363 A **A tale of two termini: profiling mRNA 5'-3' interactions in vivo.**
Stuart Archer, Meghna Sobti, Claus Hallwirth, Jenni Yuan, Traude Beilharz, Thomas Preiss

364 B **Translation of the human erythropoietin transcript is regulated by an upstream open reading frame in response to hypoxia**
Cristina Barbosa, Luísa Romão

365 C **Nanos recruits the CCR4-NOT complex to induce degradation of mRNA targets**
Dipankar Bhandari, Tobias Raisch, Elisa Izaurralde

366 A **Study of PTBP1-RRM12 in complex with an RNA-stemloop of the EMCV-IRES**
Georg Braach, Christophe Maris, Frédéric H.-T. Allain

367 B **CPEB3-controlled translation regulates memory consolidation**
Hsu-Wen Chao, Li-Yun Tsai, Yi-Ling Lu, Pei-Yi Lin, Wen-Hsuan Huang, Hsin-Jong Chou, Wen-Hsin Lu, Ping-Tao Lee, Yi-Shuian Huang

368 C **DDX3 Regulates Rac1 Translation, Modulates Wnt Signaling and Is Required for Cancer Cell Metastasis**
Hung-Hsi Chen, Hsin-I Yu, Wei-Chih Cho, Woan-Yuh Tarn

369 A **Inactivation of the mTORC1-eIF4E Pathway alters Stress Granules Formation**
Laëtitia Coudert, Marie-Josée Fournier, Pauline Adjibade, Rachid Mazroui

370 B **Identification of DDX6 as a cellular modulator of VEGF expression under hypoxia**
Sebastian de Vries, Isabel S. Naarmann-de Vries, Henning Urlaub, Hongqi Lue, Jürgen Bernhagen, Antje Ostareck-Lederer, Dirk Ostareck

371 C **Molecular characterization of SMG1-UPFs complexes**
Aurelien Deniaud, Marcello Clerici1, Simonas Masiulis, Volker Boehm, Niels Gehring, Stephen Cusack, Christiane Schaffitzel

372 A **Changes in polysomal profiles in cells with aberrant RNA degradation**
Zuzana Feketova, Dmytro Ustianenko, Hana Konecna, Zbynek Zdrahal, Stepanka Vanacova

373 B **Homeostatic regulation of AMPA receptors in synaptic plasticity: a posttranscriptional interplay between Caspr1 and the RNA-binding protein ZBP1.**
Dominique Fernandes, Luís Ribeiro, Sandra Santos, Ana Luísa Carvalho

374 C **When small non-coding RNAs meet the ribosome: Tuning the translational machinery**
Jennifer Gebetsberger, Marek Zywicki, Norbert Polacek

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375 A **Observation of mRNA Surveillance in Living Yeast by Ribosome Profiling**
Rachel Green, Nicholas R. Guydosh

376 B **The connection between mRNA binding by the DEAD-box helicase Ded1p and the kinetics of translation initiation.**
Ulf-Peter Guenther, Frank Tedeschi, Akshay Tambe, Sarah Geisler, Jeff Coller, Eckhard Jankowsky

377 C **Splicing factor SRSF3 represses the translation of Programmed Cell Death 4 mRNA by associating with the 5'UTR**
Sunjoo Jeong

378 A **Activation of HRI Kinase and Translation Control by Oxidative Stress**
Bogdan Jovanovic, Georg Stoecklin

379 B **Structural basis for translation termination by archaeal RF1 and GTP-bound EF1A complex**
Kan Kobayashi, Kazuki Saito, Ryuichiro Ishitani, Koichi Ito, Osamu Nureki

380 C **Translation of HTT mRNA with expanded CAGrepeats is regulated by the MID1–PP2A proteincomplex**
Sybille Krauss, Nadine Griesche, Ewa Jastrzebska, Changwei Chen, Désiree Rutschow, Clemens Achmüller, Stephanie Dorn, Sylvia M. Boesch, Maciej Lalowski, Erich Wanker, Rainer Schneider, Susann Schweiger

381 A **Posttranscriptional control of the DNA damage response through TIAR**
Vanesa Lafarga, Johanna Schott, Georg Stoecklin

382 B **Human DDX3 interacts with the HIV-1 Tat protein to facilitate viral mRNA translation**
Ming-Chih Lai, Shaw-Jenq Tsai, H. Sunny Sun

383 C **Exploring the role of the GW182 protein, Gawky (Gw) during early Drosophila melanogaster embryogenesis**
Jing Li, Andrew Simmonds

384 A **Global translational control during norovirus infection**
Nicolas Locker, Elizabeth Royall, Lisa Roberts, Majid Al-Sailawi, Azimah Abdul-Wahad, André Gerber

385 B **Cap-independent translational regulation of mammalian target of rapamycin (mTOR)**
Ana Marques-Ramos, Alexandre Teixeira, Rafaela Lacerda, Luísa Romão

386 C **NALM-6 acute lymphoblastic leukaemia cell line has elevated level of the subunit b of translation initiation factor 3**
Silvia Mrvova, Katerina Mocova, Martin Pospisek, Tomas Masek

387 A **Caspase-3 cleaves hnRNP K in erythroid differentiation**
Isabel S. Naarmann-de Vries, Henning Urlaub, Dirk H. Ostareck, Antje Ostareck-Lederer

388 B **Translational regulation of human hemojuvelin expression via upstream open reading frames**
Cláudia Onofre, Cristina Barbosa, Luísa Romão

389 C **Gene silencing using artificial small RNAs derived from a natural RNA scaffold in Escherichia coli**
Hongmarn Park, Geunu Bak, Sun Chang Kim, Younghoon Lee

390 A **Translation of human LAT2 mRNA is controlled by a short upstream open reading frame**
George Perdrizet, Donghui Zhou, Allison Wu, Mariana Pavon, Tao Pan

391 B **Regulation of mRNA translation in late-phase activated macrophages**
Sonja Reitter, Johanna Schott, Georg Stoecklin

392 C **Two Retinoblastoma associated SNVs in RB1 form a RiboSNitch.**
Wes Sanders, Matt Halvorsen, Justin Ritz, Joshua Martin, Alain Laederach

393 A **Regulation of translation of the most abudant protein in human body, type I collagen.**
Branko Stefanovic, Lela Stefanovic, Azariyas Challa, Zarko Manojlovic

394 B **Regulation of translation of collagen mRNAs by STRAP**
Lela Stefanovic, Milica Vukmirovic, Branko Stefanovic

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395 C **Dissecting the regulation of vFLIP expression, a Kaposi's Sarcoma-associated Herpesvirus tumorigenesis factor**
Mariam Sulaiman, Margaret Carter, Zulkefley Othman, David Blackbourn, Nicolas Locker, Lisa Roberts

396 A **The role of human RLI in cell proliferation and translational regulation**
Marina Toompuu, Kairi Kärblane, Cecilia Sarmiento, Erkki Truve

397 B **Dom34-mediated dissociation of non-translating ribosomes allows efficient restart of translation after stress****
Antonia van den Elzen, Bertrand Séraphin

398 C **Unravelling the role of dimerization for the STAR-domain RBP, GLD-1**
Jane Wright, Lea Dümpelmann, Sanda Mülhäuser, Heinz Gut, Rafal Ciosk

399 A **Tdrd7, a RNA binding protein, acts to restrict Epha2 protein synthesis in space and time during lens development**
Ying Zhang, Richard Maas

3' end processing

Abstracts 400–414

400 B **Alternative polyadenylation in CD2 expression**
Inês Boal-Carvalho, Mafalda Pinto, Juliana Miranda, João Relvas, Teresa Summavielle, Alexandre Carmo, Alexandra Moreira

401 C **Post-transcriptional regulation of COX-2**
Ashley Cornett, Carol Lutz

402 A **Assessing the "geometry" of the TRAMP and exosome complexes.**
Clémentine Delan-Forino, Stepanka Vanacova, David Tollervey

403 B **Role of mRNA 3' processing in the progression of the DNA damage response (DDR)**
Emral Devany, Xiaokan Zhang, Mirjana Persaud, Frida Kleiman

404 C **Transcriptional and translational profiles in stimulated T lymphocytes**
Andreas Gruber, Georges Martin, Philipp Müller, Nitish Mittal, Alexander Schmidt, Walter Keller, Jean Pieters, Mihaela Zavolan

405 A **Shifting targets: microRNA variants and alternative polyadenylation in cardiac hypertrophy**
Carly J. Hynes, David T. Humphreys, Nicola J. Smith, Hardip R. Patel, Robert M. Graham, Jennifer L. Clancy, Thomas Preiss

406 B **Towards the understanding of the CTD-code**
Karel Kubicek, Olga Jasnovidova, Jakub Macosek, Richard Stefl

407 C **Enhancer of RNA Interference -1-LIKE-1: One More Player in the RNA Processing Game of the Chloroplast**
Glykeria Mermigka, Ioannis Vlatakis, Eugenia Vamvaka, Jutta Helm, Heiko Schumacher, Kriton Kalantidis

408 A **FUS protein interacts with U7 snRNP and plays a role in replication-dependant histone genes expression**
Katarzyna Dorota Raczynska, Marc David Ruepp, Zofia Szweykowska-Kulinska, Artur Jarmolowski, Daniel Schümperli

409 B **Mammalian mRNA 3'end formation: is this the end? New insight on CstF64**
Valentina Romeo, Daniel Schümperli

410 C **The U7 snRNP revisited: a complex of the core U7 snRNP, FLASH and multiple polyadenylation factors controls 3' end processing of histone pre-mRNAs in vertebrates and invertebrates**
Ivan Sabath, Aleksandra Skrajna, Xiao-cui Yang, Michal Dadlez, William F. Marzluff, Zbigniew Dominski

***Abstract presented as an oral in Plenary Session 1 - Ribosome biogenesis and translation*

411 A **A search for new factors involved in 3' end processing of histone pre-mRNAs: proteins interacting with a complex of the conserved stem-loop and the Stem-Loop Binding Protein (SLBP) in Drosophila.**
Aleksandra Skrajna, Xiao-cui Yang, Ivan Sabath, Michal Dadlez, William F. Marzluff, Zbigniew Dominski

412 B **Enhancer of RNA Interference -1-LIKE-1: The ERI1 Plant Homologue Involved in the RNA Processing Game of the Chloroplast**
Ioannis Vlatakis, Glykeria Mermigka, Jutta Helm, Heiko Schumacher, Eugenia Vamvaka, Kriton Kalantidis

413 C **A triple helix structure is able to functionally replace a poly(A) tail**
Jeremy Wilusz, Laura Lu, Phillip Sharp

414 A **FPA, a regulator of alternative polyadenylation, is closely associated with cleavage and polyadenylation factors in vivo**
Vasiliki Zacharaki, Katarzyna Rataj, Gordon Simpson

RNA Turnover

Abstracts 415–446

415 B **Regulation of stress granules formation during calicivirus infection**
Majid Al-Sailawi, Nicolas Locker, Elizabeth Royall, Lisa Roberts

416 C **Exploring the role of mouse DEAH helicase, skiv2l2, in processing and degradation of non coding RNAs in neuronal N2A cells using high throughput pA-seq analysis**
James Anderson, Jane Dorweiler, Fengchao Wang, Jun Zhu, Stephen Munroe

417 A **Molecular genetic exploration of the yeast DEAH helicase, Mtr4, arch domain**
James Anderson, Yan Li, Joseph Burclaff

418 B **mRNA degradation on the ribosome in Drosophila cells**
Sanja Antic, Anna Skucha, Silke Dorner

419 C **The NOT2/NOT5 Module of the CCR4-NOT Complex is Required for Assembly of the Exosome**
Olesya Bukach, Virginie Ribaud, Geoffroy Colau, Martine Collart

420 A **Adipogenesis is efficiently regulated by SMG1 via staufen1-mediated mRNA decay**
Hana Cho, Kyoung Mi Kim, Sisu Han, Yoon Ki Kim

421 B **Control of mRNA metabolism by deadenylation**
Jeff Coller

422 C **The structural and functional organization of the TRAMP complex**
Sebastian Falk, John Weir, Peter Reichelt, Jendrik Hentschel, Fabien Bonneau, Elena Conti

423 A **Control of mRNA decay by Puf proteins regulates ribosome biogenesis**
Anthony Fischer, Wendy Olivas

424 B **Transcriptome analysis reveals thousands of targets of nonsense-mediated mRNA decay that offer clues to the mechanism in human, fish, and fly**
Courtney French, Gang Wei, Angela Brooks, Thomas Gallagher, Li Yang, Brenton Gravely, Sharon Amacher, Steven Brenner

425 C **Retroviral strategies for NMD evasion**
Zhiyun Ge, Stacey L. Baker, J. Robert Hogg

426 A **Poly(A)-specific ribonuclease (PARN): Mechanisms of processivity and catalysis**
Niklas Henriksson, Mikael Nissbeck, Per Nilsson, Magnus Lindell, Samuel Flores, Santhosh Dhanraj, Hongbing Li, Yigal Dror, Anders Virtanen

427 B **DHX34 activates NMD by promoting the transition from the SURF to the DECID complex.**
Nele Hug, Oscar Llorca, Javier Caceres

428 C **Identification of novel UPF1 target transcripts by direct determination of whole transcriptome stability.**
Naoto Imamachi, Hidenori Tani, Kazi Abdus Salam, Rena Mizutani, Takuma Irie, Tetsushi Yada, Yutaka Suzuki, Nobuyoshi Akimitsu

429 A **RT-qPCR Reference Genes and Potassium Stress Responses in the Haloarchaeon, Haloarcula marismortui**
Matthew W. Jensen, Scott Matlock, Carlene H. Reinheimer, Andrea Gorrell

430 B **Investigation of premature termination codon recognition in nonsense-mediated mRNA decay**
Raphael Joncourt, Andrea Eberle, Oliver Mühlemann

431 C **Expression of nonsense-mediated decay factors is controlled by conserved feedback loops and responds to salt stress in plants**
Anil Kesarwani, Gabriele Drechsel, Andreas Wachter

432 A **High-resolution characterization of regulatory sequences within a mammalian 3' UTR**
Katla Kristjansdottir, RaeAnna Wilson, Elizabeth Fogarty, Andrew Grimson

433 B **Direct Visualization of Alternative RNA Substrate Recruiting Pathways in Yeast Exosome**
Jun-Jie Liu, Ailong Ke, Hong-Wei Wang

434 C **SMG7 recruits the CCR4-NOT complex for degradation of NMD targets**
Belinda Loh, Stefanie Jonas, Elisa Izaurralde

435 A **The mRNA quality control factors Ski7 and Hbs1 evolved from an alternatively spliced gene that produced Ski7-like and Hbs1-like proteins.**
Alexandra N. Marshall, Maria Camila Montealegre, Claudia Jiménez-López, Michael C. Lorenz, Ambro van Hoof

436 B **Functional analysis of IMP3, a RNA-binding protein**
Rena Mizutani, Yutaka Suzuki, Nobuyoshi Akimitsu

437 C **Global analysis of exosome target introns**
Elina Niemelä, Ali Oghabian, Ger Pruijn, Mikko Frilander

438 A **A Novel Role for the Arginine Methyltransferase CARM1 in Nonsense Mediated Decay: Implications for Spinal Muscular Atrophy**
Gabriel Sanchez, Jocelyn Cote

439 B **Assessing differential susceptibility of mRNAs to NMD**
Christoph Schweingruber, Oliver Mühlemann

440 C **Structural insights into the Dhh1-Pat1 interaction**
Humayun Sharif, Sevim Ozgur, Elena Conti

441 A **Acetylation of CAF1a and BTG2 accelerates general mRNA degradation**
Sahil Sharma, Georg Stoecklin

442 B **Mpn1, mutated in poikiloderma with neutropenia protein 1, unveils cellular surveillance of catalytic spliceosomal small nuclear RNAs.**
Vadim Shchepachev, Charlotte Soneson, Claus M. Azzalin

443 C **MKT1: a hub in a post-transcriptional regulatory network**
Aditi Singh, Igor Minia, Dorothea Droll, Abeer Fadda, Esteban Erben, Christine Clayton

444 A **Structural basis for the multiple roles Edc3 plays in mRNA degradation**
Remco Sprangers, Simon Fromm, Charlotte Meylan, Niklas Hoffmann, Anna-Lisa Fuchs, Vincent Truffault

445 B **CBP1 mRNA is cleaved and produces nonstop mRNA in a tRNA splicing endonuclease activity dependent manner**
Tatsuhisa Tsuboi, Yutaka Suzuki, Tohru Yoshihisa, Toshifumi Inada

446 C **Enriched density of UPF1 in 3' untranslated regions results from its translation-dependent displacement from coding sequences**
David Zünd, Andreas Gruber, Mihaela Zavolan, Oliver Mühlemann

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RNA Editing and Modification

Abstracts 447–473

447 A **Activation of RNA cap methylation by CDK1**
Michael Aregger, Victoria H. Cowling

448 B **An emerging role for double-stranded RNA binding domains: regulating the sub-cellular localization of proteins**
Pierre Barraud, Silpi Banerjee, Stephan Emmerth, Weaam Ibrahim, Katrina Woolcock, Marc Bühler, Michael Jantsch, Frédéric Allain

449 C **Revealing the full scope of Alu editing - over a hundred million genomic sites are subject to primate-specific A-to-I RNA editing**
Lily Bazak, Ami Haviv, Michal Barak, Jasmine Jacob-Hirsch, Karen Kesarcas, Gideon Rechavi, Jin Billy Li, Erez Y. Levanon, Eli Eisenberg

450 A **A Hundred million sites located in majority of human genes are subject to A-to-I RNA editing**
Lily Bazak, Ami Haviv, Michal Barak, Jasmine Jacob-Hirsch, Karen Kesarcas, Gideon Rechavi, Jin Billy Li, Erez Y. Levanon, Eli Eisenberg

451 B **MODOMICS: a database of RNA modification pathways - new developments**
Janusz Bujnicki, Magdalena Machnicka, Kaja Milanowska, Kristian Rother, Mark Helm, Henri Grosjean, Elzbieta Purta, Okan Osman Oglou4 Malgorzata Kurkowska, Anna Olchowik, Witold Januszewski, Stanislaw Dunin-Horkawicz, Pawel Piatkowski, Sebastian Kalinowski

452 C **Alu inverted repeats induce human specific site selective A-to-I RNA editing**
Chammiran Daniel, Gilad Silberberg, Marie Öhman

453 A **Identifying the function of highly conserved residues for pseudouridine formation through a combination of in silico and in vitro studies**
Jenna Friedt, Fern Leavens, Evan Mercier, Hans-Joachim Wieden, Ute Kothe

454 B **Identification of the last rRNA methylase YhiR E.coli using a new technique**
Anna Golovina, Margarita Dzama, Petr Sergiev, Olga Dontsova

455 C **RAM: a novel and essential component of RNA cap methylation**
Thomas Gonatopoulos-Pournatzis, Victoria H. Cowling

456 A **Conserved circuitry in eukaryotes for crucial Trm7 modifications of the tRNA anticodon loop**
Michael Guy, Eric Phizicky

457 B **Structural and Functional Analysis of Archaeal ATP-dependent RNA Ligase.**
Kiong Ho, Huiqiong Gu, Yuko Takagi, Katsuhiko Murakami

458 C **Consequences of FilaminA editing**
Mamta Jain, Maja Stulic, Dieter Pullirsch, Michael Jantsch

459 A **2´-SCF3 Modified Pyrimidine Nucleosides as Labels for Probing RNA Structure and Function by 19F NMR Spectroscopy**
Marija Košutic, Katja Fauster, Christoph Kreutz, Ronald Micura

460 B **A Quantitative Atlas of RNA Editing in Mammals Reveals Dynamic Spatiotemporal Regulation**
Jin Billy Li, Meng How Tan, Robert Piskol

461 C **A single-molecule study on the molecular mechanism of microRNA uridylation**
Luuk Loeff, Kyu-Hyeon Yeom, Mohamed Fareh, V. Narry Kim, Chirlmin Joo

462 A **Structural features of Cbf5 and guide RNA involved in the functions of archaeal box H/ACA RNP complexes**
Mrinmoyee Majumder, Ramesh Gupta

463 B **The role of ADAR1 in the innate immune response**
Niamh Mannion, Sam Greenwood, Xianghua Li, Mary O'Connell

464 C **Functional implications from the atomic model of the poly(U) polymerase Cid1**
Paola Munoz-Tello, Caroline Gabus, Stephane Thore

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465 A **tRNA wobble uridine hypomodification decreases the decoding efficiency of cognate codons *in vivo***
Danny D. Nedialkova, Sebastian A. Leidel

466 B **Localization of ADAR1 to cytoplasmic stress granules**
Siew Kit Ng, Rebekka Weissbach, Deirdre Scadden

467 C **A Comprehensive Analysis of RNA Modifying Enzymes in Zebrafish**
Marion Pesch, Erez Raz, Jana Pfeiffer, Ursula Jordan, Sebastian Leidel

468 A **Differential expression of Human ADAT subunits**
Adrián Gabriel Torres, Adélaïde Saint-Leger, Francisco Miguel Torres, Eva Maria Novoa, Anna Tor, Lluís Ribas de Pouplana

469 B **RNA methylation: a mechanism for post-transcriptional regulation that is deregulated in cancer?**
Tennille Sibbritt, Hardip Patel, Brian Parker, David Humphreys, Jeffrey Squires, Susan Clark, Thomas Preiss

470 C **Engineered guideRNA-Dependent Deaminases - A Tool to Modify mRNA**
Thorsten Stafforst, Marius Schneider

471 A **Impact of inverted SINEs on gene expression**
Mansoureh Tajaddod, Konstantin Licht, Florian Huber, Michael Jantsch

472 B **RNA-binding proteins regulate substrate-specific changes in A to I editing patterns**
Aamira Tariq, Wojciech Garncarz, Oliver Pusch, Ales Balik, Michael Jantsch

473 C **Enzymes involved in human cap structure formation: their structure and function**
Maria Werner, Miroslaw Smietanski, Elzbieta Purta, Katarzyna H. Kaminska, Janusz Stepinski, Edward Darzynkiewicz, Marcin Nowotny, Janusz M. Bujnicki

Splicing Mechanisms

Abstracts 474–488

474 A **Genetic analysis of the Prp28-bypass mutant reveals further insights on U1 snRNP/5' splice site interaction**
Shang-Lin Chang, Tien-Hsien Chang

475 B **Chemical tools for investigating alternative RNA splicing**
Sara De Ornellas, Ian Eperon, Glenn Burley

476 C **Biophysical characterization of the recombinant S. cerevisiae Lsm2-8 complex**
Elizabeth Dunn, Trushar Patel, Richard Fahlman, Calvin Yip, Sean McKenna, Stephen Rader

477 A **smFRET studies of U6 during spliceosome activation in budding yeast**
Megan Mayerle, John Abelson, Christine Guthrie

478 B **Role of U2 stem IIb in splicing progression**
Alberto Moldon, Charles Query

479 C **Conserved Slu7 motif confers the preference for distal splice sites in yeast**
Eva Nicova, Katerina Abrhamova, Ondrej Gahura, Vanda Munzarova, Frantisek Puta, Petr Folk

480 A **Prp8-substrate interactions in yeast spliceosomes**
Christine Norman, Andy Newman, Kiyoshi Nagai

481 B **Identification of new natural compounds that modulate splicing in vitro and in cells**
Andrea Pawellek, Ursula Ryder, Angus Lamond

482 C **A new role for U2 snRNA in alternate 3' ss selection**
Jorge Pérez-Valle, Josep Vilardell

483 A **BRAF branch point mutation confers Vemurafenib resistance**
Maayan Salton, Ty Voss, Poulikos Poulikakos, Tom Misteli

484 B **Conservation of U2 protein – branch site interactions between yeast and human, as investigated by UV crosslinking**
Cornelius Schneider, Klaus Hartmuth, Dmitry Agafonov, Patrizia Fabrizio, Reinhard Lührmann

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485 C **Identification of small molecule pre-mRNA splicing inhibitors using a stage-specific, high-throughput in vitro splicing assay**
Anzhela Sidarovich, Timur R. Samatov, Sonja Sievers, Cindy L. Will, Henning Urlaub, Reinhard Lührmann, Herbert Waldmann

486 A **Localization of the pre-mRNA path in the activated yeast spliceosome by immuno-EM**
Chengfu Sun, Norbert Rigo, Patrizia Fabrizio, Berthold Kastner, Reinhard Luehrmann

487 B **Functional and structural analysis of Cwc25 required for first-step splicing**
Hui-Fang Wang, Chi-Kang Tseng, Ting-Wei Chiang, Soo-Chen Cheng

488 C **The G patch protein Spp2 couples Prp2-mediated ATP hydrolysis to catalytic activation of the yeast spliceosome**
Zbigniew Warkocki, Jana Schmitzová, Claudia Höbartner, Patrizia Fabrizio, Reinhard Lührmann

Splicing Regulation

Abstracts 489–529

489 A **Chromatin affects the splicing efficiency by regulating the U2snRNP activity**
Eric Allemand, Michael P. Myers, Jose Garcia-Bernado, Annick Harel-Bellan, Adrian R. Krainer, Christian Muchardt

490 B **The Evolutionary Landscape of Alternative Splicing and its Regulation in Vertebrate Species**
Nuno Barbosa-Morais, Qun Pan, Manuel Irimia, Serge Gueroussov, Eesha Sharma, Emil Nachman, Hui Xiong, Leo Lee, Valentina Slobodeniuc, Claudia Kutter, Stephen Watt, Recep Çolak, TaeHyung Kim, Christine Misquitta-Ali, Michael Wilson, Philip Kim, Duncan Odom, Brendan Frey, Benjamin Blencowe

491 C **TNF influences alternative stop codon usage in DAPK mRNA**
Natalya Benderska, Stefan Stamm, Regine Schneider-Stock

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

493 B **The many lives of Co-transcriptional Splicing**
Mattia Brugiolo, Lydia Herzel, Karla Neugebauer

494 C **Alternative splicing in the regulation of the barley circadian clock**
Cristiane Calixto, Robbie Waugh, John Brown

495 A **The Nuclear Matrix Protein Matrin 3 is a Regulator of Alternative Splicing**
Miguel Coelho, Melis Kayikci, Nicolas Bellora, Jernej Ule, Eduardo Eyras, Christopher Smith

496 B **Damage-induced alternative splicing in MDM2: Identifying cis elements and trans factors**
Daniel Comiskey, Ravi Singh, Aixa Tapia-Santos, Dawn Chandler

497 C **H3K9me3 and its role in pre-mRNA splicing.**
Eva Duskova, Martina Huranova, Fernando Carrillo Oesterreich, David Stanek

498 A **Is Prp16 remodelling of helix I during the two steps of pre-mRNA splicing carried out through the Nineteen Complex protein Cwc2?**
Rogerio de Almeida, Dharshika Pathiranage, Rebecca Hogg Hogg, Raymond O'Keefe

499 B **Selective constraint on mRNA splicing pattern by protein structural requirement**
Jean-Christophe Gelly, Hsuan-Yu Lin, Alexandre G. de Brevern, Trees-Juen Chuang, Feng-Chi Chen

500 C **Alternative splicing and gene expression in cardiomyocytes and cardiac fibroblasts during development**
Jimena Giudice, Zheng Xia, Marissa Ruddy, Wei Li, Thomas A. Cooper

501 A **Assessing influence of mutations in first nucleotides of exons on splicing of the BTK and SERPING1 genes.**
Lucie Grodecká, Pavla Lockerová, Barbora Ravcukova, Emanuelle Buratti, Francisco Baralle, Tomas Freiberger

502 B **Investigating the role of PTBP1 alternative exon 9 in the evolution of lineage-specific alternative splicing in vertebrates**
Serge Gueroussov, Jean-Philippe Lambert, Nuno Barbosa-Morais, Anne-Claude Gingras, Benjamin Blencowe

503 C **Prp45 affects early stage of spliceosome assembly and pre-mRNA abundance in yeast**
Martina Hálová, Ondrej Gahura, Zdenek Cit, Tomáš Dráb, Anna Valentová, Katerina Abrhámová, František Puta, Petr Folk

504 A **SR proteins regulate from cellular environment dependent splicing**
Egle Jakubauskiene, Laurynas Vilys, Lorenz Poellinger, Yuichi Makino, Arvydas Kanopka

505 B **High-resolution Rbfox2 binding patterns predict widespread splicing regulation in mouse embryonic stem cells**
Mohini Jangi, Paul Boutz, Phillip Sharp

506 C **Elucidation of UP1 binding to RNA substrates: Does RNA structure matter?**
Jeffrey Levengood, Carrie Rollins, Michele Tolbert, Blanton Tolbert

507 A **Thailanstatins: New Pre-mRNA Splicing Inhibitors and Potent Antiproliferative Agents Discovered from Burkholderia thailandensis MSMB43**
Xiangyang Liu, Sreya Biswas, Michael Berg, Christopher Antapli, Gideon Dreyfuss, Yi Q. "Eric" Cheng

508 B **Effects of SR Protein Expression on HIV-1 Splicing**
Le Luo, Alan Cochrane, Blanton Tolbert

509 C **Real-time kinetics of human pre-mRNA splicing**
Robert Martin, José Rino, Célia Carvalho, Tomas Kirchhausen, Maria Carmo-Fonseca

510 A **Sex-specific alternative splicing in the head of Drosophila melanogaster and its underlying regulatory mechanisms**
Carmen Mohr, Marco Blanchette Blanchette, Britta Hartmann

511 B **Structural investigation of hnRNP G interaction with SMN RNA**
Ahmed Moursy, Antoine Clery, Frederic Allain

512 C **A highly conserved GC-rich element regulates alternative splicing of mRNA for the variant thyroid hormone receptor TRα2**
Stephen Munroe

513 A **The centrosomal kinase NEK2 is a novel splicing factor kinase**
Chiara Naro, Federica Barbagallo, Paolo Chieffi, James Stevenin, Maria Paola Paronetto, Claudio Sette

514 B **Reconstructing alternative splicing of SMN exon 7 by NMR, SRM-Mass-Spectrometry and mathematical modeling**
Yaroslav Nikolaev, Martin Soste, Paola Picotti, Dagmar Iber, Frederic Allain

515 C **Comprehensive Mapping of the Splicing Regulatory Circuitry Involved in Cell Proliferation and Apoptosis**
Panagiotis Papasaikas, Juan Ramon Tejedor, Luisa Vigevani, Juan Valcarcel

516 A **Functional characterization of the RNA-binding protein Acinus: its role in pre-mRNA processing and apoptosis**
Julie Rodor, Magdalena Maslon, Javier Caceres

517 B **Widespread regulatory functions of Polypyrimidine Tract-Binding Proteins in splicing and development of Arabidopsis thaliana**
Christina Rühl, André Kahles, Gabriele Wagner, Gabriele Drechsel, Gunnar Rätsch, Andreas Wachter

518 C **hnRNP A1 promotes exon 6 inclusion of apoptotic Fas gene.**
Haihong Shen

519 A **Unusual evolutionary insertion of G-tracts creates splice variants of distinct localization and function in human cells**
Muhammad Sohail, Wenguang Cao, Sam Kung, Lisheng Wang, Eftekhar Eftekharpour, Jiuyong Xie

520 B **Mechanisms regulating alternative splicing of Dscam**
Matthias Soller, Irmgard Haussmann, Yash Hemani, Pinar Ustaoglu

521 C **A Role for the Polyadenosine Binding Protein, Nab2, in Splicing and Quality Control**
Sharon Soucek, Megan Bergkessel, Deepti Bellur, Christine Guthrie, Jonathan Staley, Anita Corbett

522 A **Prp40p WW Domain is Critical for Splicing of Introns Containing Non-canonical Branch Site Sequences in Saccharomyces cerevisiae**
Luh Tung, Chung-Shu Yeh, Fu-Lung Yeh, Hsuan-Kai Wang, Jeffrey A Pleiss, Tien-Hsien Chang

523 B **Muscleblind and Fox proteins cooperate to change a splicing program involved in stem cell differentiation and maintenance.**
Julian P Venables, Laure Lapasset, Gilles Gadea, Philippe Fort, Roscoe Klinck, Emmanuel Vignal, Panagiotis Prinos, Benoit Chabot, Sherif Abou Elela, Pierre Roux, Jean-Marc Lemaitre, Jamal Tazi

524 C **SWI/SNF regulates alternative trans-splicing of the mod(mdg4) gene**
Johan Waldholm, Simei Yu, Stefanie Böhm, Neus Visa

525 A **Splice-sensitive array profiling suggests a role for STAR proteins and PTB in control of smooth muscle cell alternative splicing.**
Selina Xiao Wang, Martina Hallegger, Clare Gooding, Adrian Buckroyd, Miriam Llorian, Nicolas Bellora, Eduardo Eyras, Melis Kayikci, Jernej Ule, Christopher Smith

526 B **EJC can regulate alternative splicing in mammalian cells**
Zhen Wang, Valentine Murigneux, Hervé Le Hir

527 C **The Ribosome-OME II: Alternative Splicing for Ribosomal Proteins?**
Jonathan R Warner

528 A **hnRNP A1 and Secondary Structure Coordinate Alternative Splicing of Mag**
Ruth Zearfoss, Emily Johnson, Sean Ryder

529 B **Regulation of alternative splicing by QKI protein in lung cancer**
Fengyang Zong, Xing Fu, Feng Wang, Wenjuan Wei, Lijuan Cao, Hongbin Ji, Jingyi Hui

RNA-Protein Interactions

Abstracts 530–579

530 C **Site-Specific Intercalation of Doxorubicin Disrupts the Iron-Responsive Element RNA – Iron Regulatory Protein Interaction**
Luigi Alvarado, Kyle Eggleston, Sam Lin, Virginia Smith, Adegboyega Oyelere, Kwaku Dayie

531 A **Defining a eukaryotic core mRNA interactome: the landscape of RNA-binding proteins in yeast and its conservation in mammals**
Benedikt Beckmann, Alfredo Castello, Bernd Fischer, Rastislav Horos, Claudia Strein, Katrin Eichelbaum, Sophia Föhr, Thomas Preiss, Lars Steinmetz, Jeroen Krijgsveld, Matthias Hentze

532 B **Regulation of human telomerase by the helicase RHAU, a quadruplex resolvase.**
Evan Booy, Markus Meier, Trushar Patel, Ryan Howard, Natalie Okun, Oksana Marushchak, Jörg Stetefeld, Sean McKenna

533 C **In vivo dynamics of SR protein-RNA interactions**
Serena Capozi, Eugenia Basyuk, Antoine Cléry, Frédéric Allain, Edouard Bertrand

534 A **GOLLD: a large, structured, noncoding RNA from bacteria and bacteriophages**
Andy Chen, Ronald Breaker

535 B **Dynamic transition upon protein-RNA complex formation: PTB RRM1 interaction with an IRES stem-loop**
Fred F. Damberger, Christophe Maris, Frédéric H.-T. Allain, Sapna Ravindranathan

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536 C **AURA 2.0: empowering post-transcriptional regulatory networks discovery**
Erik Dassi, Toma Tebaldi, Angela Re, Sara Leo, Daniele Peroni, Luigi Pasini, Alessandro Quattrone

537 A **The QUA2 domain of GLD-1 recognizes an additional nucleotide and modulates RNA binding affinity**
Gerrit Daubner, Stefan Gerhardy, Frédéric Allain

538 B **Control of Mammalian Germ Cell Differentiation by the RNA-Binding Protein DAZL**
Renaud Desgraz, Katherine Romer, David C Page

539 C **Structural and Dynamic Investigation on ETR-3 RRMs and their Interaction with AU-rich RNAs**
Nana Diarra dit Konte, Frederic Allain

540 A **Interactions between RBFOX2 and pre-microRNA-20b terminal loop**
Afzal Dogar, Julian Zagalak, Moritz Stoltz, Harry Towbin, Michel Erich, Frédéric Allain, Jonathan Hall

541 B **RNA-binding protein EWSR1 regulates CCDC6**
Sujitha Duggimpudi, Erik Larrson, Arndt Borkhardt, Jessica Hoell

542 C **Dead End, a protein counteracting miRNA-mediated repression of tumour suppressor genes, contains non-canonical RNA binding domains**
Malgorzata Duszczyk, Frédéric Allain

543 A **Potential substrates of the RNase MRP complex in cell cycle regulation**
Franziska Eckmann, Anika Wehrle, Anika Salfelder, Romy Keppler, Ekkehart Lausch, Bernhard Zabel

544 B **Post-transcriptional regulation of SMN2 expression by hnRNP G and LARP Family proteins**
Ana Miguel Fernandes, Ana Luísa Gomes, Mariana Oliveira, Gonçalo Costa, Isabel Peixeiro, Ana Margarida Matos, Carlos Cordeiro, Margarida Gama-Carvalho

545 C **Characterization of the potential role for RNA-binding protein FUS/TLS in DNA damage response: A quantitative proteomic approach**
Giuseppe Filosa, Marc-David Ruepp, Carolina Lenzken, Alessia Loffreda, Oliver Mühlemann, Angela Bachi, Silvia Barabino

546 A **A novel PAR-CLIP based approach using RRM mutations reveals RNA recognition mechanisms of HuR**
Matthew Friedersdorf, Jack Keene

547 B **The Runt domain of AML1 (RUNX1) binds a sequence-conserved RNA motif that mimics a DNA element**
Junichi Fukunaga, Yusuke Nomura, Ryo Amano, Yoichiro Tanaka, Taku Tanaka, Yoshikazu Nakamura, Gota Kawai, Tomoko Kozu, Taiichi Sakamoto

548 C **Predicting RNA-Protein Interactions: The Hunt for the Code of Recognition**
Christian Garde, Jan Gorodkin, Christopher T. Workman

549 A **Testing Protein Sequestration Candidates for RNA-Mediated Disease**
Marianne Goodwin, Apoorva Mohan, Maurice Swanson

550 B **Computational study of interactions between amino acids and nucleobases in aqueous solvent**
Matea Hajnic, Juan Osorio Iregui, Thomas Malzac, Bojan Zagrovic

551 C **Codon-usage effects and functional characterization of physicochemical complementarity between mRNA and cognate protein sequences**
Mario Hlevnjak, Lily Chan, Anton A. Polyansky, Bojan Zagrovic

552 A **Establishment of a Fluorescence Cross Correlation Spectroscopy (FCCS) based assay to measure Argonaute – siRNA interaction**
Leonhard Jakob, Michael Hannus, Stefan Hannus, Gunter Meister

553 B **Post-transcriptional control of macrophage activation by HuR.**
Panagiota Kafasla, Orsalia Hazapis, Martin Reczko, Dimitris Kontoyiannis

554 C **Repetitive RNA unwinding of a single RNA helicase A**
Hye Ran Koh, Li Xing, Lawrence Kleiman, Sua Myong

555 A **The RNA-binding Protein Repertoire of Embryonic Stem Cells**
S. Chul Kwon, Hyerim Yi, Katrin Eichelbaum, Sophia Föhr, Bernd Fischer, Kwon Tae You, Tuan Anh Nguyen, Alfredo Castello, Jeroen Krijgsveld, Matthias W. Hentze, V. Narry Kim1

556 B **Drosophila Gemin5 binds to UsnRNAs and another specific group of ncRNAs**
Sheila SK Li, Jonathan PY Lau, Tinyi Chu, Brian Qin, Ting-Fung Chan, Terrence CK Lau

557 C **RBP atlas: an exploration of interactions between mRNA and proteins and their impact on cardiomyocyte biology**
Yalin Liao, Alfredo Castello, Sophia Foehr, Stefan Leicht, Rastislav Horos, Jeroen Krijgsveld, Matthias Hentze, Thomas Preiss

558 A **Multifunctional interleukin-6 receptor aptamers**
Eileen Magbanua, Ulrich Hahn

559 B **Neurodegenerative diseases: quantitative predictions of protein-RNA interaction**
Domenica Marchese, Davide Cirillo, Gian Gaetano Tartaglia

560 C **RNA helicase function in yeast ribosome biogenesis**
Roman Martin, Philipp Hackert, Maike Ruprecht, Stefan Simm, Enrico Schleiff, Markus Bohnsack

561 A **Knock-out mice and HITS-CLIP reveal that the SGs assembly factor G3BP preferentially binds intron-retaining transcripts and controls their stability in the brain**
Sophie Martin, Juan Gonzáles-Vallinas, Nicolas Bellora, Manuel Irimia, Latifa Zekri, Alexandra Metz, Karim Chebli, Michel Vignes, Eduardo Eyras, Javier F Caceres, Ben J Blencowe, Jamal Tazi

562 B **A co-evolution network of binding contacts between protein L25 and 5S rRNA**
Zhichao Miao, Eric Westhof

563 C **Mechanism of action of the CCCH zinc finger protein TbZC3H11 upon heat shock**
Igor Minia, Dorothea Droll, Aditi Singh, Christine Clayton

564 A **Sequence specific code in RRM–RNA interactions**
Martyna Nowacka, Stanislaw Dunin-Horkawicz, Kama Wojcik, Janusz Bujnicki

565 B **Regulation of mRNA metabolism by U2AF65 splicing factor - novel mechanisms for the coordination of gene expression?**
Isabel Peixeiro, Samuel Casaca, Margarida Gama-Carvalho

566 C **Division of labor: separation of loading and unwinding units in an oligomer of the DEAD-box helicase Ded1p**
Andrea Putnam, Huijue Jia, Fei Liu, Eckhard Jankowsky

567 A **Manipulating endogenous RNAs with synthetic RNA-binding proteins**
Oliver Rackham, MF Razif, KK Nygård, TS Chia, ME Hibbs, SM Davies, I Small, A Filipovska

568 B **Imbalance of Zfp36l2-RNA-binding protein results in female infertility**
Silvia Ramos

569 C **Structure-specific RNase footprinting in multiple cell types reveals protein-binding sites throughout the human transcriptome**
Ian Silverman, Fan Li, Anissa Alexander, Daniel Beiting, Nur Selamoglu, Fevzi Daldal, Brian Gregory

570 A **Architecture of catalytic complexes essential for synthesis and co-translational insertion of selenocysteine in humans**
Miljan Simonovic, Rachel Schmidt, Malgorzata Dobosz-Bartoszek

571 B **hnRNP A1 Interacts with Conserved RNA IRES Elements in Enterovirus 71**
Michele Tolbert, Jeffery Levengood, Mei-Ling Li, Blanton Tolbert

572 C **Human DIS3L2 exonuclease is involved in the processing of tRNA-derived small RNAs**
Dmytro Ustianenko, Biter Bilen, Katerina Chalupnikova, Zuzana Feketova, Georges Martin, Mihaela Zavolan, Stepanka Vanacova

573 A **HIV Nucleocapsid Protein Precursors are Effective Nucleic Acid Chaperone Proteins**
Wei Wang, Mithun Mitra, Robert Gorelick, Zhengrong Wu, Karin Musier-Forsyth

574 B **Tethered Domains and Flexible Regions in tRNase ZL, the Long Form of tRNase Z**
Christopher Wilson, Emmanuel Chang, Louis Levinger

575 C **Poly(A) binding protein 1 and formation of processing bodies in human**
Jingwei Xie, Guennadi Kozlov, Kalle Gehring

576 A **Crystal structure of the active conformation of the Shigella flexneri VapC toxin at 1.9 Å resolution**
Kehan Xu, Emil Dedic, Patricia Cob-Cantal, Christian Dienemann, Andreas Bøggild, Kristoffer S. Winther, Kenn Gerdes, Ditlev Brodersen

577 B **Alanine scanning of the aa-tRNA binding interface of Escherishia coli EF-Tu**
Emine Yikilmaz, Olke Uhlenbeck

578 C **Evidence of direct complementary binding between mRNAs and cognate proteins**
Bojan Zagrovic, Mario Hlevnjak, Anton Polyansky

579 A **Responses of the mRNA interactome to genotoxic stress**
Elisabeth Zielonka, Alfredo Castello, Benedikt Beckmann, Rastislav Horos, Anne-Marie Alleaume, Claudia Strein, Sophia Foehr, Bernd Fischer, Jeroen Krijgsveld, Matthias Hentze

RNP Structure, Function and Biosynthesis

Abstracts 580–598

580 B **A genome-wide RNAi screen identifies novel 40S ribosome synthesis factors in human cells**
Lukas Badertscher, Thomas Wild, Lukas Bammert, Michael Stebler, Andreas Vonderheit, Christian Montellese, Karol Kozak, Gábor Csúcs, Peter Horvath, Ulrike Kutay

581 C **Characterization and in vivo functional analysis of the Schizosaccharomyces pombe ICln gene**
Adrien Barbarossa, Henry Neel, Etienne Antoine, Thierry Gostan, Johann Soret, Remy Bordonne

582 A **Dissecting RNA-binding protein complexes**
Anne Baude, Kristin Wächter, Stefan Hüttelmaier

583 B **Reconstitution of the Uridine-rich snRNP Assembly Machinery**
Clemens Englbrecht, Nils Neuenkirchen, Ashwin Chari, Jürgen Ohmer, Utz Fischer

584 C **Ribosomal protein clusters orchestrate the hierarchical construction of eukaryotic large ribosomal subunit structural domains in vivo**
Michael Gamalinda, Uli Ohmayer, Jelena Jakovljevic, Beril Kumcuoglu, Philipp Milkereit, John Woolford, Jr.

585 A **5'-cytosine methylation of C2278 in 25S rRNA stabilizes 60S ribosomal subunit in yeast**
Andriana Halacheva, Martin Koš

586 B **Identification of a chloroplast ribonucleoprotein complex containing trans-splicing factors, intron RNAs and novel components**
Jessica Jacobs, Christina Marx, Vera Kock, Olga Reifschneider, Stephanie Glanz, Ulrich Kück

587 C **The structural organization of the box C/D sRNP**
Audrone Lapinaite, Bernd Simon, Frank Gabel, Magdalena Rakwalska-Bange, Teresa Carlomagno

588 A **Budding yeast telomerase RNA: Zooming in for more definition of a large RNA.**
Nancy Laterreur, Isabelle Dionne, Jean-François Noël, Stephanie Larose, Raymund Wellinger

589 B **Characterisation of the human UTP-B complex and its role in ribosome biogenesis**
Matthias S. Leisegang, Carmen Doebele, Ana S. Ramirez, Katherine E. Sloan, Stefan Simm, Enrico Schleiff, Michael Karas, Nicholas J. Watkins, Markus T. Bohnsack

590 C **Messenger Ribonucleoprotein Assembly Requires the DEAD-box Protein Dbp2 and Enzymatic Modulation by Yra1**
Wai Kit Ma, Sara Cloutier, Elizabeth Tran

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592 B **Structural basis of Brr2-Prp8 interaction and its implications for Retinitis Pigmentosa disease type 13 and U5 snRNP biogenesis**
Thi Hoang Duong Nguyen, Jade Li, Wojciech P Galej, Hiroyuki Oshikane, Andrew J Newman, Kiyoshi Nagai

593 C **Surveillance of spliceosomal snRNP assembly in the cell nucleus**
Ivan Novotny, Daniel Mateju, Martin Sveda, Zdenek Knejzlik, David Stanek

594 A **Implication of the SMN complex in the biogenesis and steady state level of the Signal Recognition Particle**
Nathalie Piazzon, Florence Schlotter, Suzie Lefebvre, Maxime Dodré, Agnès Mereau, Johann Soret, Aurore Besse, Martine Barkats, Rémy Bordonné, Séverine Massenet, Christiane Branlant

595 B **The rRNA methyltransferase Bud23 interacts with the DEAH-box RNA helicase Ecm16 to promote cleavage at A2**
Richa Sardana, Arlen Johnson

596 C **The 5S RNP couples ribosome production to p53 regulation**
Katherine Sloan, Nicholas Watkins

597 A **Dissecting the splicing-dependent mRNA binding of ASAP complexes**
Anna-Lena Steckelberg, Niels H. Gehring

598 B **The Ribosome-OME I: Big Data & the Ribosome**
Jonathan R Warner, Varun Gupta

Riboregulation in Development

Abstracts 599–600

599 C **Cytoplasmic RNA regulatory networks orchestrate male gametogenesis**
Ryuji Minasaki, Christina Hirsch, Christian Eckmann

600 A **PX1 regulates protoxylem cell fate via RNA processing**
Kamil Ruzicka, Ana Campilho, Sedeer El-Showk, Dominique Eeckhout, Geert De Jaeger, Jan Hejatko, Mikko Frilander, Yka Helariutta

RNA Transport and Localization

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601 B **Hepatitis B virus post-transcriptional element promotes mRNA export via the cellular mRNA export machinery TREX**
Binkai Chi, Hong Cheng

602 C **Insights into the nuclear mRNA export machinery of Trypanosoma brucei**
Anna Dostalova, Sandro Käser, Marina Cristodero, Bernd Schimanski

603 A **Assembly of TREX complex components on mRNAs**
Agnieszka M. Gromadzka, Niels Gehring

604 B **Growth cone local mRNA translation of nuclear proteins in the spatio-temporal regulation of neurite outgrowth**
Francesca Moretti, Olivier Pertz

605 C **Genome-wide identification of mRNAs associated with Survival of Motor Neuron proteins and whose axonal localization is decreased upon SMN deficiency**
Florence Rage, Nawal Boulisfane, Rihan Khalil, Henry Neel, Thierry Gostan, Remy Bordonne, Johann Soret

606 A **Tetramolecular Fluorescence Complementation for Detection of Specific RNAs in Vitro**
Anna K. Rath, Stefanie J. Kellermann, Andrea Rentmeister

607 B **Resolving conflicts between Transcription and Replication: a new potential role for the mRNA export factor Yra1, regulated by its post-translational modifications.**
Evelina Tutucci, Noël Yeh Martin, Valentina Infantino, Benoît Palancade, Françoise Stutz

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608 C **Ubiquitin and SUMO regulate Yra1: linking mRNA export factor to genome stability**
Evelina Tutucci, Benoit Palancade, Noel Yeh Martin, Valentina Infantino, Françoise Stutz

609 A **mRNA transport and translation regulate neuritogenesis**
Hsin-I Yu, Hung-Hsi Chen, Wei-Chih Cho, Woan-Yuh Tarn

RNAs in Diseases

Abstracts 610–643

610 B **S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1**
Vered Ben-Hur, Polina Denichenko, Zahava Siegfried, Avi Maimon, Adrian Krainer, Ben Davidson, Rotem Karni

611 C **Altered microRNA expression profile in ALS: Role in the regulation of NFL mRNA levels**
Danae Campos-Melo, Cristian Droppelmann, Kathryn Volkening, Michael J. Strong

612 A **Novel TAL1 targets beyond protein coding genes: identification of TAL1-regulated microRNA genes in T-cell acute lymphoblastic leukemia**
Nádia C. Correia, Francisco J. Enguita, Frank Speleman, João T Barata

613 B **Retinitis pigmentosa mutations of hBrr2 reduce splicing fidelity**
Zuzana Cvacková, Daniel Mateju, David Stanek

614 C **An Exon-Specific U1 approach to correct SMN protein deficiency in spinal muscular atrophy (SMA)**
Andrea Dal Mas, Erica Bussani, Malgorzata Rogalska, Franco Pagani

615 A **Ddx5/Ddx17 RNA helicases control multiple layers of gene expression during TGFβ-induced Epithelial-to-Mesenchymal Transition (EMT)**
Etienne Dardenne, Fattet Laurent, Mathieu Deygas, Micaela Polay-Espinoza, Cyril Bourgeois, Ruth Rimokh, Didier Auboeuf

616 B **Overexpression of miR-29b and miR-122 in the invasive ductal carcinoma of the breast**
Patricia R Darin, Juliana S Zanetti, Alfredo Ribeiro-Silva, Fernando L De Lucca

617 C **The RNA binding protein Quaking regulates monocyte adhesion and differentiation**
Ruben de Bruin, Janine van Gils, Hetty de Boer, Jacques Duijs, Erik Biessen, Ton Rabelink, Lily Shiue, Manuel Ares Jr, Anton Jan van Zonneveld1 Eric van der Veer

618 A **Rho Guanine Nucleotide Exchange Factor: A Novel RNA Binding Protein Involved in the Pathology of Amyotrophic Lateral Sclerosis**
Cristian Droppelmann, Brian Keller, Danae Campos-Melo, Jian Wang, Martin Duennwald, Rob Hegele, Kathryn Volkening, Michael J. Strong

619 B **Mechanisms of Post-Trascriptional Regulation of Gene Expression in Dementias**
Francesca Fontana, Margherita Grasso, Valerio Del Vescovo, Giuseppina Covello, Michela Alessandra Denti

620 C **MicroRNAs as lung cancer biomarkers**
Margherita Grasso, Valerio Del Vescovo, Chiara Cantaloni, Leonardo Ricci, Mattia Barbareschi, Michela Alessandra Denti, Rotem Karni

621 A **hnRNP A2/B1 regulated alternative splicing of key signal transduction components and is essential for breast cancer metastasis.**
Regina Golan-Gerstl, Ilana Lebenthal-Loinger, Ben Davidson, Jasmine Jacobs, Gideon Rechavi, Rotem Karni, Michela Alessandra Denti

622 B **The role of the simtron, miR-1225, and its host gene, PKD1, in autosomal dominant polycystic kidney disease**
Mallory A. Havens, Frank Rigo, Michelle L. Hastings

623 C **Interplay of oncogenic transcription factor b-catenin with SR protein SRSF3 contributes to the alternative splicing of tumor-related genes**
Jung Hur, Sunjoo Jeong

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624 A **Exploring the in vivo functions of the mammalian tRNA ligase**
Jennifer Jurkin, Therese Kaufmann, Johannes Popow, Javier Martinez

625 B **The FUS protein is required for cell proliferation**
Silvia Carolina Lenzken, Alessia Loffreda, Monica Lupi, Sudharshan Elangovan, Marc-David Ruepp, Raffaele Calogero, Giovanna Damia, Oliver Mühlemann, Silvia Barabino

626 C **The Involvement of miRNA Dysregulation in Amyotrophic Lateral Sclerosis**
Alessia Loffreda, Marc-David Ruepp, Rémy Bruggmann, Oliver Mühlemann, Silvia Barabino

627 A **Differential LMNA splicing leads to metabolic disorders**
Isabel Cristina Lopez-Mejía, Marion de Toledo, Carine Chavey, Celia Lopez Herrera, Patricia Cavelier, François Casas, Guillaume Beranger, Ez-Zoubir Amri, Lluis Fajas , Jamal Tazi

628 B **Uncovering the role of microRNAs in SMA**
Philipp Odermatt, Daniel Schümperli

629 C **Anti-inflammatory function of miR-146a in human primary keratinocytes and atopic dermatitis**
Ana Rebane, Toomas Runnel, Alar Aab, Beate Rückert, Julia Maslovskaja, Mario Plaas, Maya Zimmermann, Lajos Kemeny, Külli Kingo, Cezmi A. Akdis

630 A **Mutation of a Zinc Finger Polyadenosine RNA Binding Protein Causes Autosomal Recessive Intellectual Disability**
Jennifer Rha, Sara Leung, Gary Bassell, Anita Corbett

631 B **A novel function for MiR-10a in the pathogenesis of atopic dermatitis**
Toomas Runnel, Maya Zimmermann, Külli Kingo, Cezmi A. Akdis, Ana Rebane

632 C **Splice variant specific blood biomarkers of Parkinson's disease**
Jose Santiago, Clemens Scherzer, Harvard Biomarker Study Group, Judith Potashkin

633 A **Hepatitis C virus induced up-regulation of miR-27 expression promotes hepatic triglyceride accumulation**
Ragunath Singaravelu, Ran Chen, Rodney Lyn, Daniel Jones, Rodney Russell, Shifawn O'Hara4 Jenny Cheng, Lorne Tyrrell, Yanouchka Rouleau, John Pezacki

634 B **Knockdown of human Dyskerin, the protein linked to Dyskeratosis congenita, blocks large ribosomal subunit production and activates p53 via RPL5 and RPL11**
Katherine Sloan, Andria Pelava, Claudia Schneider, Nicholas Watkins

635 C **Exonic splicing mutations in Mendelian disorders are more prevalent than currently estimated: the example of Lynch syndrome-associated MLH1 exon 10 variants**
Omar Soukarieh, Mohamad Hamieh, Pascaline Gaildrat, Stéphanie Baert-Desurmont, Mario Tosi, Alexandra Martins, Thierry Frébourg

636 A **RNase MRP is involved in chondrogenic differentiation**
Mandy M.F. Steinbusch, Marjolein M.J. Caron, Franziska Eckmann, Ekkehart Lausch, Bernhard Zabel, Lodewijk W. van Rhijn, Tim J.M. Welting

637 B **TDRD3 recruits FMRP and the topoisomerase TOP3ß to spliced mRNAs and provides a molecular link between schizophrenia and fragile X syndrome**
Georg Stoll, Bastian Linder, Cornelia Brosi, Aarno Palotie, Nelson Freimer, Olli Pietilainen, Oliver Ploettner, Utz Fischer

638 C **Spliceosome Integrity is Defective in the Motor Neuron Diseases ALS and SMA**
Hitomi Tsuiji, Yohei Iguchi, Asako Furuya, Kenji Sakimura, Shigeo Murayama, Gen Sobue, Koji Yamanaka

639 A **The RNA-binding protein Quaking critically regulates vascular smooth muscle cell phenotype**
Eric van der Veer, Ruben de Bruin, Adriaan Kraaijeveld, Margreet de Vries, Tonio Pera, Filip Segers, Janine van Gils, Marko Roeten, Ilze Bot, Anique Janssen, Pieter Hiemstra, Ton Rabelink, Antoine de Vries, Paul Quax, Wouter Jukema, Erik Biessen, Anton Jan van Zonneveld

640 B **Investigating the role and regulation of microRNA-10a in Acute Myeloid Leukaemia bearing the Nucleophosmin1 mutation**
Thi Thanh Vu, Catalina Palma, Adam Bryant, Dima Al Sheikha, David Ma, Vivek jayaswal

641 C **RNA binding protein FUS acts to mediate nuclear-mitochondrial communication**
Jane Wu

642 A **FUS mutations strongly promote FUS-SMN and FUS-RNAP II interactions**
Tomohiro Yamazaki, Robin Reed

643 B **Endogenous shRNA Induces the Large-scale Trans-determination of Mesenchymal Stem Cells into Hematopoietic Stem Cells with High Purity.**
James Yin

Viral RNAs

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644 C **Deep-sequencing of the Peach Latent Mosaic Viroid Reveals New Aspects of Population Heterogeneity**
François Bolduc, Jean-Pierre Sehi Glouzon, Rafael Najmanovich, Shengrui Wang, Jean-Pierre Perreault

645 A **Reduced HBsAg expression in occult HBV infection: alteration of a post-transcriptional regulatory mechanism?**
Daniel Candotti, Jean-Pierre Allain

646 B **Mechanistic insights into EMCV-IRES translation initiation**
Nathalie Chamond, Jules Deforges, Nathalie Ulryck, Audrey Brossard, Bruno Sargueil

647 C **Modulation of HIV-1 gene expression by binding of UHM-containing splicing factors to a ULM motif in the Rev protein**
Lorenzo Corsini, Michelle Vincendeau, Marta Pabis, Kostas Tripsianes, Thomas Güttler, Toby Gibson, Ruth Brack-Werner, Michael Sattler

648 A **HMGA1 interaction with HIV-1 TAR modulates basal and Tat-dependent HIV transcription.**
Sebastian Eilebrecht, Emmanuelle Wilhelm, Brendan Bell, Arndt Benecke

649 B **Thermodynamics of HIV-1 Reverse Transcriptase in action elucidates the mechanism of action of non-nucleoside inhibitors**
Bec Guillaume, Meyer Benoit, Marie-Aline Gerard, Jessica Steger, Katja Fauster, Ronald Micura, Philippe Dumas, Eric Ennifar

650 C **Domain interactions in adenovirus virus-associated RNA I mediate high-affinity PKR binding**
Katherine Launer-Felty, James Cole

651 A **Phosphorylation of hepatitis C virus RNA polymerase Ser29 and Ser42 by PRK2 regulates HCV replication**
Jae-Su Moon, Song-Hee Han, Jong-Won Oh

652 B **Structural rearrangements of HIV-2 RNA during dimerization**
Katarzyna Pachulska-Wieczorek, Katarzyna J. Purzycka, Ryszard W. Adamiak

653 C **RNA Packaging NTPase is Needed for Transcription in Double-stranded RNA Bacteriophage phi6**
Xiaoyu Sun, Markus Pirttimaa, Dennis Bamford, Minna Poranen

654 A **DNA habitat and RNA inhabitants: Relevant questions of a qualitative RNA sociology**
Guenther Witzany

Interconnections Between Gene Expression Processes

Abstracts 655–671

655 B **Distinct PPR proteins are responsible for coupling of mRNA editing, polyadenylation and translation in mitochondria of trypanosomes**
Inna Afasizheva, Lan Huang, Ruslan Afasizhev

656 C **Identification of RNA targets for the nuclear multidomain cyclophilin atCyp59 and their effect on PPIase activity**
Olga Bannikova, Marek Zywicki, Yamile Marquez, Tatsiana Skrahina, Maria Kalyna, Andrea Barta

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657 A **Impact of specific RNA Pol II CTD phosphorylation patterns**
Anne Helmrich, Daniel Schümperli

658 B **SC35 and Promoter-Associated Nascent RNA Function like HIV Tat and TAR to Regulate Transcription Pause Release**
Xiong Ji, Xiang-Dong Fu

659 C **Not5 of the CCR4-NOT complex contributes to assembly of the SAGA coactivator complex.**
Sari Kassem, Martine Collart

660 A **Paraspeckle formation during NEAT1 lncRNA biogenesis is integrated by the SWI/SNF chromatin remodeling complex**
Tetsuya Kawaguchi, Akie Tanigawa, Takao Naganuma, Tetsuro Hirose

661 B **The RNA exosome promotes a transcription termination pathway coupled to RNA decay**
Jean-Francois Lemay, Marc Larochelle, Samuel Marguerat, Jürg Bähler, Francois Bachand

662 C **Widespread regulation of mRNA steady-state levels through alternative splicing-dependent mechanisms**
Eugene V. Makeyev

663 A **Alternative splice variant of chromatin regulators drive specific transcription**
Oriane Mauger, Eric Allemand, Christian Muchardt, Eric Batsché

664 B **Traf3 alternative splicing during T cell activation: functional consequences and regulatory insights**
Monika Michel, Ilka Wilhelmi, Marco Preussner, Florian Heyd

665 C **The Bre5-Ubp3 complex links RNA surveillance to RNA Polymerase II regulation by ubiquitylation**
Laura Milligan, Sander Granneman, Grzegorz Kudla, Ross Alexander, Jean Beggs, David Tollervey

666 A **Double-stranded RNA-expressing plasmids selectively inhibit translation of exogenous mRNAs**
Jana Nejepinska, Radek Malik, Petr Svoboda

667 B **Molecular mechanisms of RNA Polymerase II termination mediated by contacts with Rat1 and CF IA**
Erika Pearson, Claire Moore

668 C **IMAGEtags for imaging Pol II activity in real time with RNA reporters**
Judhajeet Ray, Ilchung Shin, Vinayak Gupta, Jonathan Beasley, Lee Bendickson, George A Kraus, Marit Nilsen-Hamilton

669 A **A Novel Poly(A) RNA-Binding Protein Regulates a Key Subunit of ATP Synthase in Breast Cancer Cells**
Callie Wigington, Paula Vertino, Anita Corbett

670 B **Messenger RNA decay rates feedback to influence transcription and buffer gene expression in mammalian cells**
Carol Wilusz, Ju Youn Lee, Jerome Lee, Ashley Neff, Stephen Coleman, Ashton Herrington, Bin Tian, Jeffrey Wilusz

671 C **The human protein Nol12- ribosome biogenesis meets DNA damage and senescence**
Pierre-Joachim Zindy, Christian Trahan, Karen Wei, Jordi Ros Rodriguez, Marlene Oeffinger

RNA system biology

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672 A **The tissue specific and eco-responsive transcriptome of Drosophila**
James Brown, Nathan Boley, Robert Eisman, Michael Duff, Kenneth Wan, Ben Booth, Ann Hammonds, Carrie Davis, Lucy Cherbas, Piero Carninci, Thomas Gingeras, Peter Cherbas, Thomas Kaufman, Roger Hoskins, Brenton Graveley, Susan Celniker, Marcus Stoiber, Marlene Oeffinger, Peter Bickel

673 B **Modeling the RNA-binding specificity of GLD-1 suggests a function of coding region-located sites in translational repression**
Anneke Brümmer, Shivendra Kishore, Deni Subasic, Michael Hengartner, Mihaela Zavolan

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674 C **Beyond the ribosome, antagonistic functions played by a pair of ribosomal proteins paralogs**
Anne-Cecile Duc, Yong Zhang, Shuyun Rao, Xiao-Li Sun, Alison Bilbee, Michele Rhodes, Qin Li, Dietmar Kappes, Jennifer Rhodes, David Wiest

675 A **Clindamycin ribosome interactions: a molecular dynamics study**
Katarzyna Kulczycka-Mierzejewska, Joanna Trylska, Joanna Sadlej

676 B **Global gene regulation mediated by intron retention during T cell activation**
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677 C **Large-scale analysis of eukaryotic RNA-binding protein binding preferences and exploration of their roles in post-transcriptional gene regulation**
Debashish Ray, Hilal Kazan, Kate Cook, Matthew Weirauch, Hamed Najafabadi, Xiao Li, Mihai Albu, Hong Zheng, Ally Yang, Hong Na, Serge Gueroussov, Manuel Irimia, Andrew Fraser, Benjamin Blencowe, Quaid Morris, Timothy Hughes

678 A **RNase disruption of transcriptional positive auto-regulation is essential for energy-efficient phenotypic switching**
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679 B **A Potential Role for snoRNAs in PKR Activation during Metabolic Stress**
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680 C **RNAs in silico: learning from accelerated molecular dynamics**
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681 A **DNA methylation level is differentially correlated with the evolutionary features of coding exons in different genic positions**
Trees-Juen Chuang, Feng-Chi Chen, Yen-Zho Chen

682 B **Exome-Wide Computational Prediction of Evolutionarily Conserved CIS-Regulatory G-Quadruplex Motifs**
Scott Frees, Camille Menendez, Matthew Crum, Paramjeet Bagga

683 C **Determining optimal flanking regions of RNA secondary structures for experimental analysis**
Nikolai Hecker, Stefan E. Seemann, Jakob Hull Havgaard, Radhakrishnan Sabarinathan, Henrik Nielsen, Mikkel Christensen-Dalsgaard, Peter F. Stadler, Ivo Hofacker, Jan Gorodkin

684 A **EASANA: RNA-Seq and Affymetrix HTA2 data analysis, visualization and interpretation**
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685 B **Terminator: a method for precise detection of ncRNA ends and terminal stem-loops using chimeric reads from RNA-seq**
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686 C **Uncovering markers of cell identity change from transcriptome profiles**
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687 A **A new and systematic approach to analyse the population of sRNAs at the genome scale.**
Antonin Marchais, Alexis Sarrazin, Arturo Marí Ordóñez, Olivier Voinnet

688 B **Stepwise co-evolution between bacterial CRP/FNR-type transcription factors and their transcriptional networks**
Motomu Matsui, Masaru Tomita, Akio Kanai

689 C **The Conserved Structures of Right-Handed Polymerases**
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690 A **Discovering Conserved CIS-Regulatory G-Quadruplex Motifs in the Transcripts of Human CHD8 Gene Involved in Autism**
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692 C Understanding without reading: analog encoding of physico-chemical properties of proteins in cognate messenger RNA
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693 A cWords – systematic microRNA regulatory motif discovery from mRNA expression data
Simon H. Rasmussen, Anders Jacobsen, Anders Krogh

694 B Analysis of structural and functional impact of UTR single nucleotide variants identified in the non-small cell lung cancer by RNA-sequencing
Radhakrishnan Sabarinathan, Anne Wenzel, Peter Novotny, Krishna R Kalari, Jan Gorodkin

695 C Searching the coding region for microRNA targets
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696 A PyCRAC CLIP data analyses predict a prominent role for Nrd1 and Nab3 in regulation of protein coding gene expression in yeast
Shaun Webb, Ralph D Hector, Grzegorz Kudla, Sander Granneman

697 B Segmentation of Proximal RNA Binding Sites from High-throughput Sequencing Data Using a Density-Based Clustering Approach
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698 C Full-Length Transcript Sequencing: Looking Beyond the ENCODE data
Kin Fai Au, Elizabeth Tseng, Vittorio Sebastiano, Lawrence Lee, Renee Reijo Pera, Wing Hung Wong, Jason G. Underwood

699 A Comprehensive identification of RNA 5' ends in E. coli
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700 B Engineered "restriction RNases" for sequence-specific cleavage of dsRNA and RNA in DNA-RNA hybrids
Janusz Bujnicki, Krzysztof Skowronek, Agata Sulej, Dariusz Pianka, Irina Tuszynska, Marcin Nowotny, Justyna Czarnecka

701 C miR-Direct: RT-qPCR analysis of plasma microRNAs without prior RNA extraction
Anne Dallas, Sumedha Jayasena, Brian Johnston, Heini Ilves, Michael Mostachetti, Sergei Kazakov

702 A Comparing the transcriptome of mouse and human using RNA sequencing
Allissa Dillman, Melissa McCoy, Marcel van der Brug, Mark Cookson

703 B Genome-wide profiling of RNA secondary structure in planta
Yiliang Ding, Chun Kit Kwok, Yin Tang, Philip Bevilacqua, Sarah Assmann

704 C Post-transcriptional regulation of mitochondrial gene expression
Aleksandra Filipovska, Ganqiang Liu, Stefan Siira, Timothy Mercer, Anne-Marie Shearwood, Dimitar Azmanov, Stefan Davies, John Mattick, Oliver Rackham

705 A Investigating ligation bias in small RNA library construction for high-throughput sequencing and the effect of different 3' and 5' adapters
Ryan Fuchs, Zhiyi Sun, Fanglei Zhuang, G. Brett Robb

706 B tRid, a method for eliminating tRNAs without sequence information of tRNAs
Kazuki Futai, Hiroaki Suga

707 C Abstract Withdrawn

708 A Dynamic responses of the hepatocytic mRNA interactome to metabolic reprogramming
Rastislav Horos, Alfredo Castello, Bernd Fischer, Katrin Eichelbaum, Sophia Foehr, Anne-Marie Alleaume, Claudia Strein, Benedikt Beckmann, Jeroen Krijgsveld, Matthias Hentze

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709 B **Cytoplasmic mRNA Capping and the Implications of Cap Homeostasis**
Daniel Kiss, Ralf Bundschuh, Daniel Schoenberg

710 C **Screening of the RNase-sensitive subnuclear structures identified the Sam68 nuclear body that was built on RNA with novel protein components**
Taro Mannen, Naoki Goshima, Tetsuro Hirose

711 A **In vivo capture of RBPs bound to defined RNA species**
Birgit Schuster, Alfredo Castello, Benedikt Beckmann, Bruno Galy, Matthias Hentze

712 B **Discovery of gene expression inhibitors using a high throughput single-cell analysis system**
Scott Stevens, Matthew Sorenson

713 C **Imaging trinucleotide repeat RNA in live cells using Spinach2, an RNA tag with enhanced brightness and thermostability**
Rita Strack, Samie Jaffrey

714 A **Programmable sequence-specific click-labeling of RNA using archaeal box C/D RNP methyltransferases**
Migle Tomkuviene, Beatrice Clouet-d'Orval, Elmar Weinhold, Saulius Klimasauskas

715 B **A new technique for live cell RNA detection that enables single cell analysis and live cell sorting**
Don Weldon, Ronald Orallo, Alex Ko, Yuko Williams, Grace Johnston

716 C **Identification of novel post-transcriptional regulatory sequences**
Erin Wissink, Elizabeth Fogarty, Andrew Grimson

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717 B **Maintenance of adult beta–cell identity by microRNAs and transcription factors**
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Eran Hornstein, Amitai Mandelbaum, Sharon Kredo-Russo, Tal Melkman-Zehavi

718 C **Prostate cancer exosomes offering novel circulating non-coding RNA biomarkers for early cancer diagnosis and prognosis.**
Topic: **Non-coding & Regulatory RNAs**
Samuel E. Brennan, Nham Tran, Aled Clayton, Eileen M. McGowan, Paul J. Cozzi, Rosetta Martiniello-Wilks

719 C **Cold shock domain protein functions in reprogramming from differentiated cells to stem cells in the moss Physcomitrella patens**
Topic: **Riboregulation in Development**
Chen Li, Yosuke Tamada, Mitsuyasu Hasebe

720 C **Equilibrium-dependent ribosomal recoding mechanisms in RNA viruses**
Topic: **Translational Regulation**
Carolina Salguero, Michael A. Durney, Victoria M. D'Souza

721 C **Functional and Structural Characterization of a Thermostable RNA/DNA Dependent RNA Polymerase**
Topic: **RNA-Protein Interactions**
Xinlei Qian, Eugene Makeyev, Julien Lescar

722 A **Identification of novel methyltransferases, responsible for N-1 methyl-adenosine base modification of 25S rRNA in *S.cerevisiae***
Topic: **tRNA, snRNA, snoRNA, rRNA**
Sunny Sharma, Christian Peifer, Peter Kötter, Karl-Dieter Entian

723 A **Functional link between U1 snRNA 5'-end AU di-nucleotides and the mRNA cap-binding complex**
Topic: **Splicing Regulation**
Jui-Hui Chen, Chung-Shu Yeh, Jeffrey A. Pleiss and Tien-Hsien Chang

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ORAL ABSTRACTS

1 Exceptions to Canonical Decoding by the Ribosome

Venki Ramakrishnan[1]

[1]MRC Laboratory of Molecular Biology

The ribosome promotes the accuracy of selection of an aminoacyl tRNA that is cognate to the codon on mRNA as specified by the rules of the genetic code. Proper codon-anticodon pairing results in a series of conformational changes that results in GTP hydrolysis by elongation factor Tu, which allows the addition of the amino acid on the selected tRNA to the nascent peptide. However, there are notable exceptions to this rule. In one case, involving tmRNA, the role of both mRNA and tRNA is played by a protein. A second exception involves the read-through of stop codons that are modified to contain pseudouridine instead of uridine. These codons are read via a mechanism involving non-canonical base pairing previously thought to be forbidden by the genetic code. This talk will discuss how recent structures have shed light on both canonical decoding and exceptions to it.

2 Structure of the eukaryotic 40S ribosomal subunit in complex with initiation factors eIF1 and eIF1A

Melanie Weisser[1], *Marc Leibundgut*[1], *Nenad Ban*[1]

[1]Institute of Molecular Biology and Biophysics, ETH Zurich

Bacterial and eukaryotic protein synthesis differ considerably at the level of initiation. The eukaryotic small ribosomal subunit (40S) binds a large number of initiation factors necessary for scanning of messenger RNAs for the correct start codon and for assembly of the translation-competent 80S ribosome. Although biochemical and genetic studies have revealed the roles of various initiation factors involved in this process, little structural information exists on their interaction with the 40S subunit in pre-initiation complexes, which would help us understand the underlying molecular mechanisms.

Following up on our studies of the complex between the 40S ribosomal subunit and eukaryotic initiation factor 1 (eIF1) [Rabl et al., 2011] we were recently able to solve the crystal structure of a larger pre-initiation complex comprising the 40S ribosomal subunit and eukaryotic translation initiation factors eIF1 and eIF1A at a resolution of 3.7 Å. Both factors are crucial for scanning for and recognition of the correct start codon and they interact with numerous other initiation factors (eIF5, eIF3, eIF2) in the context of the larger 43S pre-initiation complex.

Our structure reveals the interactions of the two initiation factors with the small ribosomal subunit and their positions relative to each other. The eukaryotic initiation factor eIF1A binds, as expected, in the vicinity of the ribosomal A-site, and its eukaryotic-specific C-terminal domain extends towards the mRNA channel. The structure offers a good starting point for understanding the structural interactions within the 43S pre-initiation complex and the role of the initiation factors in ribosomal scanning and start codon recognition.

1. Rabl J, Leibundgut M, Ataide SF, Haag A, Ban N. (2011) Science 331, 730-6.

3 Versatile binding of eukaryotic initiation factor 3 on the small ribosomal 40S subunit and the CSFV IRES

Yaser Hashem[1], Amedee Des Georges[2], Vidya Dhote[3], Robert Langlois[2], Robert A. Grassucci[1], Tatyana V. Pestova[3], Christopher U.T. Hellen[3], Joachim Frank[1]

[1]Columbia University / HHMI; [2]Columbia University; [3]SUNY Downstate Medical Center

Protein translation initiation in most eukaryotes starts by the formation of the 43S preinitiation complex, comprising the Met-tRNAiMet, eukaryotic initiation factors 1, 1A, 2 and 3. The 43S is poised to attach the mRNA and start scanning for the start codon. Certain mRNAs possess internal ribosomal entry sites (IRESs), often at their 5′ UTR, which allows end-independent initiation to take place, circumventing canonical initiation. These IRES-containing mRNAs don't follow the same regulatory pathway that supervises the recruitment of most mRNAs to the preinitiation complex. Examples of IRES-containing mRNAs can be found in many viruses such as the Hepatitis C Virus (HCV) and the Classical Swine Fever Virus (CSFV). The interaction of these IRESs with the 40S subunit has been studied for decades by various methods and few low-resolution cryo-electron microscopy (cryo-EM) structures are already available, however their low-resolution was insufficient for atomic modeling and many aspects related to their interaction with the 40S remain unknown.

Initiation on IRES-containing mRNAs requires eIF3 that was thought to interact in a complementary fashion with the IRES on the 40S subunit, allowing it to maintain the same binding site on the 40S in the presence or absence of the IRES. Here we present several cryo-EM structures of the CSFV IRES in interaction with the 40S subunit, eIF3 and DHX29, a DExH-box protein required for scanning on structured mRNAs and was found previously to stabilize the binding of eIF3 on the 40S. Our structures show that contrarily to the current model, eIF3 binds differently in presence of an IRES and its conserved core doesn't interact directly anymore with the 40S (figure 1 below). In addition, we were able to create a convincing atomic model of the CSFV IRES into a 10Å cryo-EM map (figure 2 below), which sheds more light on the IRES contacts with the 40S subunit. Our cryo-EM reconstructions also show the versatility of eIF3 binding and capture the latter in several close orientations on the CSFV IRES. Our study poses the first structural basis of eIF3 interaction with IRES-containing mRNAs and rectifies the erroneous eIF3●IRES●40S interaction model, which we anticipate to have tremendous implications on the field.

1. Hellen et al. *Genes & Dev.* 2001
2. Hashem et al. *Cell* 2013, in press
3. Spahn et al. *Science* 2001
4. Siridechadilok et al. *Science* 2005

Figure 1

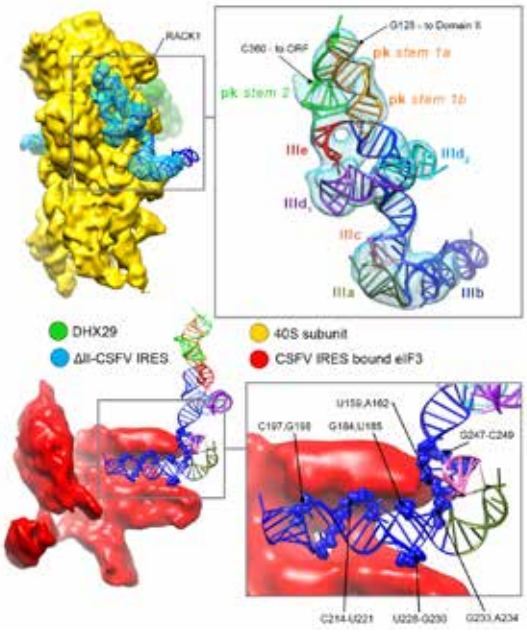

Figure 2

4 A novel strategy for protein synthesis initiation: 40S ribosomes bind to the 3′ UTR of barley yellow dwarf virus (BYDV) mRNA

Sohani Das Sharma[1], Bidisha Banerjee[1], Jelena Kraft[2], W. Allen Miller[2], Dixie Goss[1]
[1]Hunter College CUNY; [2]Iowa State University

Most gene expression in uncapped RNA viruses is regulated by either an internal ribosomal entry site (IRES) or a cap independent translation element (CITE) that are located in the 5' and 3' UTR respectively of the viral mRNA. Barley yellow dwarf virus (BYDV) mRNA, which lacks both cap and poly (A) tail, has a translation element (BTE) present in the 3' UTR that is essential for efficient translation initiation at the 5' proximal AUG. The molecular mechanism of translation initiation is not well understood. Using fluorescence anisotropy, SHAPE analysis and toeprinting, we report: 1) eIF4F binds to the 3' UTR and the binding affinity correlates with the translational efficiency of the mutant BTEs; 2) 40S ribosomes bind to the BTE first; 3) RNA structural elements in the 3' and 5' UTRs interact to transfer ribosomes to the 5' UTR; 4) Sequence interactions between 18S rRNA and 3' viral RNA BTE are required for ribosome binding. We are currently identifying eIFs required for ribosome transfer. Taken together, these results suggest a novel mechanism for eIF4F binding to the 3' UTR to recruit the 43S ribosome followed by subsequent transfer of the ribosome complex to the 5' UTR, scanning to the AUG and initiation of protein synthesis.

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5 Probing the dynamics of Ribosome biogenesis in yeast

Ralph D Hector[1], Elena Burlacu[1], Stuart Aitken[2], Atlanta Cook[3], Sander Granneman[1]
[1]Institute of Structural and Molecular Biology, Centre for Synthetic and Systems Biology (SynthSys), University of Edinburgh; [2]Computational Genomics Lab MRC Human Genetics Unit, University of Edinburgh; [3]Wellcome Trust Centre for Cell Biology, University of Edinburgh

Ribosome synthesis in eukaryotes is an incredibly complex process that, besides ribosomal proteins (r-proteins), requires the activity of ~200 ribosome assembly factors. Many of these assembly factors contain enzymatic motifs and are presumed to play crucial roles in remodeling of pre-ribosomes and rRNA folding steps. Although we have a fairly complete picture of the stages at which ribosome assembly factors bind to intermediates, we still lack detailed knowledge of the RNA folding steps that take place and the role of the putative enzymes in this process.

Inspired by impressive chemical probing work done by many groups on bacterial ribosomes, we have developed protocols for purification and chemical modification of specific yeast ribosome assembly intermediates. By combining this with high-throughput sequencing we are able to quantitatively measure structural changes and remodeling steps during ribosome synthesis in a single chemical probing reaction. Using this methodology, dubbed ChemModSeq, we discovered that a large number of ribosomal proteins that interact with the head domain of the 18S rRNA r-proteins are not in their final conformation. Our results support the notion that many ribosome assembly factors can (also) function as r-protein placeholders. This rearrangement of ribosomal proteins correlates with the presence of specific 40S ribosome assembly factors and our results show that the head domain undergoes major remodeling just before the final 18S rRNA cleavage event in the cytoplasm. Our data provide the first insights at nucleotide resolution into how assembly factors modulate the assembly of ribosomal proteins, and provide a platform for studying the role of NTPases in restructuring/remodeling during ribosome synthesis.

6 The casein kinase 1d homolog Hrr25 promotes dissociation of the ribosome assembly factor Ltv1 from nascent small ribosomal subunits to allow joining of large subunits.

Homa Ghalei[1], Katrin Karbstein[2]

[1]The Scripps Research Institute, Department of Cancer Biology, Jupiter, FL; [2]The Scripps Research Institute

Cytoplasmic small (40S) ribosome assembly intermediates are protected from premature translation initiation by assembly factors, which block recruitment of translation factors, mRNA and large subunits. Dissociation of these assembly factors is somehow coupled to a translation-like cycle, in which mature large (60S) subunits join to proofread essential activities of the maturing 40S subunit. However, how entry into this cycle is regulated and how assembly factors dissociate, remains unknown. Here we show that dissociation of the assembly factor Ltv1, located at the beak structure, initiates the cytoplasmic maturation cascade for 40S ribosomal subunits. Hrr25, the yeast homolog for casein kinase 1d, involved in many cellular processes, and linked to numerous human diseases, phosphorylates Ltv1 leading to its dissociation. Failure to release Ltv1 blocks subsequent joining of 60S subunits and entry into the translation-like cycle. We are now studying the effects from Ltv1 release on the incorporation of nearby ribosomal proteins and the 40S structure.

7 Exonucleolytic processing of the 18S rRNA precursors during nuclear export in human cells

Marie-Francoise O'Donohue[1], Milena Preti[1], Nathalie Montel-Lehry[1], Marie-Line Bortolin-Cavaille[1], Hanna Gazda[2], Pierre-Emmanuel Gleizes[1]

[1]Laboratoire de Biologie Moleculaire Eucaryote, CNRS and University of Toulouse; [2]Division of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research, Children's Hospital Boston and Harvard Medical School

Pre-ribosomal RNA maturation has long been considered a highly conserved process in eukaryotes, but recent studies have revealed evolutionary divergence between the yeast and mammalian ribosome synthesis pathways. Understanding the specifics of human ribosome biogenesis is likely to be important for elucidating pathological mechanisms in cancer and ribosomal diseases, like Diamond-Blackfan anemia, the Treacher-Collins syndrome, or the Shwachman-Diamond syndrome. Defects in ribosome biogenesis trigger stress response pathways that perturb cell proliferation and differentiation. Investigating how these signaling pathways are activated requires further understanding of the mechanisms of pre-rRNA processing in human cells.

Mutations in Diamond-Blackfan anemia, a congenital erythroblastopenia associated to mutations in ribosomal protein genes, affect ITS1 processing in a large proportion of patients. Processing of the ITS1 within the pre-40S particles starts in the nucleolus and ends in the cytoplasm in human cells as in yeast. Using loss-of-function experiments and extensive RNA analysis, we have determined that endonucleolytic cleavage E in the ITS1 takes place 78 or 81 nucleotides downstream of the 18S rRNA 3'-end. Cleavage at this site generates the 18S-E pre-rRNA, the last precursor to the 18S rRNA. Unexpectedly, we found that this endonucleolytic cleavage is followed by exonucleolytic processing of the cleavage products in both orientation. The 3'-5' exonucleoytic trimming of the 18S-E pre-rRNA occurs during nuclear export of the pre-40S particles, as revealed by detailed 3'-RACE analysis and cell frationation. The exosome may play some role in this process, but other exonucleases seem to be involved. Knockdown of several ribosomal proteins and maturation factors required for formation of the 18S rRNA results in the accumulation in the cytoplasm of a short form of the 18S-E pre-rRNA, whose final conversion into 18S rRNA requires the PIN-domain containing NOB1.

The requirement of exonucleases in the maturation of the 18S rRNA 3'-end has never been described so far in other eukaryotes, and might indicate a higher level of quality control in mammalian ribosome biogenesis. These results not only deliver a more complex picture of pre-rRNA maturation mechanisms in mammalian cells, but they also provide a mechanistic framework to further study the interplay of DBA-linked ribosomal proteins in this process. We are currently characterizing the exonucleases involved in 18S rRNA maturation, and have found that mutation of a novel DBA-associated gene encoding a large ribosomal subunit protein also affects ITS1 processing.

8 An mRNA-derived ncRNA targets and regulates the ribosome

Andreas Pircher[1], Kamilla Bakowska-Zywicka[2], Marek Zywicki[3], Norbert Polacek[1]

[1]Department of Chemistry & Biochemistry, University of Bern; [2]Institute of Bioorganic Chemistry, Polish Academy of Science, Poznan, Poland; [3]Laboratory of Computational Genomics, Adam Mickiewicz University, Poznan, Poland

Small non-protein-coding RNA (ncRNA) molecules have been recognized recently as major contributors to regulatory networks in controlling gene expression in a highly efficient manner. While the list of validated ncRNAs that regulate crucial cellular processes grows steadily, not a single ncRNA has been identified that directly interacts and regulates the ribosome during protein biosynthesis (with the notable exceptions of 7SL RNA and tmRNA). All of the recently discovered regulatory ncRNAs that act on translation (e.g. microRNAs, siRNAs, antisense RNAs) target the mRNA rather than the ribosome. This is unexpected, given the central position the ribosome plays during gene expression.

To investigate whether such a class of regulatory ncRNAs does exist we performed genomic screens for small ribosome-associated RNAs in model organisms of all three domains [1,2]. Here we focus on the functional characterisation of an 18 nucleotide long ncRNA candidate derived from an open reading frame of an annotated S. cerevisiae gene, which encodes a tRNA methyltransferase. Yeast cells lacking this tRNA methyltransferase showed clear growth defects in high salt containing media. Genetic analysis showed that the absence of the mRNA-derived ncRNA rather than the absence of the tRNA methyltransferase activity is responsible for the observed phenotype. Since we performed a screen for small ribosome-associated RNAs we examined the regulatory potential of the synthetic 18mer during translation in vitro and in vivo. Metabolic labeling experiments in the presence of the synthetic 18mer RNA revealed an inhibitory potential on the global protein biosynthesis rate. In vitro translation and northern blot analysis further strengthen the hypothesis, that this RNA is a ribosome-associated regulatory ncRNA.

Our studies in pro- and eukaryotic model organisms reveal the ribosome as a novel target for small regulatory ncRNAs in all domains of life. Ribosome-bound ncRNAs are capable of fine tuning translation and might represent a so far largely unexplored class of regulatory ncRNAs.

1. Zywicki, M., K. Bakowska-Zywicka, et al. (2012). "Revealing stable processing products from ribosome-associated small RNAs by deep-sequencing data analysis." Nucleic Acids Res 40(9): 4013-4024.
2. Gebetsberger, J., Zywicki, M., Künzi, A., Polacek, N. (2012). „tRNA-derived fragments target the ribosome and function as regulatory non-coding RNA in Haloferax volcanii" Archaea

9 Structural basis of translational regulation of msl2 mRNA by SXL and UNR during dosage compensation in Drosophila

Janosch Hennig[1], Cristina Militti[2], Grzegorz Popowicz[4], Iren Wang[1], Miriam Sonntag[1], Arie Geerlof[1], Fatima Gebauer[3], Michael Sattler[1]

[1]Helmholtz Zentrum München & TU München; [2]Centre for Genomic Regulation (CRG), Barcelona; [3]CRG Barcelona; [4]Helmholtz Zentrum München

The protein Upstream of N-Ras (UNR) is a key regulator of gene expression at the translational level in both humans and *Drosophila*. In *Drosophila*, the role of UNR in dosage compensation is well characterized. UNR and the female-specific protein Sex-lethal (SXL) bind cooperatively to the 3' UTR of *msl2* mRNA, which encodes the rate-limiting subunit of the dosage compensation complex. This interaction represses the translation of*msl2* mRNA and allows female fly viability.

We have investigated the structural basis for the assembly of the SXL-UNR-*msl2* ribonucleoprotein complex and studied the minimal relevant regions required for ternary complex formation by complementary structural biology methods, biochemistry and functional analysis. We report the crystal structure of the ternary complex at 2.8 Å resolution, which was validated by complementary data from solution NMR, SAXS and SANS. The structure reveals unprecedented insight into how the tandem RNA recognition motif (RRM) domains of SXL and UNR cold shock domains cooperate for specific the recognition of the *msl-2* RNA. Structure-based mutational analysis of protein-RNA and protein-protein interfaces reveal molecular details for translational repression by UNR during development. Our structural and functional analysis provide novel insight for an important molecular mechanism of the regulation of X-chromosome dosage compensation.

10 Circular RNAs function as efficient microRNA sponges

Jorgen Kjems[1], Thomas B. Hansen[2], Christian K Damgaard[2], Trine I. Jensen[2], Jesper B. Bramsen[2], Bettina H. Clausen[3], Bente Finsen[3]

[1]Department of Molecular Biology and Genetics, Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, Denmark; [2]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark; [3]Neurobiology Research, Institute of MolecularMedicine, University of Southern Denmark

Circular RNAs that derive from RNA splicing events across exons are abounded in mammalian cells but their function has until recently been illusive. We recently reported that the antisense transcript to the neuronally expressed CDR-1 gene is almost exclusive circular and positively regulates the expression of the CDR-1 mRNA (Hansen et al. 2011).

We have now discovered that this circular RNA, in addition, acts as a highly efficient microRNA-7 (miR-7) sponge and named it ciRS-7 (Circular RNA Sponge for miR-7; Hansen et al. In press). ciRS-7 harbours more than 70 selectively conserved putative miRNA target sites and it is highly and widely associated with Ago-proteins in a miR-7 dependent manner. While the circular RNA is completely resistant towards miRNA-mediated target destabilization, it strongly suppresses miR-7 activity resulting in elevated levels of miR-7 targets. In the mouse brain, we observe overlapping neuronal expression patterns of ciRS-7 and miR-7 in the neocortex and hippocampus, and in thalamus and substantia nigra suggesting a high degree of endogenous interaction. We also show that ciRS-7 can induce established miR-7 targets including SNCA, EGFR and IRS2, implicated in Parkinson disease, cancer and diabetes, respectively. The ciRS-7 is itself under the control of miR-671 that, unusually for a mammalian miR, cleaves the ciRS-7 and effectively removes it from the cell. Hence miR-671 may constitute a novel therapeutic reagent in a number of human diseases.

The function of circular RNAs as miR sponges appears to be a general phenomenon. We demonstrate that circular testis specific RNA, SRY, serves as a miR-138 sponge and that circular SRY expression can increase miR-138 targeted mRNAs. Thus, this serves as the first functional study of a naturally expressed circular RNA.

We are currently establishing ciRS as generic platform enabling sponging of any miR of chose by reprogramming the seed sequences.

1. Hansen, T. B., Wiklund, E. D., Bramsen, J. B., Villadsen, S. B., Statham, A. L., Clark, S. J., and Kjems, J. (2011) miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA, *The EMBO journal 30*, 4414-4422.
2. Thomas B Hansen; TB, Jensen, TI, Clausen, BH, Bramsen, JB, Finsen, B, Damgaard, CK, Kjems, J. (2013) Natural RNA circles function as efficient miRNA sponges. *Nature* (Published online 27 February 2013)

11 The regulatory circuits mediated by RNAs in Staphylococcus aureus and implication of the endoribonuclease III

Efthimia Lioliou[1], Cédric Romilly[1], Isabelle Caldelari[3], Cynthia Sharma[4], Thomas Geissmann[2], François Vandenesch[2], Joerg Vogel[4], Pascale Romby[1]

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Staphylococcus aureus is a remarkable versatile pathogen, able to cause a wide spectrum of human diseases, and is one of the main causes of community as well as hospital-acquired infections. The contribution of regulatory RNAs in the establishment of virulence in this pathogen is increasingly appreciated. Our previous data emphasize the multitude of regulatory steps affected by RNAIII in establishing a network of *S. aureus*virulence factors. We show that RNAIII and the endoribonuclease III coordinately repress the expression of numerous mRNAs that encode the transcriptional repressor of toxins, several virulence factors acting early in the infection process, and several enzymes involved in peptidoglycan metabolism. The repressor activity of RNAIII involves the formation of RNA-mRNA duplexes that results in the inhibition of translation initiation and concomitantly triggers endoribonuclease III attack. Identification of the RNA targets of the endoribonuclease III further illustrates the multiple functions of the enzyme in the regulation of RNA metabolism. Besides RNAIII, we demonstrated that the *S. aureus* genome likely encodes a high diversity of RNAs including *cis*-acting regulatory regions of mRNAs, *cis*-acting antisense RNAs, and small non-coding RNAs. We will illustrate how some of these novel non-coding RNAs have direct consequences on biofilm and capsule formation, and stress responses, and how they converged to the quorum-sensing system.

12 Messenger and long non-coding RNAs: dressed for the occasion?

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In yeast, pervasive transcription generates a heterogeneous ensemble of long non-coding RNAs (lncRNAs) as well as mRNAs and stable, structural RNAs. The mRNAs are exported to the cytoplasm for translation, whereas characterized lncRNAs perform distinct functions, such as directing chromatin modifications. LncRNAs and mRNAs share many properties including a 5' cap, poly(A) tail and transcription by Pol II, raising the question of why they have such different fates. Throughout their maturation, export and decay, mRNAs interact with a defined series of protein factors. We reasoned that analysis of the interactions of these proteins with other transcripts, such as lncRNAs, would reveal the point at which their maturation separates from that of mRNAs. We therefore determined the in vivo, transcriptome-wide targets of key protein factors in this pathway. This revealed distinct classes of lncRNAs and mRNAs, with RNP compositions tailored to the regulation and functions of transcripts within each class. Therefore, rather than undergoing a "standard" maturation process, mRNAs and lncRNAs are assembled into purpose-built RNPs. LncRNAs were abundant targets of the nuclear surveillance machinery, so are predominantly retained and degraded in the nucleus. However, further analyses revealed a subclass of stable lncRNAs that undergo cleavage and polyadenylation and are exported to the cytoplasm. In contrast, the unstable lncRNAs are subject to a distinct mode of termination. Therefore, 3' end processing is a key step in RNP biogenesis at which transcript fate is determined, and differences here explain the origin of the marked heterogeneity amongst mRNAs and lncRNAs. In support of this model, we identified dual roles for two proteins in both cleavage and polyadenylation and surveillance of lncRNAs. Unexpectedly, we also observed "lncRNA-like" mRNAs, subject to post-transcriptional regulation in the nucleus, which involves early transcription termination, upstream lncRNAs, or surveillance by the nuclear poly(A) binding protein Nab2. In yeast subjected to a short nutrient downshift there was extensive retargeting of the nuclear surveillance factor Mtr4 amongst these "lncRNA-like" mRNAs. Changes in lncRNA expression are therefore rapid and may play a widespread role in reprogramming gene expression. In conclusion, our comprehensive atlas of RNP composition effectively captures the diversity within the transcriptome and has unearthed several prominent mechanisms of post-transcriptional regulation in the nucleus.

13 Non-coding RNAs prevent spreading of a repressive histone mark

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Besides messenger RNAs (mRNAs), eukaryotic cells produce a plethora of RNAs that appear to be non-protein coding (ncRNAs). Whereas substantial progress has been made in cataloging ncRNAs, the extent of their involvement in regulatory circuits and the mechanisms through which they might act remain to be explored further. In the fission yeast *Schizosaccharomyces pombe*, ncRNAs are known to play a prominent role in the assembly and maintenance of heterochromatin. Transcription of centromeric repeat sequences results in the production of long ncRNAs (lncRNAs) that are processed by Dicer (Dcr1) into short ncRNAs (siRNAs). These are found in Argonaute (Ago1) and target the Ago1-containing RNA-induced transcriptional silencing complex (RITS) to nascent chromatin-bound lncRNAs. Subsequently, RITS recruits the histone methyltransferase (HMTase) Clr4 to methylate histone H3 at Lys 9 (H3K9), a hallmark of heterochromatin. Thus, long and short ncRNAs cooperate in the targeting of a histone modifying activity to the appropriate location in the *S. pombe*genome. In contrast to this mode of ncRNA action, we have now discovered a novel class of ncRNAs that counteract H3K9 methylation. We have identified a lncRNA residing in centromeric heterochromatin, termed BORDERLINE, which prevents the spreading of heterochromatin beyond the pericentromeric repeat region. Our results demonstrate that the production of RNA is sufficient to demarcate an epigenetically distinct chromosomal domain, irrespective of the underlying DNA sequence. In contrast to the recurring theme that ncRNAs function to recruit or guide proteins to chromatin, we show that RNA can also counteract chromatin association.

14 Single cell and genome-wide analysis to dissect antisense RNA-mediated gene silencing and pervasive transcription in S. cerevisiae

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The *S. cerevisiae* genome codes for a myriad of intergenic and antisense (AS) transcripts, some of which are unstable and degraded by the exosome component Rrp6 [1, 2]. Loss of Rrp6 results in the accumulation of long *PHO84* AS RNAs and repression of sense transcription through a process that involves *PHO84* promoter deacetylation by the Hda1/2/3 histone deacetylase (HDAC) complex [3]. Here, we use single molecule resolution fluorescent in situ hybridization (smFISH) [4] to investigate the mechanism of PHO84 antisense-mediated transcription regulation in single cells. We show that PHO84 AS acts as a bimodal switch, where continuous low frequency PHO84 AS transcription represses sense transcription within individual cells. Surprisingly, AS RNAs do not accumulate at the *PHO84* gene but are exported to the cytoplasm. Furthermore, loss of Rrp6, rather than stabilizing *PHO84* AS RNA, promotes AS elongation by reducing its early transcription termination by Nrd1/Nab3/Sen1. These observations suggest that PHO84 silencing results from low frequency but constant AS transcription through the promoter rather than its static accumulation at the repressed gene. To investigate the generality of this regulation we profiled various histone modification mutants in a D*rrp6* strain using high-density tiling arrays. We confirm a widespread occurrence of antisense-dependent gene regulation and identify three classes of genes that accumulate asRNA in the absence of Rrp6, which differ in whether their genes are silenced by the asRNA and whether the repression involves HDACs and HMT. Distinguishing features between functional and non-functional antisense RNAs include sensitivity to early termination, extension into the promoter region, or the promoter structure of the repressed gene. The data indicate that histone-modifying enzymes are particularly important for antisense-mediated silencing of highly regulated genes subjected to extensive chromatin remodeling.

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15 Telomeric non-coding RNA acts as a scaffold for telomerase high-order organization at short telomeres

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Telomerase, which is composed of both protein and RNA, maintains genome stability by replenishing telomeric repeats at the ends of chromosomes. On short telomeres, several molecules of telomerase are recruited, leading to the formation of telomerase foci or clusters, which reflects the distributive extension of short telomeres by this enzyme. How these telomerase clusters are formed on short telomeres is still unknown. Herein, we show that telomeric non-coding RNA is involved in the nucleation of telomerase clusters at short telomeres.

Telomeres are transcribed in a strand specific manner, giving rise to a G-rich telomeric-repeat containing RNA (TERRA). In mammalian cells, TERRA is nuclear and accumulates to some extent at telomeres. In yeast, TERRA expression is negatively regulated by the 5'-3' exonuclease Rat1, which actively degrades TERRA transcripts. Although several functions for TERRA have been proposed in mammalian cells, direct evidence for a specific role for TERRA in yeast is still missing. We developed a live-cell imaging assay based on the MS2-GFP system to study endogenous TERRA expression from a unique telomere at the single cell level in yeast. We show that TERRA expression is induced when its telomere shortens, leading to the accumulation of TERRA molecules into a single perinuclear focus. Live-cell imaging of a GFP-labeled TERRA and its RFP-labeled telomere revealed that a TERRA focus associates specifically but transiently with its telomere of origin in S phase, which was confirmed by chromatin immunoprecipitation.

Furthermore, an interaction between TERRA and the yeast telomerase RNA (*TLC1*) was detected *in vivo* by co-immunoprecipitation. Surprisingly, by simultaneously imaging TERRA-GFP and *TLC1* RNA-CFP, we captured spontaneous events of nucleation of *TLC1* RNA molecules on TERRA foci in S phase, suggesting that a TERRA focus acts as a scaffold for the recruitment of telomerase and triggers the formation of a telomerase cluster. Simultaneous imaging of telomere 6R- or telomere 1L-TERRA, *TLC1* RNA and telomere 6R revealed that a TERRA-*TLC1* RNA cluster forms in early S phase, and is later recruited preferentially to the telomere from which TERRA molecules originate. This association depends on factors involved in the recruitment of telomerase at short telomeres, such as Mre11, Tel1 and yKu70. We propose that a short telomere expresses non-coding RNA to nucleate telomerase molecules into a cluster in order to promote the simultaneous recruitment of several telomerase molecules on this telomere.

16 Human α satellite derived transcripts interact with the active site of RNApolII

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Whether repetitive regions of the human genome have a function is a very intriguing question arising from two recently reported facts, firstly 80 % of the human DNA is transcribed into RNA, and secondly two-thirds of the genome is repetitive or repeat-derived. Human α satellites consist of 171 bp monomers arranged tandemly in a head-to-tail manner, organized into arrays of higher order repeats spanning hundreds of kilobases to megabases. They are predominantly located near centromeres on every human chromosome, where they assure the proper chromosome segregation being a site for the spindle attachment.

Here, we present that α satellite arrays are transcribed into large transcripts (more than 8 kb) in both orientations (direct and reverse complement) to the consensus α satellite sequence. Their expression is more pronounced in cellular stress conditions and restrained to the S phase of the cell cycle. Transcription of α satellites is sensitive to α amanitin treatment, indicating that they are RNApolII transcripts. Unlike most RNApolII transcripts, they are not polyadenylated and remain in the nucleus. Intriguingly, analysis of the 5' termini of RNAs reveals that only transcripts synthesized in the direct orientation possess cap structure while reverse complement are not capped. Driven by this finding we investigated α satellites interaction with RNApolII. In genomic SELEX against RNApolII, we had isolated several aptamers derived from α satellite repeats. Moreover, we found that α satellite RNAs interact with the active core of RNApolII resulting in DRB (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole) sensitive 3' labeling of the RNA or/ and to the second strand synthesis, what in turn implies that RNApolII might act as RNA dependent RNA polymerase (RdRP) on endogenous RNAs. Using HeLa cells nucleofection with chimeric transcripts combining α satellite with an artificial sequence, we observed RNA dependent RNA synthesis activity on genomic derived RNA template *in vivo*.

17 Methylated mRNA recognition by the YTH domain

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Methylation of adenosine at the N6 position is considered the most abundant messenger RNA modification and known since nearly 40 years. Methylation is assumed to be important based on the severe phenotypes of methylase knockouts in several organisms. Recently also the demethylase has been postulated to be a disease risk gene. In 2012 two reports [1,2] were published investigating with high throughput sequencing this process and expanded the repertoire of known sites and lead to a better definition of the consensus sequence for methylation. One of these recent studies [1] investigated, which proteins bind preferentially to the methylated form of a RNA compared to the unmodified form. Two of the three hits in the top confidence category were YTH domain containing proteins (Ydf2,Ydf3).

We have previously shown that the YTH domain of YT521-B is a RNA binding domain [3] and performed NMR titrations using partially randomized nucleotides to better define the binding preferences of this domain, since the SELEX derived sequences were quite degenerate. This approach yielded a better defined binding sequence and a preliminary structure of this complex could be obtained.

Both studies [1,2] on N6-methylation of adenosine reported a consensus sequence for an A to get methylated, which resembles our NMR derived sequence. The methylated adenosine is mostly flanked 5' by a G and 3' by a C. This prompted us to perform a titration experiment using the NMR derived sequence containing a N6-methyladenosine. The addition of this methyl group shifted the exchange regime of this complex from fast/intermediate to slow indicating much higher affinity. We determined the structure with methylated RNA, which represents the first structure of this domain in complex with RNA.

We were able to obtain 14 InterNOEs between the protein and the methyl group, which is contacted by two tryptophan residues via CH-π interactions. This resembles the recognition of methylated arginines and lysines of histones by their respective binding domains [4]. The two tryptophan residues are strictly conserved in all YTH domains [5]. Furthermore our experiments were carried out with the domain of YT521-B and not the domains of YDF2 or YDF3.

The structure reveals, how the YTH domain is able to recognize a methylated adenosine and it is tempting to assign the YTH domain, which is on a molecular and functional level not well characterized, the role of recognizing mRNAs containing N6-methylated adenosines. YT521-B regulates alternative splicing and the presence of the YTH domain is crucial for this [3]. Our results might therefore hint at a role of mRNA methylation in the control of splicing.

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18 Determination of N6-methyladenosine RNA modification status at single nucleotide resolution and the application to a long non-coding RNA-protein interaction

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N^6-methyladenosine (m^{6}A) is the most abundant modification in eukaryotic mRNA and long non-coding RNA, but m^{6}A studies have been hindered by the lack of methods for its precise identification. Here we develop a method, named Site-specific Cleavage And Radioactively-labeling followed by Ligation-assisted Extraction and TLC (SCARLET), that identifies m^{6}A and its modification fraction in mRNA/lncRNA at single nucleotide resolution. We applied SCARLET to locate four m^{6}A sites on the nuclear-localized MALAT1 (metastasis associated lung adenocarcinoma transcript), an abundant lncRNA known to regulate alternative splicing and chromatin remodeling.

We further identified nuclear-localized proteins that selectively bind to two m^{6}A modified sites in MALAT1. Gel shift using the synthetic m^{6}A-containing RNA oligos from MALAT1 and the HeLa nuclear extract showed that m^{6}A-containing RNA oligos can recruit 2-5 fold more proteins compared with the unmodified RNA. Further, RNA pull down followed by LC-MS/MS show that heteronuclear ribonucleoprotein G (hnRNP-G) and hnRNP-C selectively bind to one of the two m^{6}A-modified MALAT1 sites; both hnRNP proteins are known to be involved in RNA splicing and transport. We also demonstrate that hnRNP-G and hnRNP-C prefer m^{6}A modified sites *in vivo*. The identification of m^{6}A-selective proteins indicates that a primary role of m^{6}A modification is to recruit specific proteins or protein complexes to the modification site to modulate the function or activity associated with these RNA-protein complexes.

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19 Inosine-mediated modulation of RNA sensing by innate immune sensors

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RNA-specific adenosine deaminase (ADAR)-mediated adenosine to inosine (A-to-I) editing is a critical arm of the antiviral response. However, mechanistic insights into how A-to-I RNA editing affects viral infection are lacking. We posited that inosine incorporation into RNA facilitates sensing of non-self RNA by innate immune sensors, and accordingly investigated the impact of inosine-modified RNA on Toll Like Receptor (TLR) 7/8 sensing. Inosine incorporation into synthetic ssRNA potentiated TNF-α or IFN-α production in human PBMCs, in a sequence-dependent manner, indicative of TLR7/8 recruitment. The effect of inosine incorporation on TLR7/8 sensing was restricted to immunostimulatory ssRNAs, and was not seen with inosine-containing short dsRNAs, nor with a deoxy-inosine-modified ssRNA. Inosine-mediated increase of self-secondary structure of an ssRNA resulted in potentiated mouse Tlr7 activation, as established through the use of *Tlr7*-deficient cells. There was a correlation between hyper-editing of influenza A viral ssRNA and its ability to stimulate TNF-α, independent of 5'-triphosphate residues. Furthermore, A-to-I editing of viral ssRNA directly enhanced mouse Tlr7 sensing, when present in proportions reproducing biologically relevant levels of RNA editing. Thus we demonstrate for the first time that inosine incorporation into immunostimulatory ssRNA can potentiate TLR7/8 activation. Our results suggest a novel function of A-to-I RNA editing, which is to facilitate sensing of phagocytosed viral RNA by innate immune sensors TLR7/8.

20 Impact of ADARs on abundance and sequence of miRNAs and other non-coding RNAs.

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Adenosine deaminases that act on RNA (ADARs) bind double-stranded RNAs and deaminate adenosines to inosines. Inosines are recognized as guanosines and thus editing changes sequence information but also the structure of RNAs. A to I editing is required for normal life and development. Besides mRNAs and repetitive elements, ADARs also target miRNA precursors and affect their processing efficiency or alter their target specificity. Previous studies on the impact of editing of miRNAs only focused on few specific miRNAs. However, the genome wide effect of editing on miRNAs had not been firmly established.

Using transgenic mouse embryos lacking the editing enzymes ADAR, ADARB1, or both we compared relative abundance and sequence composition of mature miRNAs to wild type mice by NextGen sequencing. Deficiency of ADARB1 leads to a reproducible change in abundance of several miRNAs, which seem to be unrelated to editing events. The additional lack of ADAR has little impact on the deregulation of mature miRNAs, indicating that proper miRNA processing is primarily dependent on ADARB1. A to G transitions reflecting A to I editing events can be detected at few sites and at low frequency during the early embryonic stage investigated. Again, most editing events are ADARB1 dependent with only few editing sites being specifically edited by ADAR.

Due to the strong impact of ADARB1 on miRNA abundance and the known higher editing levels in adult mice we applied the same experimental setup to miRNAs from adult mouse brain in wild type and ADARB1 mutant mice. ADARB1 deficient mice show reproducible changes in abundance of specific miRNAs, of which again many are unrelated to A to I editing events. Thus, binding of miRNA precursors by ADARB1, without editing, influences their abundance. Unlike in the embryo, A to G transitions are found in many sites and with a frequency up to 80% in the mature miRNA sequence. More than half of the editing events are primarily caused by ADARB1. Moreover, also previously unrecognized editing sites in miRNAs were identified. Interestingly, 64% of editing events occur in the seed region of miRNAs, allowing a retargeting of the edited miRNA.

Lack of ADARs also affects other non coding RNAs such as snoRNAs, snRNAs, and transcripts of repetitive elements. Understanding the consequences of these changes is a current focus of our studies.

21 ADAR proteins suppress activation of antiviral signaling by cellular RNA.

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Mutations in the gene encoding the RNA editing enzyme ADAR1 have recently been shown to cause the rare autoimmune disorder Aicardi-Goutières syndrome (AGS). AGS is a clinical mimic of in utero-acquired infection and is associated with increased production of the antiviral cytokine interferon (IFN) alpha. *Adar1*-null mice also have aberrant expression of Type-1 IFN, interferon-stimulated genes (ISGs) and pro-inflammatory cytokines, yet the phenotype is more severe with embryonic lethality around E12.5. Therefore it has been proposed that ADAR1 is required to suppress antiviral responses.

Genetic crosses revealed that mice lacking both ADAR1 and the interferon receptor can survive to E16.5, suggesting the effect of the *Adar1* mutation is cell autonomous. Moreover, embryonic lethality in *Adar1*-null mice is rescued by crossing with a mutant lacking a key protein involved in antiviral signaling. These double homozygous mice have decreased IFN production, ISG expression and pro-inflammatory cytokine levels. Analysis of cellular RNA from *Adar1*-null mice revealed increased expression of transcripts encoding specific classes of repetitive elements. Surprisingly, increased expression of immune-response genes and altered levels of repetitive element transcripts are also observed in *Adar*-null *Drosophila*, supporting a conserved role for ADAR proteins as suppressors of innate immunity.

22 Mechanism of gRNA Biogenesis in Trypanosome Mitochondria

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The U-insertion/deletion mRNA editing reactions are directed by guide RNAs (gRNAs). Mature 50-60 nt gRNAs are generated from ~800 nt precursors by 3′ end nucleolytic processing and subsequent RET1-catalyzed uridylation. We previously demonstrated that TbRET1 repression in T. brucei leads to a loss of 3′ oligo(U) tails and accumulation of gRNA precursors. The former observation is consistent with TbRET1's uridylyl transferase activity; the latter finding remained puzzling because TbRET1 lacks nucleolytic activity. Here, we show that TbRET1 forms a stoichiometric complex with a 3'-5' exonuclease TbDSS1 and several proteins without any discernible motifs, which we named TbRDS (TbRET1-TbDSS1) complex. In S. cerevisiae, DSS1 interacts with SUV3 helicase to form a mitochondrial degradosome. However, in T. brucei we found no evidence of stable DSS1-SUV3 interaction. Repression of TbDSS1 led to a loss of mature gRNAs and accumulation of ~800 nt gRNA precursors indicating that TbRET1 and TbDSS1 function in the same processing pathway. Furthermore, overexpression of enzymatically inactive DSS1 triggered gRNA processing defects. In addition to minicircle-encoded gRNA precursors, TbRDS complex also targets maxicirlce-encoded messenger and ribosomal RNA precursors leading to generation of mature molecules. In vitro, TbRDS complex processively degrades single-stranded RNA to 5-6 nt oligonucleotides whereas in vivo degradation stops precisely at the 3′ ends of gRNAs, mRNAs and rRNAs. Deep sequencing of small mitochondrial RNA fraction indicated that guide RNA-sized antisense transcripts may define the 3′ boundary of mature mitochondrial RNAs. Collectively, our data indicate that nucleolytic processing of gRNA precursors by TbDSS1 and uridylation of mature gRNAs by TbRET1 are coupled by virtue of both enzymes being assembled into a stable TbRDS complex. The degradation activity of TbRDS is likely controlled by antisense RNAs thereby the 5' end of the antisense determines the 3' end of the sense strand.

23 Biogenesis and function of cyclic N6-threonylcarbamoyladenosine (ct6A) as a widely distributed tRNA hypermodification

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N^6-threonylcarbamoyladenosine (t^6A) has been recognized as a universally conserved, essential modified nucleoside found in tRNAs responsible for ANN codons in all three domains of life. t^6A plays a crucial role in maintaining decoding accuracy during protein synthesis. The presence of t^6A in cellular tRNAs has been well documented for more than four decades. However, under conditions optimized for nucleoside preparation, we detected little t^6A in tRNAs from Escherichia coli. Instead, we identified a novel modified base named "cyclic t^6A" (ct^6A) (ref1), which is a cyclized active ester with an oxazolone ring. ct^6A is widely distributed in many bacteria, fungi, protists and plants. ct^6A has a supportive role in promoting decoding efficiency of $tRNA^{Lys}$. Structural modeling indicated that ct^6A recognizes the first adenine base of ANN codon at the ribosomal A-site.

We also identified an E1-like enzyme named tRNA threonylcarbamoyladenosine dehydratase A (TcdA) which catalyzes ATP-dependent dehydration of t^6A to form ct^6A. Detailed catalytic mechanism of t^6A dehydration has been investigated by structural and biochemical approaches. In addition, we identified a cysteine desulfurase (CsdA) and a sulfur carrier protein (CsdE) to be required for efficient ct^6A formation, indicating that sulfur relay system is involved in this reaction.

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24 Unusual non-canonical editing important for tRNA processing in Trypanosomes as revealed by shallow sequencing.

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In eukaryotes, precursor tRNAs contain introns whose cleavage is mediated by an evolutionarily conserved endonuclease complex that generates fully functional mature tRNAs. The *Trypanosoma brucei* genome encodes a single intron-containing tRNA (tRNATyr), responsible for decoding all tyrosine codons; therefore, intron removal is essential for protein synthesis and consequently cell viability. In this organism, little is known about the mechanism of intron processing, but owing to its early evolutionary divergence from other eukaryotes, *T. brucei* often reveals unexpected peculiarities. Bioinformatic analyses revealed only one homolog of the four conserved canonical subunits of the multi-protein complex endonucleases required for tRNA splicing in other eukaryotes. Here we show an unprecedented number of nucleotide differences within the intron-containing pre-tRNATyr and its genomic copy. Significantly, these differences occur at the RNA level and cannot be ascribed to canonical deamination-type editing. Intron editing is required for proper pre-tRNA processing, establishing its functional significance for production of the full complement of tRNAs needed for translation. The demonstration of a novel editing mechanism required for proper function of a highly divergent splicing endonuclease in kinetoplastids has great implications to our understanding of the evolution of tRNA processing across eukaryotes.

25 Structural insights into the assembly of spliceosomal U snRNPs

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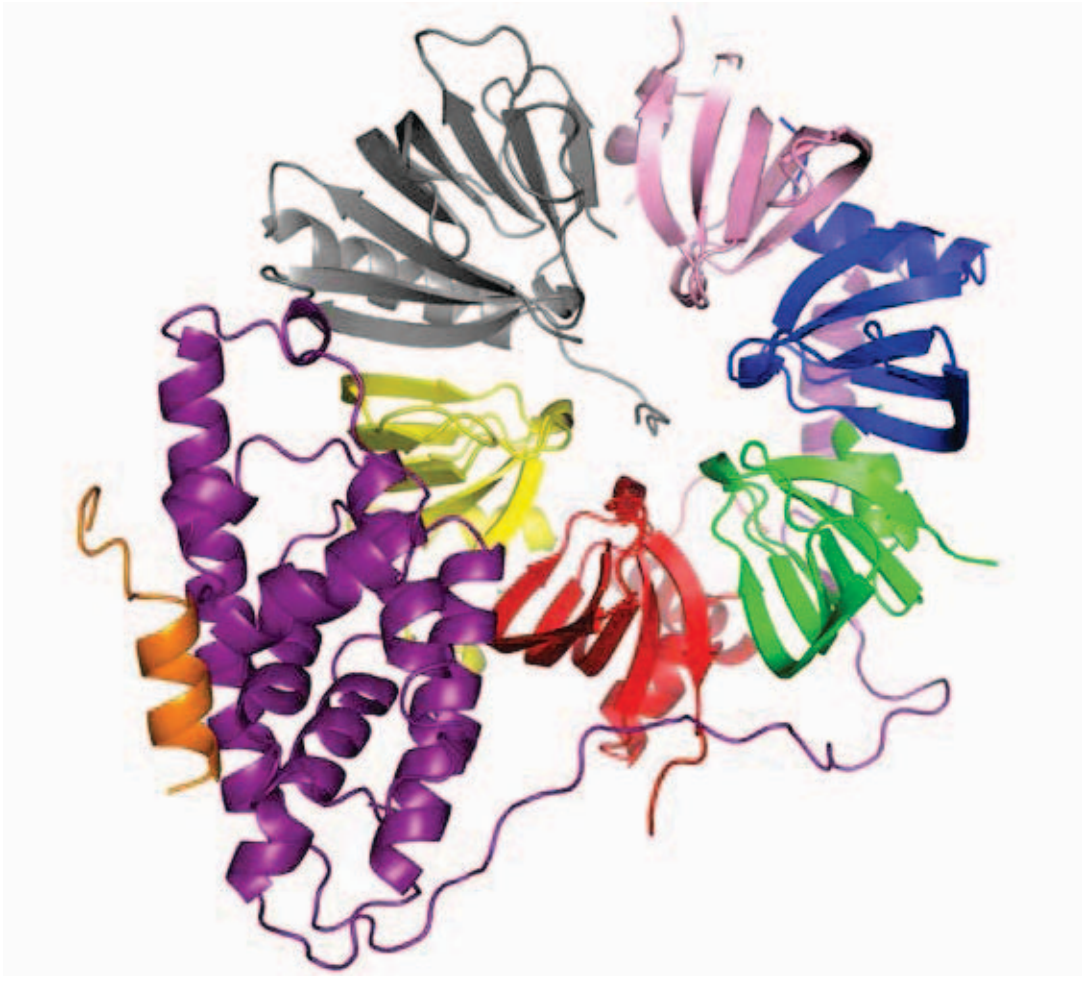
Splicing is the process in which non-coding introns are excised from the primary transcript (pre-mRNA) and the coding exons are joined. Splicing is catalyzed by the spliceosome, which consists of the four small nuclear ribonucleoproteins (snRNPs) U1, U2, U4/6 and U5 as well as a large number of other proteins. Assembly of snRNPs from RNA and protein is an essential pre-requisite for splicesome formation. In vivo, this is facilitated in a highly complex biogenesis pathway. Initially, the snRNA is transported from its site of transcription to the cytoplasm. Within this compartment, the common (Sm) proteins as well as some proteins specific for the respective U snRNP associate with the snRNA. Finally, the mature particle is transported to the nucleus and eventually incorporated into the spliceosome. Our group has identified a unique machinery that assists the assembly of spliceosomal U snRNPs (Chari et al., 2008, Cell 135, 497-509). This machinery consists of two cooperating units termed SMN-complex and PRMT5-complex, respectively. While the SMN-complex is an RNP assembler and loads Sm proteins onto the snRNA, the PRMT5 complex functions upstream in this pathway. It acts as an assembly chaperone by forcing Sm proteins into a higher order structure (termed 6S complex) required for the subsequent transfer onto the SMN-complex. We will report on the atomic structure of two key intermediates of the snRNP assembly machinery. The structure of the early 6S intermediate identifies pICln as an Sm-protein mimic, which enables the topological organization of five Sm proteins in a closed ring and prevents premature RNA binding. A second structure of the 6S assembly intermediate bound to the SMN-complex components SMN and Gemin2 (see figure below) uncovers the likely mechanism of pICln elimination and Sm protein activation for snRNA binding. These structural studies along with biochemical experiments reveal how assembly factors facilitate formation of RNA-protein complexes in vivo.



26 Crystal structure of human spliceosomal U1 snRNP at 3.3 Å resolution.

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Human spliceosomal U1 snRNP consists of U1 snRNA, seven Sm proteins and three U1-specific proteins; U1-A, U1-C, and U1-70k. The binding of U1 snRNP to the 5' splice site (5'ss) within the mRNA precursor (pre-mRNA) initiates spliceosome assembly. Numerous biochemical and genetic studies showed that U1 snRNP recognizes the 5'ss of pre-mRNA through base-pairing with the 5' end of U1 snRNA and that U1-C is important for the efficiency of the binding (1). Our previously-reported 5.5 Å crystal structure revealed that the U1-C zinc finger domain locates next to U1 snRNA 5'-end: 5'ss duplex, but we could not study this interaction in detail due to the limited resolution (2). Although the sequence of the 5'-end of U1 snRNA is completely conserved from yeast to human, the 5' splice site sequence of the pre-mRNA to which it binds shows different degrees of conservation in different species. The 5'ss is stringently conserved in *Saccharomyces cerevisiae* whereas in human only the GU di-nucleotide is strictly conserved.

In order to gain further insights into the recognition of the 5›ss by U1 snRNP, using previous structures as a guide, we designed a minimal sub-structure of U1 snRNP containing all the components necessary for 5›ss recognition. We reconstituted and crystallised this sub-structure with the seven Sm proteins, U1-C, the N-terminal domain of U1-70k, and a U1 snRNA lacking stem/loop I, II and III,.

Here we report a 3.3 Å resolution crystal structure of a U1 snRNP sub-structure to which an RNA oligonucleotide containing a 5'ss sequence is bound. The crystal structure reveals how the U1-70k N-terminal peptide stabilizes the binding of U1-C protein and how U1-C in turn facilitates the binding of the 5' splice site. The structure also shows the differences between the U1 and U4 core domain structures and provides insight into the specificity of the N-terminal peptide of U1-70k for the U1, and not the U4 core domain.

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2. Pomeranz Krummel, D. A., Oubridge, C., Leung, A. K., Li, J. and Nagai, K. Crystal structure of human spliceosomal U1 snRNP at 5.5 Å resolution. *Nature* 458, 475-480 (2009).

27 Crystal structure of Prp5p reveals intra-molecular interactions that impact splicing fidelity

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Prp5p, a DEAD-box ATPase, is essential for spliceosome assembly by facilitating U2 snRNP binding to the intron branch site (BS) region and proofreads splicing. However, the structural basis for the function of Prp5p remains unclear. Here, we report crystal structures of *S. cerevisiae* Prp5p alone and in complex with ADP at resolutions of 2.12 Å and 1.95 Å, respectively. The structures reveal that two RecA-like subdomains (D1 and D2) of Prp5p adopt an unusual twisted 'open state' conformation by extensive intra-molecular interactions with flanking sequences and the subdomain linker. Further in vivo mutagenesis in *S. cerevisiae* demonstrated that a number of *prp5* alleles, which destabilized the intra-molecular interactions, specifically inhibit splicing of suboptimal BS substrates. The mutant Prp5p proteins possess faster-than-WT ATPase activities in vitro. These observations suggest a mechanism in which the twisted 'open state' conformation of Prp5p must be remodeled into a 'closed state' conformation during branch site recognition in order to activate its ATPase activity and to commit the intron to the splicing pathway. Changes in the relative stabilities of the 'open' and 'closed' forms of Prp5p result in alteration of splicing fidelity.

28 Sequential contacts of DExD/H-box protein Prp28p with Prp8p, Brr2p, and Snu114p during splicing as captured by a chemical cross-linking approach

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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 RNPases to dissociate proteins from RNA or to remodel RNA-protein complexes. Precisely how the latter can be achieved remains unknown. We have been trying to approach this issue by using yeast Prp28p as a model system. Prp28p is an evolutionarily conserved DExD/H-box splicing factor that facilitates the U1/U6 switch at the 5' splice site (5'ss) during spliceosomal assembly. We have previously shown that Prp28p can be made dispensable in the presence of specific mutations that alter U1C, Prp42p, Snu71p, Cbp80p, and Ynl187p. These data suggest a model that Prp28p counteracts the stabilizing effect by those proteins on U1 snRNP/5'ss interaction. 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 and some of these cross-linked products are 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 treatment. Using mass-spec and Western blotting analysis, we found that Prp28p-K136^{BPA} cross-links to Prp8p and Prp28p-K82^{BPA} contacts both Prp8p and Snu114p. Furthermore, Prp28p-K27^{BPA} and Prp28p-K41^{BPA} were cross-linked to Brr2p and Snu114p, respectively. These data are consistent with the structural study of the U5–U4/U6 tri-snRNP, which placed Snu114p next to Prp8p, which is in turn adjacent to Brr2p. ChIP analysis revealed that alanine substitutions at Prp28p-K136 region reduce the binding of Prp28p to spliceosome and delay the release of U1 snRNP from spliceosome, consistent with our genetic analysis suggesting that Prp28p works in concert with Prp8p. Finally, mixing experiments suggested that Prp28p makes contact with Prp8p and then with Brr2p. Taken together, our data suggest a working model that Prp28p approaches the complex spliceosomal milieu by making prior contact with Prp8p to effect U1 snRNP dissociation and then with Brr2p (and perhaps Snu114p), which may transmit the signal to couple U1 dissociation with the U4/U6 unwinding, a key step in spliceosomal remodeling.

29 Functional spliceosome assembly without stable U4/U6 snRNA pairing

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The cycle of spliceosome assembly, intron excision, and disassembly involves large-scale snRNA structural rearrangements. U6 snRNA toggles between a U2 snRNA-bound state in the catalytically active spliceosome and a U4 snRNA-bound state during spliceosome assembly. The function of U4/U6 pairing is not clear, but is presumed to be essential since it occurs in all eukaryotes examined. Here we report the surprising finding that stable U4/U6 pairing is dispensable for splicing in yeast. Single point mutations in U4 and U6 that disrupt their pairing are lethal in combination, but are rescued by a second mutation in U6 that extends U2/U6 Helix II and stabilizes the U2/U6 complex. The resulting triple mutant strain lacks detectable U4/U6 snRNP, and accumulates a novel U2/U6 snRNP. NMR analysis of wild-type and mutant U2/U6 RNA constructs reveals that either single mutation in U6 disrupts the wild-type three-helix junction structure, but the two mutations together restore and further stabilize the U2/U6 three-helix junction. We conclude that, in the presence of a stabilized U2/U6 snRNP, stable U4/U6 pairing is not essential for spliceosome assembly and function. Nevertheless, U4 snRNA is still required for cell viability, and a U4.U5.U6 tri-snRNP accumulates even in the absence of U4/U6 pairing. We propose that, in the triple-mutant strain, the U2 snRNP acts as a chaperone for assembly of the tri-snRNP, as has previously been suggested for the human U4/U6 snRNP.

30 A group II intron-like catalytic triplex in the U6 snRNA forms during spliceosome activation

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Catalysis of pre-mRNA splicing and group II intronic RNA self-splicing has been proposed to occur by a two-metal ion mechanism. The crystal structure of a group II self-splicing intron shows the catalytic domain V in a configuration where five non-bridging oxygens of the phosphate backbone coordinate two metal ions. We have recently provided evidence that the corresponding five residues in the spliceosomal U6 snRNA are bona fide metal ion ligands and one of these ligands - the bulged U80 in the U6 ISL - plays a direct catalytic role by binding metal ions that stabilize the leaving groups during both steps of splicing. In the group II intron, the metal binding configuration of domain V depends on formation of three RNA base-triple interactions that function during both steps of splicing, and it has been suggested that similar interactions may occur between within the U6 snRNA between the ACAGAGA, the AGC triad, and U80. Consistent with this prediction we found that mutations in the AGC triad are suppressed in vivo by mutations that restore the base-triple interactions. Furthermore, we show by in vitro molecular genetics that the U6 base triples function at the catalytic stage and are required for efficient exon ligation.

Unexpectedly, by site-directed cross-linking, we found that U6 base triples form before Prp2-dependent catalytic activation of the spliceosome and require the NTC complex, suggesting that the catalytic core forms already at the pre-catalytic stage. Preliminary evidence also suggests that Cwc2 promotes formation of the base triples, supporting previous proposals that Cwc2 may stabilize formation of catalytic structures in U6 by substituting in the spliceosome for RNA structures that in the group II intron induce the catalytic configuration of domain V.

Our findings indicate that the U6 snRNA adopts a group II intron-like tertiary conformation to catalyze pre-mRNA splicing, providing further evidence that the spliceosome and group II introns share common catalytic mechanisms and evolutionary origins.

31 3D Cryo-EM structure of the yeast activated spliceosome (Bact) and localisation of functionally important regions

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The spliceosome, which catalyses the removal of the intron from nuclear pre-mRNAs, assembles initially into a pre-catalytic ensemble, termed complex B, which contains the snRNPs U1, U2 and the U4/U6.U5 tri-snRNP and numerous non-snRNP proteins. For catalytic activation the spliceosome undergoes a major structural rearrangement; this yields the B^{act} complex and entails the dissociation of U1 and U4 RNPs and the formation of a new RNA–RNA interaction network involving U6, U2 and the pre-mRNA, which lies at the heart of the emerging catalytic centre. At the same time the Prp19 complex proteins (NTC) and ca. 10 NTC-related proteins are stably integrated into the B^{act} complex.

We have used single-particle electron cryomicroscopy to reconstruct the 3D structure of purified yeast B^{act} complexes at a resolution of 20–25 Å. Consistently with the dramatic change in biochemical composition that accompanies its formation, the structure of the B^{act} complex differs greatly from that of the pre-catalytic B complex. The reconstituted 3D structure of the B^{act} complex from *S. cerevisiae* has a mushroom-like appearance. The "mushroom cap" (the main body) consists of a relatively compact, prolate body about 37 nm long and 28 nm wide. From it, the "mushroom stalk" is seen to emerge as a slightly tapering, 15-nm-long protuberance. At the two ends of the main body large clefts and tunnels are seen, while its centre is clearly more solid. The protuberance is roughly perpendicular to the ellipsoid's main axis at its base, but is slightly curved.

We have begun to map the position of selected proteins such as Cwc2, Prp2, Prp19, Brr2 and Prp8 and some U2 proteins. These data provide initial insights into the location of the emerging catalytic centre and other functionally important sites in the B^{act} complex.

Finally, we have located certain U5- and U4/U6-specific proteins at the surface of purified pre-catalytic B complexes, which – in combination with our earlier electron-microscopic localisation studies of proteins in the purified U4/U6.U5 tri-snRNP (NSMB 2008; 15: 1206) – allows us to fit the tri-snRNP structure into the B complex at an unambiguously defined position.

32 A conformational switch in PRP8 mediates metal ion coordination that promotes pre-mRNA exon ligation

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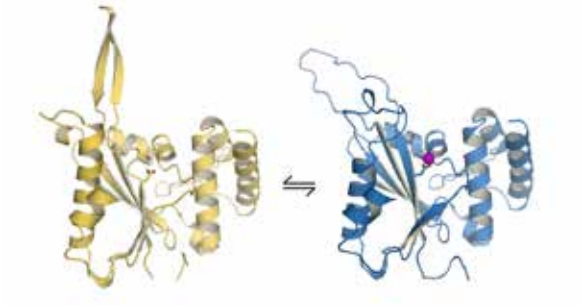
The spliceosome is a large and complex RNA-protein machine that removes introns during maturation of pre-mRNAs by a mechanism involving two sequential phosphotransfer reactions. A wealth of evidence shows that the spliceosome is a dynamic structure undergoing a series of conformational changes as it assembles on a pre-mRNA substrate, catalyzes two the two splicing reactions, and disassembles/recycles for subsequent rounds of catalysis.

The catalytic center of the splicing machinery includes an RNA structure comprised of the U2 and U6 spliceosomal snRNAs with divalent metal binding by U6 snRNA implicated in catalysis of both steps of splicing. A wealth of genetic and biochemical evidence places the protein PRP8 at the heart of the splicing machinery during spliceosome assembly through to catalysis, and suggests that it undergoes a conformational change between the two steps of splicing.

We have obtained structural and functional evidence that the RNase H domain of PRP8 undergoes a conformational change between the two transesterification steps of splicing rationalizing yeast *prp8* alleles that promote either the first or second step. We have further discovered that this conformational switch unmasks a metal-binding site involved in the second transesterification reaction (see Figure 1 below). Together these data establish that PRP8 is a metalloprotein that promotes exon ligation within the spliceosome.



33 Versatile reaction catalyzed by the Spliceosome in a competitive manner

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The spliceosome is a large and dynamic ribonucleoprotein complex, which undergoes components exchange and structural rearrangements throughout the entire splicing pathway. During catalytic steps, Prp16 is required to facilitate conformational change from step one to step two by destabilization of Yju2 and Cwc25, which are required for the first catalytic reaction. Despite of the energy requirement of such structural changes for the progression of the reaction, both steps of the splicing reaction can reverse on the purified spliceosome under appropriate conditions without having to replace splicing factors. In addition, the spliceosome can catalyze hydrolytic spliced-exon reopening (SER) reaction when arrested in step-two conformation, and debranching of lariat-intron-exon 2 (DBR) when arrested in step-one conformation. The SER reaction is in competition with the reverse reaction of step-two splicing (R2), whereas DBR is in competition with the reverse reaction of step-one splicing (R1). Recently we have found that changes in the N-terminal structure of Yju2 and Cwc25 biased the reaction toward R1 and strongly inhibited DBR. Cwc25 with epitope tagged at the N-terminus was destabilized from the spliceosome after the first catalytic reaction. Such spliceosomes could promote high efficiency of R1 and also promote the second reaction (F2) at a low level. Adding back Cwc25 inhibits the F2 reaction. These results indicate that the spliceosome can bypass the requirement of Prp16 and other step-two factors to promote the second reaction under the condition of Prp16-independent Cwc25 destabilization. The F2 reaction that occurs in the absence of step-two factors requires KCl and is inhibited by the presence of Cwc25, which instead promotes the debranching reaction. Our studies provide novel mechanistic insights into the molecular mechanism of the splicing reaction.

34 An unexpected role of the nuclear periphery for mRNA export in yeast

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The export of mRNAs from the nucleus to the cytoplasm is one of the many steps along the gene expression pathway and reflects only a short time period within the lifetime of an mRNA. However, mRNA export cannot be seen as an isolated process, as it has been functionally linked to different upstream and downstream processes, in particular the localization of the gene within the nucleus, transcription, mRNA processing and quality control. In the yeast *S cerevisiae*, many active genes are tethered to the nuclear periphery through interactions with the nuclear pore complex; the role of this perinuclear localization however is not fully understood. As many genes showing perinuclear localization are regulated by stress, NPC anchoring has been hypothesized to ensure fast and efficient export of newly synthetized RNAs under these conditions.

The NPC itself has to fulfill a dual function in mRNA transport, as it not only has to allow for fast export of mRNAs, but also ensure that only mature mRNPs are exported to the cytoplasm. Indeed, quality control steps have been suggested to occur at the NPC, such as the retention of unspliced pre-mRNAs. To better understand how the interconnection of gene localization, RNA processing and quality control affects the efficient export of mRNAs, we use single molecule resolution real-time microscopy to follow individual mRNAs from their site of transcription on their path towards to cytoplasm. We show that, different to current models, gene tethering to the periphery does not lead to the export of mRNAs through an adjacent nuclear pore complexes, as mRNAs are released into the nucleoplasm where they reach the nuclear periphery by diffusion within less then a second. Once reaching the nuclear periphery, they are not immediately exported but start scanning along the nuclear periphery, likely to find a nuclear pore that allows export. Interestingly, deleting specific components of the nuclear basket significantly reduces the time mRNPs scan the nuclear periphery and result in frequent release of mRNPs back into the nucleoplasm. This suggests that the nuclear basket not only acts as a landing platform for mRNPs at the nuclear pore but also has the capacity to maintain mRNAs at the periphery, possibly to allow sufficient time for quality control steps to occur without releasing the mRNPs back into the nucleoplasm.

35 NMD3 regulates mRNA nuclear export via an Xpo1-linked mechanism

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The RBP NMD3 (Upf3) has been implicated in mRNA Nonsense Mediated Decay and rRNA processing and export. Interestingly, we have also discovered that NMD3 RNAi generates a strong upregulation of a specific subset of transcripts. RNAseq transcriptome analysis of NMD3-depleted cells show that this effect is highly specific whereas reporter assays demonstrate that this effect is *UTR*-dependent and restricted to the mRNA level (no protein elevation occurs). ChIP analysis confirmed the transcript upregulation is not mediated by enhanced locus-specific transcription, nor is it a consequence of translational inhibition resulting from the effects of NMD3 depletion on rRNA maturation. Importantly, depletion of the 7SL RNA nuclear export factor, XPO1, recapitulates the effects of NMD3 depletion on specific mRNAs. Moreover, *in situ*hybridisation analyses demonstrate a bulk nuclear accumulation of all mRNAs in response to NMD3 depletion. This invokes a novel mRNA regulatory mechanism involving the NMD3- and XPO1-dependent nuclear export of mRNA cargos, with specific mRNA accumulation resulting from nuclear sequestration and hence protection from rapid cytoplasmic turnover. This novel function for NMD3 may be conserved in other systems.

36 RNA recognition and architectural activity of Zipcode Binding Protein 1

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IGF2 mRNA binding protein 1 (IMP1)/Zipcode binding protein 1 (ZBP1) is an oncofetal protein expressed at very low level in most adult tissues. ZBP1 is important for cell motility and its expression in cancer cells has been linked to tumor dissemination. ZBP1 is an RNA-binding protein that mediates the transport of a set of mRNAs from the nucleus to the cell periphery. It contains two RRM and 4 KH domains organised in three di-domain units. An important and well studied target of ZBP1 is the β-actin mRNA, and it has been shown that ZBP1 binds to β-actin mRNA in the nucleus and mediates its transport to the cell edge in a translationally repressed state. Here ZBP1 is phosphorylated by Src and the mRNA is released and translated. ZBP1-mediated transport takes place in large protein-RNA particles and the structure of the KH3 and KH4 domains, the key domains for the recognition of the β-actin RNA, suggest that ZBP1 may play an architectural role. We have solved the high resolution structure of ZBP1 KH3-KH4 in complex with the β-actin RNA target. We discuss a novel mode of KH-RNA binding and a mechanistic model for ZBP1 recruitment and ZBP1 architectural activity.

37 Identification and analysis of Staufen2 target RNAs from rat brain

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In neurons, RNA-binding proteins (RBPs) play an important role in directing RNA translation to subregions of the cell, including synapses. This process is known to critically contribute to synaptic plasticity. The double-stranded RBP, Staufen2 (Stau2), has been implicated in cell fate determination as well as in dendritic RNA localization and synaptic plasticity in mammalian neurons.

In order to better elucidate the role of Stau2 in neurons and its contribution to RNA localization, we have identified Stau2 target RNAs in the rat brain by immunoprecipitation of endogenous soluble Stau2 particles. Many of the Stau2 target RNAs encode functionally related proteins involved in G-protein-coupled receptor (GPCR) and small GTPase-mediated signaling pathways. Additionally, 77% of the Stau2 targets that are expressed in the hippocampus overlap with a new dataset of neuronal process-localized mRNAs (Cajigas *et al.*, Neuron, 2012) indicating most Stau2 RNPs can localize away from the cell body. Interestingly, the activity of extracellular signal-regulated kinases-1 and -2 (ERK1/2) is reduced upon Stau2 knockdown, suggesting Stau2 affects intracellular signaling pathways in the brain. Furthermore, we present evidence that Stau2 stabilizes the expression of one of the GPCR pathway components, the Regulator of G-protein Signaling 4 (*Rgs4*) mRNA. This effect is mediated via the *Rgs4* 3'-UTR since downregulation of Stau2 in neurons leads to reduced expression of a luciferase reporter. We hypothesize that the regulation of ERK1/2 and Rgs4 may underlie the involvement of Stau2 in dendritic spine morphogenesis and synaptic plasticity. Taken together, our data provide new insight into the role of Stau2 in neurons.

38 The order of assembly and disassembly of nuclear ASH1-mRNPs

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mRNA localization is a universal feature in eukaryotes, requiring the assembly of motor-dependent transport particles. The cytoplasmic transport of mRNAs is preceded by the nuclear assembly of pre-mRNPs. A transit of such nuclear pre-mRNPs through the nucleolus has been reported. One example is the *ASH1* mRNA, where the nucleolar protein Loc1p is required for efficient cytoplasmic *ASH1* mRNA localization, most likely by remodeling the nuclear mRNP. Although immunoprecipitation experiments already revealed that Loc1p forms co-complexes with other nuclear components of the transport complex, it remains unclear how Loc1p is recruited into this mRNP. Because genomic deletion of *LOC1* also results in impaired ribosome biogenesis, it has been suggested that impaired *ASH1* mRNA localization might be only an indirect effect.

Here we show that Loc1p interacts directly and specifically with the *ASH1* mRNA-binding protein She2p, indicating a direct role of Loc1p in mRNA localization. Our data suggest that the Loc1p interaction with She2p and RNA results in the formation of a stable ternary complex, in which both proteins directly contact the RNA. A second nucleolar factor co-immunoprecipitated with She2p and *ASH1* mRNA is Puf6p. We do not find a direct interaction of Puf6p with Loc1p and She2p, suggesting that these factors do not form a joint protein complex. After nuclear export, myosin-bound She3p joins the complex of ASH1 mRNA, She2p, and Puf6p. Since Loc1p remains in the nucleus, it must be removed from the complex prior or during export. Our competition experiments show that She3p is able to displace Loc1p from the *ASH1* complex. Together these findings imply an ordered process of nuclear assembly and disassembly, and suggest that Loc1p and She3p encounter each other in the vicinity of the nuclear pore. Furthermore our data indicate that the mutual recruitment of Loc1p and She2p to the *ASH1* mRNA enables She2p to escort the *ASH1* mRNA through the nucle(ol)us. It confirms an integral role of Loc1p in *ASH1* mRNP biogenesis.

39 Novel players and novel mRNAs transported by the Bic-D / Egl / Dynein RNA localization machinery

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Many key cellular processes in eukaryotes depend on mRNA localization. In *Drosophila*, the mRNA transport machinery consisting of Bic-D, Egl and the dynein motor is used at many different developmental stages and in many cell types to localize key mRNAs required for oocyte differentiation, embryonic axis formation and patterning. Through the dynein / dynactin motor the mRNA cargo is moved on microtubules to distinct cellular compartments. While Egl links these motors and Bic-D with mRNA cargo, it is unclear, which additional proteins are required to confer mRNA target specificity and translational control during the transport. Using immunopurification of Bic-D and Egl complexes we have identified several novel components of the Bic-D / Egl transport machinery. Amongst them are the poly(A) binding protein (Pabp) and the insulin growth factor mRNA binding protein (Imp). Through a polyA independent activity, Pabp localizes *osk* mRNA in the female germ line, while *Drosophila* Imp is found to co-localize with the Bic-D/*hairy* mRNA–RNP complexes in embryos. Despite this, our functional analysis revealed that *imp* plays at most a redundant role in localizing this mRNA. Instead, it seems to function in repressing translation of *hairy* mRNA during transport. Two additional proteins were shown to interact with Bic-D and novel findings regarding them will be presented at the meeting. Encouraged by the success of the isolation of protein partners, we also screened for novel mRNAs present in complexes with Bic-D and Egl. We immunoprecipitated these proteins, purified the bound mRNAs and quantified the transcripts using high-throughput cDNA sequencing. The *in situ* localization patterns of the IP-enriched mRNAs, the presence of the known targets of the Bic-D localization machinery in these complexes and the dependence of their localization patterns on *Bic-D* revealed a rewarding success rate for this approach. Such targets and their analyses will be presented in Davos, too.

40 An RNA biosensor for imaging translation of single mRNAs in living cells.

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After transcription, an mRNA's fate is determined by an orchestrated series of events that is regulated both temporally and spatially within the cell. The regulation of translation is crucial for control of gene expression. While measurements of ribosome occupancy of mRNAs have provided a genome-wide view of translation, the cellular context has been lost. Fluorescent microscopy can complement these global approaches because it allows interrogation of gene expression with single-molecule resolution in intact cells. We have developed an RNA biosensor that allows individual untranslated mRNAs to be distinguished from mRNAs that have already been translated in living cells.

Most approaches for imaging translation have relied on either detecting the newly synthesized polypeptide or identifying actively translating ribosomes within the cell, which are inherently limited by low signal-to-noise. We have chosen to consider translation from the perspective of the mRNA because imaging single-molecules of RNA has been well established. We have utilized the orthogonal MS2 and PP7 RNA-protein complexes to label an mRNA in the coding sequence (PP7-GFP) and 3′ UTR (MS2-RFP) with spectrally distinct fluorescent proteins. When this mRNA is untranslated it will be labeled with both fluorescent proteins, however, once the mRNA encounters the ribosome for the first time, the PP7-GFP signal will be displaced from the transcript resulting in only MS2-RFP labeled mRNAs. This simple strategy allows untranslated mRNAs (yellow) to be readily distinguished from translated ones (red). We refer to this technique as translating mRNA imaging by coat protein knock-off (TRICK).

The TRICK assay has been used to characterize the translation of a reporter mRNA in U2 O-S cells. We find no evidence for translation of the reporter mRNA in the nucleus. In the cytoplasm, however, almost every mRNA has been translated at least once. By combining fixed and live cell measurements, our data suggests that the average mRNA takes approximately two minutes once exported to the cytoplasm to be bound, scanned and have translation initiated by the ribosome.

41 Single molecule systems biology of RNA silencing

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Imagine we could use eukaryotic cells as reaction vessels in which we directly "see" physiologically low numbers of RNA molecules that individually go about their biological functions within their natural "habitat", under the constant influence of molecular crowding amongst all known and unknown interaction partners. This arguably ultimate goal of a quantitative biology for the 21st century has only just come within reach, opening entirely new avenues towards understanding cellular life and disease – after all, "seeing is believing". To realize this vision, we recently developed iSHiRLoC, or intracellular Single molecule, High-Resolution Localization andCounting, as an innovative probe concept optimized for detecting single, biologically functional RNA molecules inside live human cells. For iSHiRLoC, we microinject an RNA of interest, inconspicuously labeled with a single fluorophore, into cultured human cells. Microinjection allows us to keep the number of molecules low so as not to overwhelm the cellular RNA processing machinery, while enabling intracellular single RNA molecule detection during low-background highly inclined laminar optical sheet (HILO) microscopy. At defined time points after microinjection, we then perform one of two experiments – single particle tracking in the live cell or, upon formaldehyde fixation, counting of the number of photobleaching steps per particle as a fingerprint of RNA assembly – giving us the unique opportunity to directly watch and quantify the dynamics of intracellular RNA pathways. In its first successful implementation, we applied iSHiRLoC to gene regulation by microRNAs (miRNAs)1, key effectors of the evolutionarily conserved RNA silencing pathway, with profound significance for human health as a master regulator of gene expression. We showed that microinjected, 3' fluorophore labeled microRNAs retain full functionality in RNA silencing and display two kinetically distinct assembly pathways that we propose are sequential translational inhibition and degradation of the targeted messenger RNAs (mRNAs). We have recently extended iSHiRLoC to two colors to co-track and count pairwise combinations of miRNAs and their target mRNA and protein interaction partners. To further interpret these data, we are performing systems biology modeling through Monte-Carlo simulations in the molecular environment of the cell, using our experimental iSHiRLoC data as input to predict the cellular output. To deeply integrate our experimental and computational approaches into the envisioned Single Molecule Systems Biology, we experimentally measure the extent of gene repression by miRNAs, then compare the computational prediction with experimental reality iteratively to derive a fully converged mechanistic description of RNA silencing.

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42 GRSF1 regulates RNA processing in mitochondrial RNA granules

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RNA molecules sometimes assemble into macromolecular structures called RNA granules. These large ribonucleoparticles, known as "stress granules", "P-bodies" or "neuronal RNA granules" have many RNA-related functions such RNA storage, protection, degradation or transport.

We will describe the existence of RNA granules within mitochondria[1]. These mitochondrial RNA granules contain nascent RNA molecules together with specifically associated proteins including the G-rich RNA sequence binding factor 1 (GRSF1), the Fas-Activated Serine/Threonine kinase (FASTK), RNAse P, and the helicase hSuv3p. We found that GRSF1 is required for the processing of long polycistronic mitochondrial transcripts into mature mRNAs, tRNAs and rRNAs, which is an essential aspect of mitochondrial gene expression that remains poorly understood. Impairment of RNA processing delays the release of newly synthesized RNA from mitochondrial RNA granules, indicating that these structures may correspond to factories where primary RNA transcripts converge and are processed before release.

Based on these results, we hypothesize that mitochondrial RNA granules are involved in several aspects of post-transcriptional regulation of mitochondrial gene expression and that dysfunction of key components of these RNA granules may lead to severe pathologies.

1. *Alexis A. Jourdain, Mirko Koppen, Mateusz Wydro, Chris D. Rodley, Robert N. Lightowlers, Zofia M. Chrzanowska-Lightowlers, Jean-Claude Martinou. GRSF1 regulates RNA processing in mitochondrial RNA granules. Cell Metabolism. In press.*

43 Virus-Induced Dysregulation of Cellular mRNA Decay and Alternative Polyadenylaton – Implications for Pathogenesis

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RNA viruses must successfully interface with the cellular RNA decay machinery in order to maintain the stability of their transcripts and promote a productive infection. We have determined that alphaviruses interact with the cellular HuR protein through high affinity binding sites in their 3' UTR to stabilize viral transcripts. Furthermore, alphaviruses cause a redistribution of the cellular HuR protein from the nucleus to the cytoplasm during infection. Transfection studies have revealed that this is due to the abundant cytoplasmic viral transcripts acting as molecular sponges for the protein. This decrease of HuR protein in the nucleus causes alterations in patterns of alternative polyadenylation in the cell. To our knowledge this is the first time that a cytoplasmic RNA virus has been shown to influence patterns of nuclear poly(A) site choice. The sequestration of HuR protein by alphaviruses also dramatically reduces the stability of cellular mRNAs that normally depend on the protein for regulated stability or to prevent miRNA targeting. Many of these mRNAs encode mediators of innate immune functions, cytokines or apoptosis regulators that can directly impact on the outcome of a virus infection. A similar story is also evolving from our flavivirus studies. All arthropod-borne flaviviruses produce a small non-coding sfRNA from their 3' UTR as a decay intermediate from Xrn1 exoribonuclease digestion. Xrn1 remains associated with sfRNA and becomes functionally repressed during infection. This effectively shuts down a major pathway of cellular mRNA decay during flavivirus infection, resulting in stability of viral transcripts and significant dysregulation of the stability of cellular mRNAs. Thus we believe that disruption of regulated mRNA stability during infections with either alphaviruses or flaviviruses likely plays an important and underappreciated role in cytopathology and viral pathogenesis.

44 A dengue virus 2 non-coding RNA downregulates translation of antiviral interferon-stimulated mRNAs through interaction with host RNA binding proteins.

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Flaviviruses, as other viruses, has evolved powerful strategies to counteract the antiviral action of type I interferon (IFN). In particular, it was recently shown that a short, abundant, highly structured non-coding RNA termed sfRNA was required for evasion of the IFN response. Although the mechanism of action of the sfRNA remains to be elucidated, several non-coding RNAs of viral origin have been proposed to act as sponges for host RNA-binding proteins (RBPs) to modulate host cell function. To gain insight in the role of sfRNA we used dengue virus 2 (DENV-2) sfRNA, whose interactome was recently characterized in our laboratory, as a model. We found that DENV-2 sfRNA specifically downregulated translation of IFN-stimulated genes (ISGs) mRNAs, interfering with the establishment of the antiviral state. DENV-2 sfRNA was able to interact with host RBPs G3BP1, G3BP2 and CAPRIN1, which we showed were required for antiviral action of IFNs. Strikingly, this activity was independent from their previously described role in cellular stress response but they were required for translation of ISG mRNAs. Finally, we established that the ability of DENV-2 sfRNA to decrease ISG induction was conditional on G3BP1, G3BP2 and CAPRIN1 binding, suggesting that the sfRNA sequesters the host RBPs to counteract their activity. Taken together, this study identifies G3BP1, G3BP2 and CAPRIN1 as novel cellular regulators of the IFN response, highlight translation as a previously underappreciated, important layer of regulation of immune responses and provide the first mechanism of action for DENV-2 sfRNA.

45 RNA/RNA interactions govern selective packaging of influenza A genomic segments

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The genome of influenza A viruses (IAV) is split into eight viral RNAs (vRNAs) that are encapsidated as viral ribonucleoproteins (vRNPs). The existence of a segment-specific packaging mechanism is well established, but the molecular basis of this mechanism remains to be deciphered. Selective packaging could be mediated by direct interactions between the vRNA packaging regions, but such interactions have never been demonstrated in virions. We showed that the eight vRNAs of a human H3N2 IAV (MO) form a single interaction network *in vitro* that involves regions of the vRNAs known to contain packaging signals in the most studied human H1N1 IAV strains. When we applied the same strategy to an avian H5N2 IAV (EN), we observed that the eight EN vRNAs of ENG also form a single network of interactions *in vitro*, but the interactions and the regions of the vRNAs they involve differed compared to the MO virus. Electron tomography also revealed significant differences in the interactions between vRNPs in ENG and MO viral particles. All together, these data suggest that the packaging signals are not conserved between IAVs. We identified at the nucleotide level the sequences involved in several interactions, and in a few cases, we confirmed the interactions using silent compensatory mutations in the interacting sequences. Then, we focused on the interaction between the PB1 and NS EN segments, which is inhibited *in vitro* by silent mutations in either vRNA, and restored when combining the two mutated segments. Using reverse genetics, we produced the wild type, the two single mutants, and the double mutant viruses. Quantification of the vRNAs by Q-RT-PCR and observation of the viral particles by electron microscopy indicated that this interaction also exists in infected cells and/or in viral particles and revealed a global defect in vRNA packaging in single mutant viruses. In addition, competition experiments between wild type and mutant vRNAs for packaging supported a role of this interaction in the selective packaging of these genomic RNA segments. To our knowledge, these results constitute the first direct evidence of an interaction between two vRNAs involved in the selective packaging of IAV vRNPs.

46 Interplay between retroviral genomic RNA packaging and mRNA translation

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During retroviral RNA encapsidation, two full-length genomic (g) RNAs are selectively incorporated into assembling virions. Packaging involves a cis-acting packaging element (Ψ) within the 5′ untranslated region of unspliced HIV-1 RNA genome. However, the mechanism(s) that selects and limits gRNAs for packaging remains uncertain. Using a dual complementation system involving bipartite HIV-1 gRNA, we observed that gRNA packaging is additionally dependent on a cis-acting RNA element, the genomic RNA packaging enhancer (GRPE), found within the gag p1-p6 domain and overlapping the Gag-Pol ribosomal frameshift signal (1). Deleting or disrupting the structure of the two conserved GRPE stem loops diminished gRNA packaging and infectivity >50-fold, while deleting gag sequences between Ψ and GRPE had no effect. Our studies allowed us to conclude that only the HIV-1 RNAs employed for Gag-Pol translation may be specifically selected for encapsidation, possibly explaining the limitation of two gRNAs per virion. The basis for this mechanism will be presented.

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47 HIV1, Antisense RNA and ADAR editing

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The HIV1 genome consists of a 9kB “single stranded” mRNA. Structure analysis using SHAPE and other methods have shown a complex HIV1 genome secondary structure with several regions that are potential targets for ADAR editing. It has previously been reported that HIV1 can be edited by ADAR1, as well as by ADAR2. Several recent studies have also demonstrated the presence of natural antisense HIV1 RNA. We have studied HIV1 inhibition mediated by a therapeutic 937nt long antisense (AS) HIV1 RNA as a model system for the potential effects of HIV1 AS RNA . We have previously shown that the surprisingly potent inhibition that is observed with this therapeutic AS RNA, requires that it traffics on the CRM1 export pathway through Rev and the HIV1-RRE. If the RNA is redirected to the NXF1 pathway (using RevM10Tap), efficient AS inhibition is abolished. To further investigate the mechanism, we sequenced the AS target region in individual RT-PCR clones from cells expressing HIV1 +/- RRE-AS RNA. When the RRE-AS RNA was present, we observed several A to G changes, consistent with ADAR editing. However, in most clones, only a few residues were altered, with only 1-8 percent of the A residues modified, rather than the 50% that would be expected in a long perfect sense/antisense RNA region. The changes were present throughout the AS target region, with different A residues changed in the different clones. These results suggest that the RNA had been subject to regulated ADAR editing. In the absence of AS RNA, or when the AS RNA was redirected to the NXF1 pathway, most clones contained zero or one modified A, demonstrating that the regulation was specific for the Rev/RRE pathway.

When ADAR2 was over-expressed, AS inhibition was significantly relieved and even less inhibition was seen with a cytoplasmic mutant of ADAR2 (with aa 1-72 deleted). RT-PCR analysis of the AS target region did not show any increase in editing when ADAR2 was expressed. However, catalytic and ds-RNA binding domain mutants of ADAR2 failed to relieve inhibition, indicating a role for editing. These results suggest a complex role for ADAR in the regulation of AS inhibition at the cytoplasmic level. Interestingly, expression of ADAR2 led to significant editing in other regions of the HIV1 genome with or without the therapeutic AS-RNA (in TAR and the RRE). Taken together, our results indicate that HIV1 is regulated by ADAR at many levels. Editing of specific residues in TAR and the RRE is likely to be involved in post-transcriptional RNA regulation of HIV1, whereas expression of natural HIV1 AS RNA could serve to regulate HIV1 at the translational level, potentially through miRNAs generated from ADAR edited AS RNA. Most intriguingly, natural AS RNA expression and regulated ADAR editing may also be a strategy used by the virus to increase the rate of HIV1 evolution to help the virus evade the immune system (in conjunction with editing by ApoBec3G regulated by the viral Vif protein).

48 The Identification of a novel posttranscriptional regulatory element in gamma retroviruses

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Posttranscriptional regulatory mechanisms of several simple retroviruses and retroelements have been elucidated with the only exception being the gamma retrovirus family, e.g. MuLV. We identified a novel ~1500 nt PostTranscriptional regulatory Element (PTE) located in *pro-pol* (nt 3184-4652) of MuLV and XMRV, being transferrable and promoting potent expression of an HIV *gag* reporter and the cat DM128 reporter transcript. Deletion of *pro-pol* and *env* from the provirus failed to produce Gag particles, whereas *env* deletion produced Gag particles, pointing to a critical role of the *pro-pol* sequence. We showed that the *pro-pol*sequences could be replaced by heterologous RNA export elements e.g. CTE. We found that the RNA encoding γ-?retroviral *gag* only has a reduced half-life and this defect can be counteracted upon insertion of a *pro-pol* or CTE elements acting in cis. SHAPE technology was applied to the *pro-pol* RNA, with particular emphasis on the PTE, which revealed a very complex stem-loop structure. Comparisons of *pro-pol* sequences from MuLV, XMRV and related γ-retroviruses showed that despite sequence divergence in the 3' end of pol, their RNA structures show little variability, indicating that the RNA structure is invariant in PTE. We conclude that complex structural components are critical for function, in addition to sequences representing interaction sites for export factors. With our discovery of the PTE, we now have a complete picture of posttranscriptional regulation of simple retroviruses. Collectively, these viruses contain negative-acting sequences embedded in *gag* mediating RNA instability, hence impair expression. These viruses also contain positive-acting RNA elements, essential for promoting Gag expression. These RNA elements do not share sequence or structural features, their location within the full-length retroviral RNA can vary, but they depend on the cellular NXF1 export machinery.

49 Shunting revisited.

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Viruses must manage their genome sustainably and efficient. As a consequence they developed ways to control their replication, transcription, splicing, and translation. For efficient translation, viruses use various types of polycistronic translation, alternative translation starts and stops, internal ribosome entries and bypass of sequences by shunting.

During shunting caulimovirus leaders of about 600nt are bypassed by the scanning ribosome, as originally observed with Cauliflower mosaic virus (CaMV). The CaMV leader is highly structured and contains several small open reading frames. Of importance is just the first one, which ends six to nine nucleotides in front of the root of a strong stem structure. After translation of this sORF the ribosome is not dissociated but jumps across the base of the stem structure, continues scanning and reinitiates at the main ORF. The positioning of the sORF and the strength of the stem-structure, but not their primary sequences, are important for successful shunting. Interestingly, this shunt structure is preserved in the in the six genera of plant pararetroviruses (caulimoviridae). One of these, Rice tungro bacilliform virus (RTBV), infects rice plants together with the Rice tungro spherical (Waika)virus (RTSV), a RNA virus. Interestingly, not only RTBV, but also the accompanying RTSV have a related shunt structure. It is an interesting question whether this double occurrence is coincidence or of mutual benefit for both of the viruses.

It seems puzzling that the long leader, which seemingly constitutes a basic hindrance for translation, has evolved. One reason might be that it harbours a series of signals, i.e. for enhancement, polyadenylation, splicing, packaging, but those signals could cope with much less space. Recently, we found another function. The leader spawns huge amounts of siRNAs of all size classes. They make up half of the total load of sRNAs present in the infected plant. The coding region of the CaMV genome is represented by a much lower amount of siRNAs.

The RNA-form of the CaMV genome is reverse transcribed within viral particles to yield open circular dsDNA. Since repair- and ligase enzymes are missing within particles and cytoplasm, the termini of the DNA strands are not joined, until the DNA enters the nucleus. The DNA minus strand is just interrupted between leader and coding region and if transcription is initiated before repair and ligation, the RNA falls off and is consequently not polyadenylated. This aberrant ("8S") RNA, which covers just the leader gives rise to the production of dsRNA, the substrate for dicers and consequently the source of the massive amounts of leader derived siRNAs.

Due to its strong secondary structure the CaMV leader itself is a poor substrate for the set of leader-derived siRNAs, rsp. RISC complexes and we argue that this set acts as decoy out-titrating the AGO proteins and thereby protecting the CaMV transcripts.

50 Inquiry into the variability of HCV IRES and its impact on function by developing and evaluation of a large-scale mutation database that also unfolds potential of some new nucleotides.

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Synthesis of the hepatitis C virus polyprotein is fully controlled by an IRES located within 5'UTR of the viral RNA. We developed a new flow-cytometry-based approach allowing us to monitor patient to patient differences in IRES activities of their whole viral populations. Simultaneously we employed more conventional methods comprising cloning of PCR fragments, DGGE, TGGE, sequencing and bicistronic reporter assay for finding new HCV IRES mutations and measuring their activities. Sequence data from patients' samples along with analysis of migration patterns by DGGE and/or TGGE allowed us to estimate the variation that may have persisted over time within one or group of patients individually and collectively. We also compiled from the literature a comprehensive database comprising ~1300 IRES mutations further categorized by frequency, structural and functional behavior, experimental parameters, clinical data etc. Some IRESs bearing multiple mutations in various domains displayed either higher or lower levels of efficacy than expected after simple counting of known effects of the individual mutations. These differences in IRES activities raise the question of how some mutations are capable of sustaining an IRES WT activity. Could it be a long-range interaction between mutated nucleotides, and if yes, then how much is the extent of structure adaptability that IRES may need to go through for conservation of its function. We also collected data from published studies dealing with induced therapeutic response of interferon and ribavirin and its possible outcome in individuals with reference to occurrence of mutations in HCV IRES. By mapping out these mutations in sustained and non-sustained responders we observed no specific distribution that may conclude a direct impact in determining the therapy response between the two populations. We also illustrate impact of ~25 novel mutations found in our patients on the HCV function.

51 Novel Insights from Structural Analysis of Lentiviral and Gammaretroviral Reverse Transcriptases in Complex with RNA/DNA Hybrids.

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Structures of HIV-1 reverse transcriptase (RT) have been reported in several forms, but only one contains an RNA/DNA hybrid, the conformation of which has been controversial. We have been successful in obtaining three structures of HIV-1 RT complexed with a non-nucleoside RT inhibitor (NNRTI) and an RNA/DNA hybrid[1]. In the presence of an NNRTI, our RNA/DNA structure differs from all prior nucleic acid bound to RT including the previously-reported RNA/DNA hybrid derived froom the polypurine tract. The enzyme structure observed in our cocrystals also differs from all previous RT-DNA complexes. As a result, the hybrid has ready access to the ribonuclease H (RNase H) active site. These observations collectively reinforce previous proposals that an RT-nucleic acid complex may be required to adopt independent structural states competent for DNA synthesis and the other for RNA degradation. RT mutations that confer drug resistance but are distant from the inhibitor-binding sites map to the unique RT-hybrid interface that undergoes conformational changes between two catalytic states. Structural features of the nucleoprotein complex, including drug resistance mutations, have been verified by site-directed mutagenesis, and will be presented.

Although the single-subunit RT of Moloney murine leukemia virus (Mo-MLV) has been extensively characterized biochemically, structural information is lacking that describes the substrate binding mechanism for this RT species. We also present data on the first crystal structure of a complex between an RNA/DNA hybrid and the 72 kDa single-subunit RT from the related xenotropic murine leukemia virus-related virus (XMRV)[2]. A comparison of this structure with its HIV-1 counterpart shows that substrate binding around the DNA polymerase active site is conserved but differs between the two enzymes in their thumb and connection subdomains. Small-angle X-ray scattering (SAXS) was used to model full-length XMRV RT, demonstrating its flexible RNase H domain becomes ordered in the presence of substrate, a key difference between monomeric and dimeric RTs.

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52 RNAi dependent and independent control of LINE1 mobility and accumulation in mouse ES cells

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In most mouse tissues, long-interspersed elements-1 (L1s) are silenced *via* methylation of their 5'-untranslated regions (5'-UTR). A gradual loss-of-methylation in pre-implantation embryos coincides with L1 retrotransposition in blastocysts, generating potentially harmful mutations. Here, we show that Dicer- and Ago2-dependent RNAi restricts L1 accumulation and retrotransposition in undifferentiated embryonic stem cells (mESCs), derived from blastocysts. RNAi correlates with production of Dicer-dependent 22-nt small RNAs mapping overlapping sense/antisense transcripts produced from the L1 5'-UTR. However, RNA-surveillance pathways simultaneously degrade these transcripts, consequently confounding the anti-L1 RNAi response. In *Dicer-/-* mESC complementation experiments, L1 silencing was rescued in cells strongly depleted of microRNAs, which proliferated and differentiated normally unlike their non-complemented counterparts. These results shed new light on L1 biology and, possibly, on the differentiation defects of *Dicer-/-* mESCs.

53 Role of Telomeric Repeat-containing RNA in Alternative Lengthening of Telomeres

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The large majority of cancer cells maintain their telomeres indefinitely by re-activation of the specialized reverse transcriptase telomerase. However, certain tumors especially osteosarcomas, gastric and bladder carcinomas, and cancers of the central nervous system bypass telomerase re-activation and maintain telomere length by the so called 'alternative lengthening of telomeres' (ALT) pathway, which relies on homologous recombination between telomeric sequences. Recent studies have revealed that ALT cells can also originate from telomerase-positive cancers treated with telomerase inhibitors. Despite the relevance of ALT in cancer biology, the molecular mechanisms behind ALT activation and progression remain poorly defined. A few years ago we demonstrated that human telomeres are transcribed into long non-coding nuclear transcripts ranging in size between approximately 500 and more than 9000 bases and named 'telomeric repeat-containing RNA' (TERRA). We show here that ALT cells are characterized by extremely elevated rates of TERRA transcription as compared to telomerase positive cancer cells and primary fibroblasts. TERRA co-localize with telomeres and PML within ALT-associated PML bodies (APBs), which are thought to be the nuclear compartments where telomeric homologous recombination takes place. Strikingly, we also find that ALT cells, but not telomerase positive cancer cells and primary fibroblasts, express shorter telomeric transcripts comprised between 300 and 36 bases (small TERRRA: smTERRA). Down-regulation of Dicer leads to a decrease in smTERRA cellular levels and concomitant accumulation of longer transcripts. On the contrary, depletion of Drosha does not affect smTERRA levels, suggesting a non-canonical role for Dicer in processing TERRA into short RNA species. Our data reveal an unexpected scenario where different TERRA species might support ALT establishment and/or maintenance, and should pave the way towards the development of therapies to specifically cure or prevent ALT tumors.

54 RNA and DNA Targeting CRISPR-Cas Immune Systems of Pyrococcus furiosus

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CRISPR-Cas systems are recently discovered, small RNA-based immune systems that control invasions of viruses and plasmids in archaea and bacteria. Prokaryotes with CRISPR-Cas immune systems capture short invader sequences within the CRISPR loci in their genomes, and small RNAs produced from the CRISPR loci (CRISPR (cr)RNAs) guide Cas proteins to recognize and degrade (or otherwise silence) the invading nucleic acids. There are multiple variations of the pathway found among prokaryotes, each mediated by largely distinct components and mechanisms that we are only beginning to delineate.

Using the hyperthermophile *Pyrococcus furiosus*, we have delineated several key steps in CRISPR-Cas invader defense pathways. *P. furiosus* has 7 transcriptionally active CRISPR loci distributed throughout the genome that together encode a total of ~200 crRNAs. The 27 Cas proteins in this organism represent 3 distinct pathways and are primarily encoded in two large gene clusters. The Cas6 protein "dices" large precursor CRISPR transcripts to generate the individual invader-targeting crRNAs. The mature crRNAs include a signature sequence element ("5' tag") derived from the CRISPR repeat that is important for function. crRNAs are tailored into distinct species and integrated into three distinct crRNA-Cas protein complexes that are likely immune effector complexes. The complex formed by the Cmr (type IIIB) system proteins cleaves complementary target RNAs and can be programmed to cleave novel target RNAs in a prokaryotic RNAi-like manner. The other two CRISPR-Cas systems in *P. furiosus*, Csa (type IA) and Cst (type IB), target invaders at the DNA level. Recent work will be presented on the structure and function of CRISPR-Cas immune effector complexes from this organism. The results of our work in *P. furiosus* have revealed mechanisms of crRNA biogenesis and silencing for 3 of the diverse CRISPR-Cas pathways, and reveal that organisms like *P. furiosus* possess an arsenal of multiple RNA-guided mechanisms to resist diverse invaders.

55 Involvement of TERT-RdRP in heterochromatin maintenance

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Constitutive expression of telomerase in human cells prevents the onset of senescence and crisis by maintaining telomere homeostasis. Recent evidence suggests that the human telomerase catalytic subunit (hTERT)contributes to cell physiology independent of its ability to elongate telomeres. We have recently reported that hTERT form an RNA dependent RNA polymerase (RdRP) and produce double-stranded RNAs that can be processed into small interfering RNA. These observations identify a mammalian RdRP composed of hTERT (Maida et al. Nature 2009).

In fission yeast, centromeric heterochromatin is maintained by an RNA-directed RNA polymerase complex (RDRC) and the RNA-induced transcriptional silencing (RITS) complex in a manner that depends on the RNAi pathway. In association with hTERC, the telomerase reverse transcriptase (TERT) forms telomerase and counteracts telomere attrition; however, TERT has also been implicated in the regulation of heterochromatin at locations distinct from telomeres. Here, we describe a complex comprising human TERT (hTERT), Brahma-related gene 1 (BRG1), and nucleostemin (NS) that contributes to heterochromatin maintenance at centromeres and transposons. Acting as an RNA dependent RNA polymerase (RdRP), this complex produced double stranded RNAs that were processed into small interfering RNAs targeted to heterochromatin regions. These small interfering RNAs promoted heterochromatin assembly and mitotic progression in a manner dependent on the RNA interference (RNAi) pathway. These observations implicate the hTERT/BRG1/NS (TBN) complex in heterochromatin assembly at particular sites in the mammalian genome.

56 The role of the Arabidopsis exosome complex in siRNA-independent silencing of heterochromatic loci

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To maintain genomic stability and prevent expansion of invasive genomic sequences such as transposable elements (TEs), eukaryotes have evolved defensive mechanisms to control them. Here, we examine the role of the*Arabidopsis* exosome complex in such mechanisms. Depletion of the exosome allows some repetitive sequences to escape from silencing. Most of these transcripts emanate from centromeric and pericentromeric chromosomal regions and other heterochromatic loci, and many derive from repetitive and transposable elements [1]. In plants, TEs are targeted for *de novo* DNA methylation by smRNA-mediated pathways. To investigate whether exosome interacts with RdDM, we profiled Arabidopsis smRNAs by deep sequencing in exosome and RdDM mutants and also analyzed RdDM-controlled loci. We found that exosome loss had a very minor effect on global smRNA populations, suggesting that, in contrast to fission yeast, in Arabidopsis the exosome does not control the spurious entry of RNAs into smRNA pathways. Exosome defects resulted in decreased histone H3K9 dimethylation at RdDM-controlled loci, without affecting smRNAs or DNA methylation. Exosome also exhibits a strong genetic interaction with RNA Pol V, but not Pol IV, and physically associates with transcripts produced from the scaffold RNAs generating region. We also show that two *Arabidopsis rrp6* homologues act in gene silencing [2]. Our data suggest that Arabidopsis exosome may act in parallel with RdDM in gene silencing, by epigenetic effects on chromatin structure, not through siRNAs or DNA methylation. We also propose that the *Arabidopsis* exosome may coordinate the transcriptional interplay of different RNA polymerases to modulate repression of some repetitive sequences. Results of further examination of exosome involvement in gene silencing in *Arabidopsis* will be presented.

1. Chekanova et al., (2007) A hidden component of the transcriptome and specialization of function of the exosome complex in *Arabidopsis*. *Cell*, 131(7), pp.1340-1353.
2. Shin et al., (2013) "The role of the *Arabidopsis* exosome in siRNA-independent silencing of heterochromatic loci". *PLoS Genetics*, accepted.

57 Involvement of the novel complex consisting of the splicing factor Prp14p/DHX38 RNA helicase and centromeric non-coding RNAs in the regulation of chromosome segregation

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prp14, of which the responsible gene encodes RNA helicase with the DEAH box motif, is one of the pre-mRNA splicing mutants in fission yeast *Schizosaccharomyces pombe*. In fission yeast, formation of heterochromatin at the centromere is induced by the RNAi-mediated system. Interestingly, *prp14* shows defective formation of the centromeric heterochromatin, resulting in a high incidence of lagging chromosomes during anaphase. *prp14* accumulates the unprocessed centromeric noncoding RNAs and shows decreased level of H3K9 methylation and Swi6p localization at the centromere, suggesting that the Prp14p helicase is involved in not only the splicing reaction, but also the RNAi-mediated heterochromatin assembly at the centromere. We found the mRNA-type intron in the *dg* non-coding RNA transcribed from the centromere region (Chinen et al, 2010). The IP analyses demonstrated that Prp14p interacts with Cid12p, a subunit of RDRC (RNA-directed RNA polymerase complex), implying that the spliceosome or sub-spliceosome assembled on the *dg* ncRNA intron works as a platform for recruitment of RDRC. To examine a role of the *dg* intron in the formation of centromeric heterochromatin, we constructed a minichromosome containing the centromere with or without the*dg* intron. Interestingly, the minichromosome without the *dg* intron showed the decreased H3K9 methylation comparing the minichromosome with the *dg* intron, supporting that the *dg* intron plays an important role in the methylation process of H3K9.

We also tested the involvement of the human homologue of Prp14p (DHX38) in the process of the chromosome segregation. Knockdown of DHX38 in HeLa cells caused the abnormal chromosome segregation, due to the defective attachment of microtubles to the kinetochores at metaphase. No significant defects were observed in splicing of tested pre-mRNAs in the knockdown cells. Interestingly, knockdown of satellite RNAs, non-coding RNAs transcribed from the human centromere, resulted in the similar defects in the chromosome segregation. In addition, the RIP assay showed that DHX38 associates physically with the satellite ncRNAs, suggesting that the DHX38/satellite ncRNA complex plays an essential role in the control of chromosomal segregation in mammalian cells.

58 A genome-wide RNAi screen draws a genetic framework for transposon control and primary piRNA biogenesis in Drosophila

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A large fraction of our genome consists of mobile genetic elements. Governing transposons in germ cells is critically important, and failure to do so compromises genome integrity, leading to sterility. In animals, the piRNA pathway is the key to transposon constraint, yet the precise molecular details of how piRNAs are formed and how the pathway represses mobile elements remain poorly understood. In an effort to identify general requirements for transposon control and novel components of the piRNA pathway, we carried out a genome-wide RNAi screen in Drosophila ovarian somatic sheet cells. We identified and validated 87 genes necessary for transposon silencing. Among these were several novel piRNA biogenesis factors. We also found a novel gene, which we named *asterix*, to be essential for transposon silencing, most likely by contributing to the effector step of transcriptional repression. Asterix loss leads to decreases in H3K9me3 marks on certain transposons but has no effect on piRNA levels.

59 New Approaches in RNA Chemical Biology

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Many types of oligonucleotide-based tools have advanced our knowledge of RNA biology, including microRNA (miRNA) mimics, LNAs and TaqMan probes. Other examples are functionalities employed for imaging RNAs, or identifying binding partners in cells. One of the limitations of RNA tools for chemical biology applications is their length: RNA synthesis using standard reagents is mostly limited to <80 nucleotides (nt). Beyond this, success is uncertain and characterization of the molecules by for example mass spec. is challenging. The length of pre-miRNAs typically spans 50-90 nt. MiRNA hairpin precursors are increasingly appreciated as functional RNAs with their own regulatory elements. For example, a variety of RNA binding proteins (RBPs) has been shown to bind to conserved sites in the terminal loop regions of miRNA precursors and regulate their biogenesis. We recently synthesized a library containing a subset of biotin-labeled pre-miRNAs mostly with phylogenetically-conserved terminal loop regions. We used it to screen for interactions with RBPs Lin28, hnRNP A1, KSRP and others, in their native state, i.e. using cell lysates. We showed that Lin28 as well as hnRNP A1 bind pre-let-7 family members with remarkable selectivity and that KSRP binds prominently to pre-miR-1-2 (1,2). Furthermore, we measured the affinities of these interactions in the presence of cellular factors. In a chemistry program, we used a biotin-labeled pre-miR-122 in a surface plasmon resonance assay to show that miravirsen, an LNA-based drug in clinical trials inhibits biogenesis of miR-122(submitted).

At the heart of these approaches are the chemical methods used to synthesize the RNAs. We have now developed protocols to label RNA of up to 75 nt with single and double functionality. Groups can be positioned so that they protrude into the major and minor grooves of RNA duplexes. A variety of groups with orthogonal functions have been examined. One particular combination of functions is that of biotin-psoralen, which we have used for cross-linking and deep sequencing experiments. Considerable experimentation was required to position groups so that the RNAs would be incorporated into functional RISC complexes. These RNAs were delivered into cells and under mild cross-linking conditions captured their RNA targets, including known and new targets.

1. Loughlin et al., Nat Struct Mol Biol. 2012, 22, 109; Towbin et al., Nucleic Acids Res 2013, 41, e47. Funding: SNF [CRSII3_127454; 205321_124720]

60 Resolving functional RNA dynamics by NMR

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The research focus in our group lies on the dynamic characterization of RNA by solution NMR spectroscopy. In the field of protein structural biology it is well established that biological function is often encoded in transiently populated conformational states, which are hard to characterize by conventional biochemical and biophysical experiments. Only recently the dynamic nature of ribonucleic acid was also recognized to be a crucial aspect in manifold functions carried out by RNA. For example, such dynamics are presumed to be of special importance in ligand recognition and/or in catalytic processes controlled by recently discovered noncoding RNAs.

For that purpose we develop sophisticated isotope labeling protocols for RNA relying on the site-specific introduction of ^{13}C-reporter spins by chemical and enzymatic methods. We then use state-of-the-art NMR experiments that exploit the particular features of the site-specific ^{13}C-modified RNAs to address functionally important dynamics occurring at the milli- to microsecond time frame. In detail, we used 6-^{13}C-modified pyrimidine labels to address the excited state structure of the HIV-1 TAR RNA further corroborating the conformational selection mechanism of this RNA to recognize the ligand argininamide. The approach is currently applied to RNAs with enzymatic function and biologically relevant RNAs involved in protein free gene regulation (i.e. riboswitches)

61 RNA-based fluorescent biosensors for live cell imaging of second messengers cyclic di-GMP and cyclic AMP-GMP.

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Cyclic di-nucleotides are a newly expanded class of signaling molecules in bacteria that regulate important processes including biofilm formation, motility, and virulence. Furthermore, cyclic di-nucleotides have been shown to trigger the mammalian innate immune response upon bacterial secretion of these molecules into the host cell cytosol. However, many of the components of cyclic di-nucleotide signaling remain poorly understood, so we aim to create tools for monitoring their role and regulation *in vivo*. We have designed fluorescent RNA biosensors for cyclic di-GMP and cyclic AMP-GMP through fusion of the Spinach aptamer to variants of a natural GEMM-I riboswitch. In live cell imaging experiments, these biosensors demonstrate fluorescence turn-on in response to cyclic di-nucleotides, and they were used to confirm *in vivo* production of the newly discovered natural molecule, cyclic AMP-GMP. Ongoing work is focused on applying these sensors to different cell types and on studying other phylogenetic variants of the GEMM-I riboswitch class for biosensor optimization. It is envisioned that these cyclic di-nucleotide biosensors will help elucidate how the different second messengers are integrated and transduced in bacterial and mammalian signaling.

62 Sequence Specific Modulation of G-Quadruplex Folding

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G-quadruplexes (G4) are highly stable non canonical structures that can be adopted by guanine rich nucleic acids. Over the past years, many important biological roles have been attributed to DNA as well as RNA G4. Hence, many research studies have focused on targeting G4 with chemical compounds that specifically bind these structures and either prevent or enhance their folding. Although these compounds are able to discriminate between G4 and other nucleic acid structures, they are not able to recognize one particular G4 sequence. Since there are more than 370 000 potential G4 sequences in the human genome, off-target effects remain a major issue. The aim of this project was to target specific G4 sequences with chemically modified RNA oligonucleotides. The specificity is obtained using Watson-Crick base pairing between the oligonucleotide and the targeted G4. To ensure that there would not be any off-target effects, we focused on G4 harboring a particular topology, namely a long loop 2. Oligonucleotides with specific chemical modifications (i.e. 2'O-methylated and LNA) were used for their high affinity and their great stability in human cells. By using *in line* probing, we showed that the oligonucleotides could modulate the folding of G4 either positively or negatively, depending on where the oligonucleotide is bound. This modulation was obtained on artificial G4 sequences as well as on sequences present in the 5' UTR of human genes. By inserting them in the 5'UTR of a luciferase reporter gene, we also demonstrated that long loop 2 G4 motif can decrease translation in human cells. We showed that this translation inhibition can be either increased or decreased by co-transfecting oligonucleotides with the luciferase gene. To our knowledge this is the first report of targeting a specific G4, and it also paves the way for a new kind of therapeutic tool that could modulate the expression of specific genes.

63 Structural stabilization of toxic CUG repeats reverses mis-splicing associated with myotonic dystrophy

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Myotonic dystrophy type 1 (DM1) is a dominant RNA gain of function disease caused by a trinucleotide repeat (CTG) expansion in the 3' untranslated region of the DMPK gene. When transcribed, these expanded CUG repeats sequester the muscleblind (MBNL) family of RNA binding proteins (MBNL1, MBNL2 and MBNL3). The MBNL proteins are regulators of alternative splicing, and sequestration to the CUG repeats results in the mis-splicing of MBNL target pre-mRNAs, leading to some of the disease symptoms.

Model CUG repeat RNA substrates and purified MBNL1 protein were used to determine how modifying the CUG repeats with pseudouridine and 2' O-methyl would affect the interaction between MBNL1 and the toxic RNA. A reduction or full elimination of MBNL1 binding to the CUG repeats was observed with modification. The higher number of modifications correlated with increased inhibition of protein binding to the RNA. Thermal melting studies demonstrated that pseudouridine and 2' O-methyl modifications significantly stabilized the CUG repeats in a helical conformation. Crystallographic and molecular dynamic simulations of CUG repeats containing pseudouridine suggest that helix stability is the result of a reduction in the dynamics of the U-U mismatch in the helical CUG repeats.

Cell and animal DM1 models were used to determine if modification of CUG repeats could reduce molecular and phenotypic consequences. In the DM1 cell model no mis-splicing defects were observed when CUG repeats containing 50% and 100% pseudouridylation were used. The RNA lacking modification resulted in the sequestration of MBNL proteins and mis-splicing in the cell model. Preliminary experiments in a DM1 zebrafish model showed that pseudouridylation of CUG repeats increased viability compared to unmodified RNA. Motor defects (spontaneous coiling and touch evoked escape) observed in the CUG repeat fish were reduced in the fish containing CUG repeats with pseudouridines. Our studies suggest that stabilization of toxic RNA with modifications or others means is a strategy that could lead to new therapeutic approaches for myotonic dystrophy and other diseases with toxic RNA components.

64 A chemo-enzymatic approach for selective modification of the RNA cap

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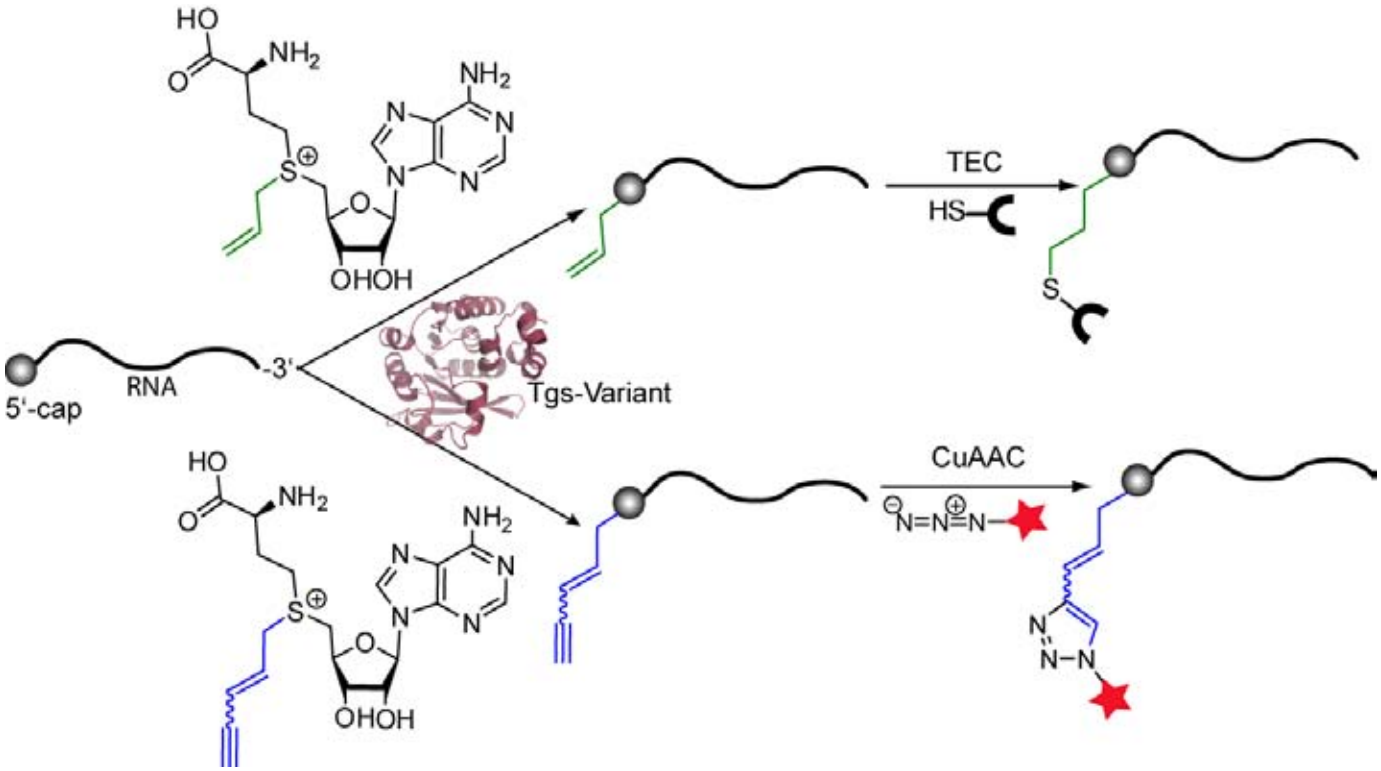
The 5'-cap is a hallmark of eukaryotic mRNAs and methods for selective modification of this particular biomolecule are highly sought in order to produce labeled capped RNA or to isolate mRNA from total RNA. Presently, there are no methods for selective covalent modification of natural cap-bearing RNAs, although these are the most important study object in terms of expression and also subcellular localization. We developed a two-step strategy, combining an enzymatic and a chemical conversion to achieve this goal. It is selective for capped RNAs and site-specific for the exocyclic N^2-position of the guanosine in the cap. We used enzymes that specifically recognize and hypermethylate the 5'-cap, so-called trimethylguanosinsynthases (Tgs), and engineered variants that successfully transfer alternative alkyl groups suitable for bioorthogonal chemistry. Although wildtype enzymes showed no or very low promiscuous activity on the AdoMet-analogs, we can now produce site-selectively modified caps with up to 91 % yield. We demonstrated further chemical modification by thiol-ene and copper-catalyzed click chemistry and introduced biotin as well as a fluorescent dye. Importantly, the chemo-enzymatic modification was also possible in cell lysate.

Potential applications of our approach include isolation of mRNAs from total RNA of eukaryotic cells for further use in RNA sequencing, which has become increasingly important to study gene expression in various cell types and states. Commonly, isolation proceeds via the 3'-end harboring a poly(A)-tail. However, the length of this poly(A)-tail itself is regulated and interesting to study. Our method might allow to isolate mRNAs via the 5'-end via a covalent or non-covalent interaction with the matrix.



65 Bromomethylcoumarins as selective reagents for RNA labeling

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Selective alkylation of RNA nucleotides is an important field of RNA biochemistry, e.g. in applications of fluorescent labeling or in structural probing experiments, yet detailed structure-function studies of the labeling agents are rare. Here, bromomethylcoumarins as reactive dyes for fluorescent labeling of RNA are developed as an attractive scaffold on which electronic properties can be modulated by variegated substituents. Using semi-quantitative LC-MS/MS analysis, the selectivity of various coumarin derivatives in labeling reaction with respect to the four major and a number of minor, i.e. modified nucleotides was assessed. This allowed the development of coumarins (i) as adaptor modules carrying an azide or alkyne function permitting further labeling by click chemistry; (ii) as photocrosslinking probes at uridine moieties, (iii) tagging the naturally occurring nucleotide modification 4-thiouridine with a selectivity of four orders of magnitude. This establishes bromomethylcoumarins as versatile reagents with a wide range of applications in RNA science.

66 Somatic spliceosomal factor mutations in bone marrow neoplasms lead to alterations in alternative splicing patterns that relate to the splicing mechanism

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Frequent somatic mutations of spliceosomal proteins such as U2AF1, SF3B1, SRSF2 and PRPF8 have been identified in myeloid malignancies. These mutations are usually single heterozygous missense mutations at highly recurrent sites. While all of these mutations affect proteins in the common splicing pathway, their downstream consequences may be diverse and involve distinct oncogenic pathways. To identify affected genes and gain mechanistic insight into the effects of these mutations, we have analyzed the tumor transcriptomes of multiple AML patients with and without spliceosomal factor mutations. For U2AF1, we analyzed 6 tumors with U2AF1 mutations and 14 tumors that had no known splicing factor mutations. Focusing on cases of alternative exon inclusion, we identified 35 exons in 35 genes whose inclusion was altered using stringent statistical cutoffs. Of these, 8 exons were more included while 27 were more excluded in the U2AF1 mutant tumors. We examined the splice site signals flanking the alternative exons and noted a highly unusual sequence pattern adjacent to the 3' AG dinucleotide which is the recognition site of U2AF1. The sequence at this position showed a mutually exclusive pattern upon comparing the excluded to the included exons and this pattern also differed from the consensus 3' splice site sequence. We speculate that the RNA recognition activity of U2AF1 is altered by these mutations. We also found that these splicing changes are not seen in tumors expressing low levels of unmutated U2AF1 nor are the patterns the same in similar tumors with mutations in splicing factors SF3B1, SRSF2 or PRPF8.

The core splicing factor PRPF8 is also often mutated or is expressed at low levels in myeloid malignancies. We identified 9 different missense mutations in AML patients. Eight of these positions were conserved in yeast and the analogous mutations were introduced into the Prp8 gene. None of these mutations produced a growth, temperature sensitive or cold sensitive phenotype. Most showed weak suppression of a subset of splice site mutations. The mutations map to a surface of Prp8 that appears to associate with Brr2. Transcriptome analyses of tumors from mutant and low PRPF8 expressers showed characteristic alterations in exon inclusion. There was a low overlap between the genes most affected in the mutant cases compared to the low expression cases suggesting that the mutant PRPF8 has an altered function.

67 Mnk2 alternative splicing inactivates its tumor suppressor activity as a modulator of the p38-MAPK stress pathway

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The kinase Mnk2 is a substrate of the MAPK pathway and phosphorylates the translation initiation factor eIF4E. In humans, *MKNK2*, the gene encoding for Mnk2 is alternatively spliced yielding two splicing isoforms with differing last exons: Mnk2a, which contains a MAPK binding domain and Mnk2b which lacks this domain. We found that the Mnk2a isoform is downregulated in breast, lung and colon tumors and is tumor suppressive. Mnk2a directly interacts with, activates and translocates p38α-MAPK into the nucleus, leading to activation of its target genes, increasing cell death and suppression of Ras-induced transformation in vitro and in vivo. Alternatively, Mnk2b is pro-oncogenic and does not activate p38-MAPK, while still enhancing eIF4E phosphorylation. Using mutants of Mnk2a that localized either to the cytoplasm or the nucleus we show that Mnk2a colocalization with p38α-MAPK in the nucleus is both required and sufficient for its tumor suppressive activity; induction of p38α-MAPK target genes and apoptosis. Thus, Mnk2a downregulation by alternative splicing is a new tumor suppressor mechanism, which is lost in some breast, colon and lung tumors.

68 Multiple myeloma-associated hDIS3 mutations cause perturbations in cellular RNA metabolism and suggest potential drug targets

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Dis3 is a human nuclear exosome catalytic subunits which contains exonucleolytic (RNB) and endonucleolytic (PIN) active domains. Approximately 10% of multiple myeloma (MM) cases have mutations in Dis3 which locate mostly in the conserved residues of RNB domain. Moreover, Dis3 mutations were also found in other cancers like Acute Myeloid Leukemia or Medulloblastoma.

We show that mutations found in MM patents interfere with Dis3 exonucleolytic activity leading to overall decrease of enzymatic activity, loss of processivity and inability to degrade double-stranded regions. MM Dis3 mutations when introduced into yeast Dis3 ortholog causes growth alterations and changes in nuclear RNA metabolism typical for yeast exosome dysfunction. Since the role of human Dis3 has not been intensively studied we generated conditional Dis3 knockout in chicken B-cell derived DT40 cell line and found out that Dis3 is essential for cell survival suggesting that in its function cannot be taken over by other human exosome associated nucleases: Dis3l and Rrp6. Construction of human cellular model in which endogenous WT Dis3 is replaced with MM mutants reveled substantial changes in RNA metabolism as detected by northern blot and genome wide RNA-seq experiments. For example, Dis3 mutations interfered with rRNA processing and leaded to accumulation of unstable RNAs arising from pervasive transcription. Finally, we analyzed vulnerabilities caused by Dis3 dysfunction what revealed potential drug targets for cancers bearing Dis3 mutations.

All data mentioned above point to the conclusion that hDIS3 mutations found in MM influence cell physiology, disturb exosome function and may potentially cause global defects in the posttranscriptional regulation of gene expression. Such defects are a source of vulnerabilities which can be used for cancer drug development.

69 Defective RNP Assembly in Prostate and Other Cancers

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H/ACA RNPs, one of the two major families of snoRNPs, are important for many basic cellular processes including protein synthesis, pre-mRNA splicing, and genome integrity. Consisting of only one small non-coding H/ACA RNA and four core proteins, their biogenesis is surprisingly complex depending on at least two H/ACA-specific and four general assembly factors. SHQ1 is a chaperone of the central H/ACA core protein and pseudouridine synthase NAP57, aka dyskerin and Cbf5 in yeast and archaea. Through tight interaction, SHQ1 functions in the essential first step of H/ACA RNP biogenesis protecting the inherently unstable NAP57 from degradation and aggregation. It is this NAP57-SHQ1 interaction that is the target of mutations in NAP57 causing the inherited bone marrow failure syndrome dyskeratosis congenita. Surprisingly, although mutations in other components of H/ACA RNPs have been identified in this often-fatal disease, none have been uncovered in SHQ1.

Now a prostate cancer genomics project implicates SHQ1 as a tumor suppressor in cooperation with the androgen-driven serine protease-transcription factor translocation TMPRSS2-ERG, which is observed in ~50% of prostate cancers. Indeed, knockdown of SHQ1 or NAP57 (in combination with ERG expression) in a prostate tissue recombination assay leads to intraepithelial neoplastic lesions in 4-6 weeks. Additionally, SHQ1 is the only one in a cluster of genes with copy number alterations that also carries tumor-associated mutations. Importantly, prostate cancer mutations are also identified in NAP57 implicating both proteins as tumor suppressors. Additional somatic mutations in SHQ1 and NAP57 are identified in other types of tumors, i.e., colon, lung, uterine, and glioblastoma, with most amino acid substitutions adorning the interface between the two proteins. We demonstrate that these cancer mutations, unlike SNPs or mutations of conserved amino acids required for catalysis, impair the interaction of SHQ1 with NAP57 thereby impeding overall H/ACA RNP biogenesis. We conclude that, through SHQ1 and NAP57, H/ACA RNP biogenesis is a target for inherited and somatic diseases and we will speculate on the underlying molecular mechanisms.

70 Loss of MBNL1 function impairs neuronal morphology in myotonic dystrophy type 1

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Myotonic dystrophy (DM) is the most common cause of adult onset muscular dystrophy. Cognitive deficits are found in a high percentage of individuals with DM, type 1 (DM1). The cognitive and behavioral abnormalities include mental retardation, attention deficit and hyperactivity disorder, excessive daytime sleepiness and psychiatric disorders. The genetic basis of DM1 is caused by an expansion of CTG repeats in the 3' untranslated region (UTR) of the Dystrophia Myotonica Protein Kinase (*DMPK*) gene. *DMPK* mRNA containing expanded CUG repeats accumulates in nuclear foci and affect nuclear and cytoplasmic functions of RNA binding proteins such as muscleblind like 1 (MBNL1) and CELF1 (CUGBP and ETR3 Like Factor). Dysfunction of MBNL1 and CELF1 resulting in mis-regulated alternative splicing has been known to involve in the pathogenesis of DM1 skeletal muscle and heart respectively. However the molecular mechanism of how expanded CUG RNA affects central nervous system (CNS) functions in DM1 remains unknown. We have established a brain specific DM1 mouse model, EpA960/CaMKII-Cre, in which expanded CUG RNA is specifically expressed in brain. We found that mouse brains expressing expanded CUG RNA displayed several features of DM1 brain including mis-regulation of alternative splicing and atrophy of cortex and corpus callosum. Hippocampal cultured neurons expressing expanded CUG RNA or knockdown of MBNL1 exhibited similar defects in axonal outgrowth and dendrite development suggesting that loss of MBNL1 function plays an important role in DM1 CNS pathogenesis. In studying the phenotype progression in DM1 mouse brain, we found the progressive loss of MBNL1 in the cytoplasm that is reminisced of changes in alternative splicing of Mbnl1 exon 5. Using time-lapse microscopy we found that MBNL1 cytoplasmic isoform resulting from exclusion of exon 5 is involved in trafficking in cultured hippocampal neurons. We further demonstrated that overexpression of this spliced variant enhances neurite outgrowth through mediating BDNF signaling by interacting with cytoplasmic dynein light chain. More importantly neurons expressing expanded CUG RNA fail to respond to BDNF treatment and loss of BDNF responsiveness can be rescued by expression of MBNL1 cytoplasmic isoform. Thus our results provide a novel mechanism for MBNL1 in DM1 CNS pathogenesis.

71 Mutations in the gene encoding U11/U12-65K protein leads to pituitary hypoplasia and isolated growth hormone deficiency type I

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We describe here the second human disease that is caused by mutations in a specific component of the U12-dependent spliceosome. Earlier recent work by He et al. (2011) and Edery et al. (2011) have described recessive mutations in the U4atac snRNA that cause Microcephalic Osteodysplastic Primordial Dwarfism type 1 or Taybi-Linder syndrome (MOPD1/TALS). The patients with this disease suffer from severe developmental defects and death during early childhood, most likely due to splicing defects in a subset of ~800 genes carrying U12-type introns.

Here we have studied a family with three sisters affected with severe postnatal growth retardation due to isolated GH deficiency and otherwise normal development. Brain MRI scans revealed hypoplasia of the anterior pituitary. The therapeutic response to GH replacement has been excellent to date, suggesting a diagnosis of familial isolated GH deficiency with associated pituitary hypoplasia. Mutational and segregation analyses discarded involvement of all known genes of the GH axis. Exome sequencing revealed biallelic mutations in the RNPC3 gene encoding for the U11/U12-65K protein. This protein is one of the seven unique protein components in the U11/U12 di-snRNP that recognizes the 5'ss and BPS of U12-type introns. The 65K protein is known to bind to the 3' terminal stemloop of U12 snRNA and form a molecular bridge between U11 and U12 snRNPs via interactions with 59K and 48K proteins (Benecke et al., 2005; Turunen et al., 2008). The mutations are in the second RNA recognition motif (RRM2), which binds to the U12 snRNA.

Our preliminary RNAseq analyses from patient lymphocytes have identified defects in splicing of a subset of U12-type introns, which indicate a partial loss of function and is consistent with the observed mild pathological effects of the mutations. Furthermore, glycerol gradient analyses and biotinylated 2'-O-Me RNA oligo pulldown experiments indicate significantly reduced cellular levels of U11/U12 di-snRNPs, which is consistent with predicted consequences of the RRM2 mutations. Finally, we observed unexpected ~2-fold upregulation in the cellular levels of U4atac snRNA alone and as an U4atac/U6atac di-snRNA complex, which may be a possible compensatory mechanism for the defects in the 65K function.

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72 miR-34c-5p is a novel regulator of naive T-cell activation that impacts HIV replication

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CD4+T-cells are the main orchestrators of immune responses. They patrol the body as so-called "naïve" T-cells and become activated through their specific T-cell receptor (TCR) upon encounter with a cognate antigen, a process essential for their clonal expansion and differentiation into memory and effector T-cells. These processes involve a delicate balance of cell proliferation, differentiation and death that have been shown to highly dependent upon microRNA regulation.

Activated CD4+ T-cells are the main targets of HIV infection. Moreover, HIV critically depends upon the cellular activation-state for effective integration into the host genome and productive viral replication. We hypothesize that HIV may subvert the miR profile induced by TCR stimulation.

Based on a massive parallel sequencing study of the microRnome of primary human naive CD4+T-cells, we have identified miR-34c-5p as a novel candidate regulator of T cell activation that is targeted during HIV infection.

miR-34c-5p was found to be expressed almost below detection limits in naive CD4+ T-cells but was induced as a late response to TCR stimulation. miR-34c-5p up-regulation was absent in memory cells, providing novel insights into the differential regulation of these two cell populations. Profiling of the naive cell response to HIV infection revealed that miR-34c-5p was consistently down-regulated by the virus. By generating a T-cell line stably expressing high levels of miR-34c-5p we showed that this microRNA induced a quiescent, non-proliferative phenotype and identified several relevant target genes for the regulation of T-cell function and response to HIV infection. Additionally, the ability of HIV to infect this T cell line was significantly reduced. Our results identified miR-34c-5p as a critical regulator of HIV infection and provide data regarding the mechanisms controlling the expression of this miR during cell activation and virus infection.

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73 Genome-wide mapping of RBM10 binding sites reveals its role in splicing regulation: Implications for cleft palate and TARP syndrome

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RBM10 is an RNA-binding protein of unknown function, which comprises several motifs that bind RNA, including two RNA recognition motifs (RRMs) and Zinc fingers. Mutations in human RBM10, which maps to the X-chromosome, results in a syndromic form of cleft palate, termed TARP syndrome. This syndrome comprises Pierre–Robin sequence (micrognathia, glossoptosis, and cleft palate), talipes equinovarus, atrial septal defect (ASD), and persistence of the left superior vena cava and results in pre- or postnatal lethality in affected males[1]. The loss of function of RBM10 in TARP syndrome shows that this gene is critical for normal mammalian development.

In order to understand the role of RBM10 and how its disruption results in human disease, we aimed to identify endogenous RMB10 RNA targets in a tissue that is relevant to the phenotype observed in TARP syndrome. For this, we used the Cross-Linking Immunoprecipitation protocol (iCLIP) that allows the mapping of protein-RNA interactions at an individual nucleotide resolution. Given the strong conservation between the human and the orthologous murine RBM10 protein and the pattern of expression of the mouse RBM10 gene being consistent with the human malformations of the TARP syndrome, we performed the iCLIP experiment in a mouse mandibular cell line.

We identified around 57,000 RBM10 binding sites at the genomic level, with significant enrichment in intronic regions, in agreement with a putative role for this protein in pre-mRNA splicing. We also uncovered a variety of cellular RNAs, including non-coding RNAs that are bound by RBM10. We used an exon-junction array to analyze changes in gene expression and alternative splicing in a male X-linked Rbm10 mouse deficient cell line. This analysis revealed significant changes in the expression level of approximately 1,400 genes as well as changes in 200 splicing events. Importantly, we observed that those pre-mRNAs that display changes in splicing also contain RBM10 iCLIP tags, suggesting a direct role of RBM10 in splicing regulation. Furthermore, transcriptome changes in the RBM10 KO ES cells revealed that RBM10-regulated genes are in cellular pathways that may help explain the phenotype observed in the TARP syndrome. These experiments will shed light on the pathways that are disturbed in the syndromic form of cleft palate associated with loss of function of RBM10.

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74 Muscleblind-like proteins negatively regulate embryonic stem cell-specific alternative splicing and reprogramming

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Previous investigations of the core gene regulatory circuitry that controls embryonic stem cell (ESC) pluripotency have largely focused on the roles of transcription, chromatin and non-coding RNA regulators. Alternative splicing (AS) represents a widely acting mode of gene regulation, yet its role in the regulation of ESC pluripotency and differentiation is poorly understood. Here, I identify the Muscleblind-like RNA binding proteins, MBNL1 and MBNL2, as conserved and direct negative regulators of a large program of AS events that are differentially regulated between ESCs and other cell types. Knockdown of MBNL proteins in differentiated cells causes switching to an ESC-like AS pattern for approximately half of these AS events. Among the events is an ESC-specific AS switch in the forkhead family transcription factor FOXP1 that controls pluripotency. Consistent with a central and negative regulatory role for MBNL proteins in pluripotency, their knockdown significantly enhances the expression of key pluripotency genes and the formation of induced pluripotent stem cells (iPSCs) during somatic cell reprogramming.

75 A pair of RNA binding proteins regulate neuron-subtype specific alternative splicing in C. elegans

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Alternative splicing is an important and pervasive means of increasing the diversity of the transcriptome and proteome in metazoans. An increased transcriptomic repertoire has likely played a particularly important role in the cellular diversification of the nervous system, where different classes of neurons have evolved to perform distinct functions. However, it has remained a challenge to study the mechanisms and physiological impact of alternative splicing regulation at the level of individual neuronal subtypes. Utilizing the nematode *C. elegans*, with its genetic tractability and simple but well-differentiated nervous system, we created fluorescent two-color reporters to observe alternative splicing *in vivo* and at single neuron resolution. Our results reveal a remarkable diversity of alternative splicing patterns among individual neuron types. One striking example involved differential inclusion of exon 16 in UNC-16/JIP3 transcripts between GABAergic and cholinergic neurons (the two major classes of motor neurons in the animal). We conducted a genetic screen for regulators of this neuron-type specific splicing pattern and identified two broadly conserved RNA binding proteins, UNC-75/CELF and EXC-7/ELAV, both of which facilitate inclusion of the alternative exon. Analysis of splicing patterns in mutant animals and expression studies of the two factors showed that UNC-75 and EXC-7 act combinatorially to achieve neuron-type specificity through partially non-overlapping expression patterns. We next used mRNA-Seq experiments to profile the transcriptomes of wild type and mutant animals and performed CLIP-Seq to assay which transcripts were directly bound by the splicing factors. We found several hundred differentially regulated alternative splicing events when either or both factors are absent, a substantial overlap in targets between the two RNA binding proteins, and distinct modes of combinatorial regulation. Gene Ontology analysis indicated that targeted alternative splicing events were enriched in genes associated with synaptic transmission and locomotion. We are now using knockout mutant strains to explore how perturbing the network of splice isoforms impacts behavioral phenotypes. Initial results indicate that the splicing regulatory network can be utilized to implicate both known and previously uncharacterized genes in aspects of locomotory behavior and synaptic transmission, and that the impact of individual targeted isoforms on neuronal phenotypes can be teased apart *in vivo*. Taken together, our findings suggest that the combinatorial action of splicing factors help shape the regulatory networks contributing to the identity and function of distinct cell types in the nervous system. We speculate that similar regulatory strategies have been frequently utilized in the specification and diversification of the mammalian central nervous system.

76 CFIm25 Links Global change in APA to Cell Growth Control and Glioblastoma Survival

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A growing body of evidence implicates alternative cleavage and polyadenylation (APA) as an important mechanism in regulating gene expression. In rapidly proliferating cells or tumors, widespread shortening of 3′UTRs is posited to result in the increased expression of genes important for these altered states. While this observation has been known for some time, the underlying molecular events that lead to APA in these cells are not known.

Here, we systematically depleted each member of the mammalian cleavage and polyadenylation machinery and measured APA of three test genes: Cyclin D1, Timp2, and Dicer1. Depletion of only the members of the CFIm complex led to shortening of these 3'UTRs with the most dramatic shortening after reduction in CFIm25. To determine its global targets, we developed a novel algorithm capable of identifying and quantifying APA events from standard RNA-Sequencing. Using this algorithm, we identified over 1000 genes that switched from distal to proximal PAS selection after knockdown of CFIm25 in Hela cells. Among the top genes whose APA is under CFIm25 regulation is Glutaminase (GLS), which converts glutamine to glutamate in highly proliferative tumors to support the cancer metabolic phenotype. Depletion of CFIm25 leads to significant shortening of the GLS 3′UTR that causes a profound increase in GLS protein levels. In response, cells with CFIm25 depleted not only proliferate faster but also are sensitive to glutamine withdrawal demonstrating a metabolic change in response to global 3′UTR shortening.

To investigate the biological role of CFIm25 in APA we used our algorithm on data deposited in the TCGA database. This analysis uncovered a previously unsuspected link between CFIm25 levels and survival in glioblastoma patients. Moreover, using our algorithm we compared the 3′UTR usage in RNA seq data from samples of glioblastoma patients who expressed either high or low levels of CFIm25 and identified a group of genes with 3′UTR shortening in conditions of reduced expression. Top on this list is MeCP2, which has been associated with the increased proliferation of several cancers and its altered expression in neurons leads to a spectrum of human neurological disorders. These findings identify a pivotal role of the CFIm25 in governing APA, demonstrate APA identification through standard RNA-Seq, and reveal a previously unknown link between APA and metabolic pathways important for enhanced cell proliferation.

77 Subsets of introns are abundant in poly(A)+ RNA

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Deep sequencing of poly(A)-selected RNA from mouse embryonic stem cells revealed many transcripts in which specific internal introns were significantly more abundant than the other introns within the transcript. We validated a number of these high read-coverage introns by qRT-PCR and found them to be 3-80 fold more abundant than their downstream neighbors. A computational pipeline was designed that identified thousands of high-coverage introns with a stringent statistical cutoff in both human and mouse, using our own as well as ENCODE-generated poly(A)+ RNA-seq data. These introns flank both constitutive and alternatively spliced exons, on average exhibit higher phylogenetic sequence conservation, and the genes containing them are enriched for transcription and RNA binding/processing factors as well as cell cycle and stress response genes. After transcriptional inhibition by flavopiridol (30 minutes), several measured high-coverage introns were predominantly nuclear and their abundance was mostly unchanged; flanking downstream introns were removed by this time. During longer flavopiridol treatments the high-coverage introns ultimately decayed but with significantly slower kinetics than their rapidly spliced neighbors. These results are consistent with high-coverage introns being posttranscriptionally-spliced introns within transcripts that are detained in the nucleus until all introns have been removed. We refer to these as "detained" introns (DIs) to distinguish them from "retained" introns, which are unspliced introns present in transcripts that are transported to the cytoplasm. DIs flanking cassette exons exhibited exon-specific, cotranscriptional and posttranscriptional splicing in response to knockdown of the splicing factor Fox2. A number of DIs are associated with alternative splicing events that are predicted to function as a switch between the production of nonsense-mediated decay (NMD)-substrates and protein coding mRNAs. Among four human cell lines, evidence suggests that DIs are dynamically regulated and comprise a separate pool from previously identified mRNAs that are substrates for the NMD pathway. We propose a modified exon-definition mechanism to explain how intron detention can occur, and suggest that transcripts containing DIs may function as a nuclear detained pool for rapid mobilization of coding mRNAs in response to cellular needs.

78 Genome-wide analysis of pre-mRNA splicing in budding yeast from the perspective of the intron

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In eukaryotes, genes are interrupted by non-coding introns that are spliced out in the process of pre-mRNA splicing. Monitoring pre-mRNA splicing genome-wide is vital to annotating gene structure and understanding the role of pre-mRNA splicing in gene regulation. Pre-mRNA splicing was initially analyzed genome-wide by array-based methods. However, microarrays suffer from low dynamic range and cross hybridization and they typically require prior knowledge of splicing junctions. Recently, RNA-seq, which can reveal splice junctions, has provided another opportunity to monitor splicing with unprecedented dynamic range and resolution. However, the mRNA population ,the target of array and RNA-seq approaches, is underrepresented for splicing events that yield mRNAs subject to non-sense mediated decay. Moreover, due to slow turnover, mRNA can be sluggish in reflecting changes in splicing. Additionally, mRNA sequence does not reveal the branch point, the nucleophile for 5' splice site cleavage. Notably, the limitations of focusing on mRNA to monitor pre-mRNA splicing can be overcome by an alternative approach targeting the intron. We and others have developed approaches to target the intron. In our approach, we sequence the ends of the excised intron to reveal the 5' splice site and the branch point. We have tested the utility of our method in budding yeast and confirmed the majority of annotated intron (~240 introns) that are expressed and spliced under vegetative growth conditions. Furthermore, we detected novel introns in coding regions, 3' UTRs and antisense transcripts. Additionally, given the depth of our analysis, we observed evidence for splicing errors. Finally, although alternative splice site usage is extremely rare in budding yeast, we have uncovered and validated cases of alternative 5' splice site usage and alternative branch point usage. Intriguingly, some genes that undergo alternative splicing code for splicing factors. We hypothesize that alternative splicing of these transcripts enables auto-regulation by novel regulatory mechanisms, which we are currently testing. Our study underscores the value of viewing splicing from the perspective of the intron.

79 Lariat Sequencing in a Unicellular Yeast Identifies Regulated Alternative Splicing of Exons that are Evolutionarily Conserved with Humans

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Alternative splicing is a potent regulator of gene expression that vastly increases proteomic diversity in multicellular eukaryotes, and is associated with organismal complexity. Although it is widespread in vertebrates, little is known about the evolutionary origins of this process owing in part to the absence of phylogenetically conserved events that cross major eukaryotic clades. Here we describe a lariat sequencing approach, which offers high sensitivity for detecting splicing events, and its application to the unicellular fungus, *Schizosaccharomyces pombe*, an organism that shares many of the hallmarks of alternative splicing in mammalian systems but for which no previous examples of exon-skipping had been demonstrated. Over 200 previously unannotated splicing events were identified, including examples of regulated alternative splicing. Remarkably, an evolutionary analysis of four of the exons identified here as subject to skipping in *S. pombe* reveals high sequence conservation and perfect length conservation with their homologs in scores of plants, animals, and fungi. Moreover, alternative splicing of two of these exons have been documented in multiple vertebrate organisms, making these the first demonstrations of identical alternative splicing patterns in species that are separated by over one billion years of evolution.

80 Global Analysis of Phosphorylation by SR Protein Kinases and Their Effects on Genome-wide Splicing in Schizosaccharomyces pombe

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Phosphorylation of both core splicing factors and other co-transcriptionally associated proteins has been shown to influence multiple steps in pre-mRNA splicing. Some phosphorylation events have been linked to specific kinases, but a global analysis of splicing kinases and their substrates, as well as how these events affect splicing genome-wide, is currently lacking. Known substrates of splicing kinases include the conserved serine/arginine (SR) proteins, which are vital in both constitutive and alternative splicing. *S. pombe* has two SR proteins (Srp1 and Srp2), two SR specific kinases (Dsk1 and Kic1/Lkh1), and almost 5,000 introns making it an attractive but manageable system to study the genome-wide effects of phosphorylation on splicing. In order to identify specific phosphorylation events, we took a chemical genetic approach by mutating the "gatekeeper" residue of specific kinases. This mutation provides access to a hydrophobic region at the back of the ATP binding pocket enabling selective use of bulky ATP analogs. We mapped the specific sites of phosphorylation in extract followed by LC-MS/MS for both the Srpk1 homolog Dsk1 and the Clk/Sty homolog Kic1/Lkh1. We found extensive phosphorylation within the RS domains of the SR proteins Srp1 and Srp2, as well as the SR-like proteins U2AF^{59} and Rsd1. In addition, we identified proteins that are also phosphorylated in humans, such as Srrm1 and U1-70K, even though their fission yeast homologs have a reduced RS domain. Perhaps most surprisingly phosphorylation was identified in core splicing factors such as Prp8, Bpb1, and Sap155. We next found that *dsk1-Δ* results in a defect in spliceosome complex A assembly in extracts, which further underlines the importance of these phosphorylation events in splicing. Expanding on this result, we analyzed the genome-wide effect of *dsk1-Δ* and *kic1-Δ* on splicing and found that *dsk1-Δ* has a broad splicing defect. To confirm the importance of phosphorylation on splicing in a titratable manner, we then integrated a gatekeeper mutated *dsk1* into yeast and observed splicing defects by specifically inhibiting its activity using a small molecule inhibitor. In the future, we will use varying amounts of inhibitor to analyze genetic interactions using an epistatic miniarray profile (EMAP) and *in vitro* assembly assays. We are currently investigating the *in vivo* importance of specific sites of phosphorylation by integrating alanine mutants of kinase substrates into yeast. Early analysis of these alanine mutants indicates that the Dsk1 phosphorylation of Bpb1 is required for normal growth. We now plan to use the microarrays to analyze splicing globally with the alanine mutants as well as genetic interactions via an EMAP and *in vitro* assembly assays. Our results from yeast support parallel results in humans and provide an evolutionary view of how phosphorylation is vital for splicing.

81 The Future of RiboScience

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"Big Data" and computational biology have accelerated the pace of discovery in RNA research. From riboswitches to lncRNAs to CRISPR to circular RNAs that act as miRNA sponges, new classes of functional noncoding RNAs are appearing with a dizzying frequency. The power of combining genome-wide and transcriptome-wide approaches with traditional biochemistry and cell biology is influencing the vision for new research institutions around the world. It also needs to influence how we educate our graduate and undergraduate students, so that they see the connections between physics, chemistry, computer science, engineering and biology and can harness multiple disciplines to address problems in RNA research and in biology more generally.

82 The architecture of Tetrahymena telomerase holoenzyme

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Telomerase adds telomeric repeats to chromosome ends using an internal RNA template and specialized telomerase reverse transcriptase (TERT), thereby maintaining genome integrity. Little is known about the physical relationships among protein and RNA subunits within a biologically functional holoenzyme. Here we describe the architecture of *Tetrahymena thermophila* telomerase holoenzyme determined by electron microscopy. Six of the 7 proteins and the TERT-binding regions of telomerase RNA (TER) have been localized by affinity labeling. Fitting with high-resolution structures reveals the organization of TERT, TER, and p65 in the RNP catalytic core. Among the other holoenzyme proteins, p50 has an unanticipated role as a hub between the RNP catalytic core, p75-p19-p45 subcomplex, and the DNA-binding Teb1. A complete *in vitro* holoenzyme reconstitution assigns function to these interactions in processive telomeric repeat synthesis. These studies provide the first view of the extensive network of subunit associations necessary for telomerase holoenzyme assembly and physiological function.

83 Deciphering the assembly of box C/D snoRNP complexes

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Box C/D small nucleolar Ribonucleoproteins (C/D snoRNPs) are essential complexes for gene expression as they direct post-transcriptional modifications of rRNAs in the nucleolus. Box C/D snoRNAs are assembled with a set of four core proteins. Their assembly occurs in the nucleoplasm but the mechanisms involved are not yet clearly defined although we know several assembly factors such as Nufip and the HSP90/R2TP system, which contain the key AAA+ ATPases Rvb1 and Rvb2.

Here, we described a new snoRNP assembly factor named Trip3. We showed that Trip3 dimerizes with Nufip and is important for first steps of snoRNP assembly. By quantitative SILAC proteomics, we found that Nufip/Trip3 associates with part of the R2TP complex and some snoRNP core proteins to form a complex devoid of RNA. This complex is subsequently recruited on nascent box C/D snoRNA, and this occurs concomitantly with removal of Trip3 while Nufip is removed at a later stage. By performing detailed mutagenesis coupled to NMR studies and structural modeling, we obtained a model of the interaction of a fragment of Nufip with pre-snoRNP. This model shows that Nufip keeps pre-snoRNPs in an inactive state, and that Nufip must be removed to allow formation of the active snoRNP structure. We propose that this is catalyzed by the AAA+ ATPases Rvb1/2.

84 Crystal Structure of the Bacterial Pnkp1/Rnl/Hen1 Heterohexamer: A New RNA Repair Complex

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Ribotoxins cleave essential RNAs for cell killing *in vivo*, and we have previously shown that the bacterial Pnkp/Hen1 RNA repair complex was able to repair ribotoxin-cleaved RNAs *in vitro*. Because of 2'-O-methylation by bacterial Hen1 during RNA repair, the repaired RNA resists future cleavage by the same ribotoxin that causes the original damage. Through bioinformatic analysis, we have recently found a new bacterial RNA repair complex that is related to, but also distinct from, the Pnkp/Hen1 complex. The newly discovered RNA repair complex is composed of three proteins, named Pnkp1, Rnl, and Hen1, which form a heterohexamer *in vitro*. To provide insight into the mechanism of RNA repair and shed light on potential *in vivo* RNA substrates, we solved the crystal structure of the 270 kDa Pnkp1/Rnl/Hen1 heterohexamer with cofactors (ATP, SAH, and Mg^{2+}) occupying all eight enzymatic active sites. The structure reveals the architecture of Pnkp1/Rnl/Hen1 heterohexamer as two ring-shaped Pnkp1/Rnl/Hen1 fused at Pnkp1, which forms a homodimer. Each Pnkp1/Rnl/Hen1 ring is formed through pairwise protein-protein interactions between these three proteins, but the interactions are *trans* in nature (e.g., Pnkp1a-to-Rnla-to-Hen1a-to-Pnkp1b, and vice versa). Four active sites that are required for RNA repair (kinase, phosphatase, methyltransferase, and ligase) are located on the inner rim of each ring, allowing RNA repair to be carried out efficiently. A lack of a specific RNA-binding groove/cleft and a wide opening at the center of the ring allow a variety of damaged RNAs to access the four active sites for repair, indicating that the Pnkp1/Rnl/Hen1 heterohexamer might be an all-purpose RNA repair complex.

85 Molecular basis of translation activation by the non-coding RNA RsmZ

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In bacteria, sRNAs (small regulatory/ non-coding RNAs) coordinate global changes in gene expression. The most important global post-transcriptional regulatory system responsible for bacterial virulence is the Csr/Rsm system, in which a sRNA (CsrB/RsmZ) activates translation initiation by sequestering a homo-dimeric protein (CsrA/RsmE) that is binding to the ribosome binding site of a subset of mRNAs [1, 2]. However, the mechanism of translation derepression is only partially understood on the molecular and atomic level.

Here we show for Pseudomonas fluorescens that several RsmE protein dimers are assembling sequentially, specifically and cooperatively onto the sRNA RsmZ, while binding of the third RsmE protein dimer changes RsmZ from an RNase E accessible to a protected form. Furthermore, we elucidated the 70 kDa solution structure of RsmZ bound to three RsmE proteins using a combinatorial approach consisting of nuclear magnetic resonance and electron paramagnetic resonance spectroscopy as well as multiple segmental isotope labeling of the RNA [3].

Strikingly, we discovered two similarly populated conformations in solution, which cannot directly interconvert between each other. Their interconversion requires the dissociation of all three proteins and thus, shows that the first RsmE protein binds the sRNA RsmZ by conformational selection. To our knowledge, both conformations represent a global RNA fold, which has not been described before. The structures visualize how the sRNA can bind several RsmE protein dimers with high affinity by using the helical stems as clamps which tightly grab the proteins. Furthermore, both conformations explain why the third RsmE protein dimer binding to RsmZ is protecting the RNA from RNase E mediated cleavage.

In conclusion, our findings illustrate the molecular basis of translation activation by the sRNA RsmZ and propose how the targeted proteins could ultimately be released from sRNA sequestration.

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86 Single-molecule analysis of L7Ae protein binding to a k-turn : induced fit or conformational selection ?

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When binding of a protein causes a conformational transition to occur in the target RNA this can in principle result from an active induced fit process, or a passive conformational selection. The k-turn is a commonly-occurring structural motif that introduces a tight kink into duplex RNA. In free solution it can exist in an extended form, or be folding into the kinked structure. Binding of proteins including the L7Ae family generate the formation of the kinked geometry, raising the question of whether this occurs by conformational selection of the kinked structure, or a more active induced fit process in which the protein manipulates the RNA structure.

We have devised a single-molecule FRET experiment whereby immobilized L7Ae protein binds Cy3-Cy5-labelled RNA from free solution. We find that all bound RNA is in the kinked geometry, with no evidence for transitions to an extended form at a millisecond timescale. Furthermore, real-time binding experiments provide no evidence for a more extended transient intermediate during the binding process.

The data support a passive model by which the protein selects a fraction of RNA that is already in the kinked conformation, thereby drawing the equilibrium into this form.

87 The structural basis of SRP receptor recruitment and GTPase activation by SRP RNA

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The Signal Recognition Particle (SRP) pathway is a universally conserved membrane protein targeting system, which recognizes ribosome nascent chain complexes (RNCs) exposing a SRP signal sequence (cargo). The cargo is subsequently transferred to the translocon, which mediates the co-translational transport of the emerging polypeptide across the lipid bilayer and also guides the insertion and folding of membrane proteins.

Transfer of the cargo to the translocon involves a large scale rearrangement of the SRP:receptor complex during which the SRP RNA plays a pivotal role in both receptor recruitment and GTPase activation. We have determined the crystal structure of prokaryotic SRP:receptor GTPase domain heterodimers bound to SRP RNA at the *tetraloop* and the *distal site* at 2.6 Ångström resolution.

The interactions at the *tetraloop* reveal the structural basis of receptor recruitment and rationalize previously accumulated biochemical and structural data on the dynamics of this process. Strikingly, interactions between the SRP receptor and the SRP RNA at the *tetraloop* as well as at the *distal site* involve the insertion box domain (IBD), a domain that is unique to the SRP-GTPase family.

At the *distal region*, a flipped-out base of the SRP RNA inserts into the cleft between the two proteins and stimulates hydrolysis of receptor bound GTP by reordering of a side chain provided *in trans* by Ffh. Biochemical data confirm that these fully conserved residues are essential for GTP hydrolysis and efficient protein translocation.

The structural findings combined with biochemical experiments reported in this study reveal the molecular basis of the SRP receptor recruitment to the tetraloop of the SRP RNA and allow us to suggest a possible mechanism for GTPase activation at the distal site of the SRP RNA.

88 Crystal structure of Prp8 and its implications for the spliceosomal active site

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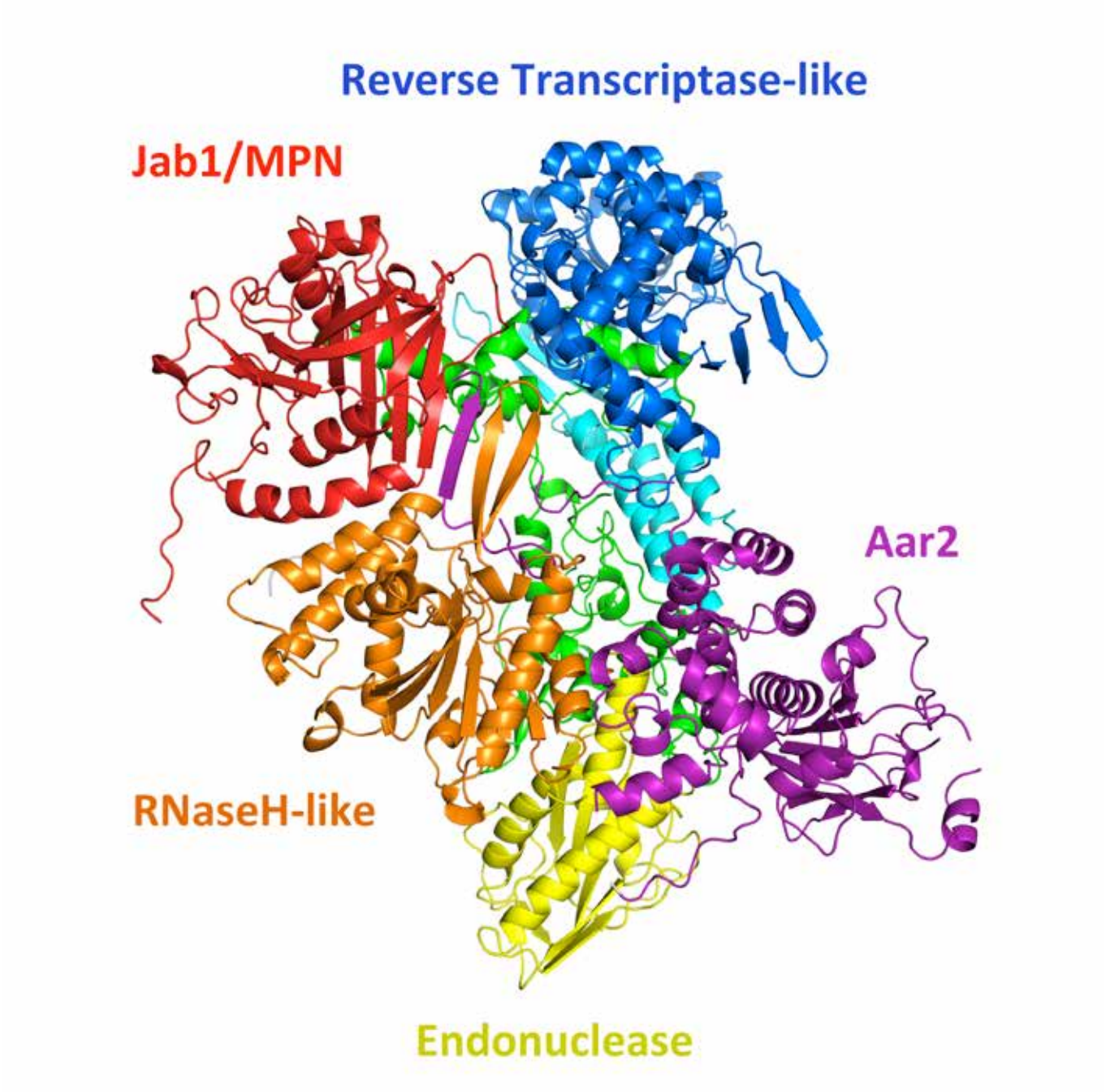
The active centre of the spliceosome consists of an intricate network formed by U5, U2 and U6 small nuclear RNAs, and a pre-messenger-RNA substrate. Prp8, a component of the U5 small nuclear ribonucleoprotein particle, crosslinks extensively with this RNA catalytic core. I will present the crystal structure of yeast Prp8 (residues 885-2413) in complex with Aar2, a U5 small nuclear ribonucleoprotein particle assembly factor. The structure reveals tightly associated domains of Prp8 resembling a bacterial group II intron reverse transcriptase and a type II restriction endonuclease (Figure 1). Suppressors of splice-site mutations, and an intron branch-point crosslink, map to a large cavity formed by the reverse transcriptase thumb, the endonuclease-like and RNaseH-like domains. Our structure provides first structural insights into the architecture of the spliceosome active site, and reinforces the notion that nuclear pre-mRNA splicing and group II intron splicing have a common evolutionary origin.



89 Structural and functional studies of pre-mRNA 5′ and 3′-end processing

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Most eukaryotic mRNA precursors (pre-mRNAs) must undergo extensive processing, which includes 5'-end capping, splicing and 3'-end cleavage and polyadenylation. We have been studying the structural basis for the organization and activity of the 3'-end processing machinery, and have reported the crystal structures of several proteins in this machinery. Histone pre-mRNAs are distinct in that they possess a stem-loop (SL) structure near the 3'-end. Stem-loop binding protein (SLBP) specifically recognizes the SL and regulates most aspects of histone mRNA metabolism. We have determined the crystal structure of a 26-nt histone mRNA SL in complex with human SLBP RNA binding domain and the human 3'-5' exonuclease 3'hExo (also known as Eri-1).

5'-end capping occurs early during transcription and it was generally believed that the capping process is always successful and no quality control mechanism was known. Our studies of yeast Rai1, a protein partner of the nuclear 5'-3' exonuclease Rat1, unexpectedly revealed that it is a novel eukaryotic enzyme with RNA 5'-end pyrophosphohydrolase (PPH) activity as well as decapping activity (but only toward unmethylated caps). These observations led us to demonstrate the presence of RNA 5'-end capping defects in yeast, and that Rai1 is a central player in a novel RNA quality surveillance pathway, promoting the degradation of such defective RNAs.

Our recent studies with Dom3Z, the mammalian homolog of Rai1, showed that it has PPH, decapping as well as 5'-3' exoribonuclease activities, and hence we have renamed it DXO (decapping exonuclease). DXO preferentially degrades defectively capped pre-mRNAs in cells, consistent with its role in 5'-end capping quality surveillance. More importantly, we found that incompletely capped pre-mRNAs are inefficiently spliced at internal introns, in contrast to current understanding where capping is linked to the splicing of only the first intron. We have also determined the crystal structures of mouse DXO in complex with substrate mimic and product RNAs at up to 1.5 Å resolution, which provide elegant insights into the catalytic mechanism of DXO and the molecular basis for its three apparently distinct catalytic activities.

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90 Analysis of eukaryotic orthologous groups reveals Archease as a crucial factor in human tRNA splicing.

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RNA ligases catalyze the ligation of RNA in eukaryotes, archaea and bacteria and play essential roles in many processes including RNA repair and stress-induced splicing of mRNA. In archaebacteria and eukaryotes, RNA ligases also join exonic sequences from transfer RNA precursors (pre-tRNAs) to generate functional tRNAs required for protein synthesis. We recently identified the RNA ligase required for tRNA splicing in humans, a multimeric protein complex having RTCB (also known as HSPC117, C22orf28, FAAP or D10Wsu52e) as the essential subunit. The human RNA ligase complex catalyzes RNA ligation only when purified from its native host indicating that additional, unknown components are required for its full activity. Here, taking advantage of clusters of eukaryotic orthologous groups (KOGs), we identified one protein of unknown function, Archease, as sharing its specific phyletic distribution with RTCB suggesting that these proteins cooperate to ligate RNA substrates. We show that Archease is required for maturation of tRNAs in human cells. Archease physically interacts with the human tRNA ligase complex, facilitating the formation of an RTCB- guanylate intermediate by the release of GMP associated after an initial round of catalysis. The widespread co-occurrence of Archease and RtcB proteins in bacterial and archaebacterial operons implies evolutionary conservation of the functional interplay between these two proteins.

91 Control of myogenesis by rodent SINE-containing lncRNAs

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Staufen1 (STAU1)-mediated mRNA decay (SMD), which also involves STAU2[1], degrades mRNAs that harbor a STAU-binding site (SBS) in their 3'-untranslated regions (3'UTRs)[2]. We have reported that human SBSs can be formed by intramolecular base-pairing, as exemplified by a 19-base-pair stem and 100-nucleotide apex within mRNA encoding human ADP ribosylation factor 1 (ARF1)[3]. More recently, we have found that human SBSs can also form by intermolecular base-pairing between an mRNA 3'UTR Alu element and an Alu element within a cytoplasmic and polyadenylated long noncoding RNA (lncRNA), called a ½-sbsRNA[4]. Such long (up to ~300-base-pair) duplexes undoubtedly bind more than one STAU1 and/or STAU2 molecule, which are known to homo- and hetero-dimerize[5] if not multimerize. Alu elements are a type of short interspersed element (SINE) and derive from 7SL RNA. Since Alu elements are confined to primates, it was unclear how SMD occurs in rodents. SMD is clearly important to rodents as evidenced by our finding that the efficiency of SMD increases during the differentiation of mouse C2C12 myoblasts to myotubes in a way that promotes the myogenic process[6]. SINEs in rodents include B1, B2 and B4 elements, which are evolutionarily unrelated. Here we mine the mouse transcriptome and report that 13.2% of annotated mouse mRNAs contain a single 3'UTR B SINE, and 28.4% of known mouse lncRNAs contain one or more B SINE. We show that SMD occurs in mouse cells via mRNA−lncRNA base-pairing of partially complementary SINEs (i.e., B1-B1, B2-B2 or B4-B4). A single mRNA can be regulated by multiple lncRNAs, and one lncRNA can regulate multiple mRNAs. We also provide evidence that mouse (m)½-sbsRNA-triggered SMD regulates C2C12 cell myogenesis. For example, the SMD of tumor necrosis factor receptor-associated factor 6 (mTraf6) mRNA by m½-sbsRNA2, whose abundance is upregulated during the differentiation of C2C12 myoblasts to myotubes, promotes myogenesis. Our findings define new roles for lncRNAs and B SINEs in mouse that undoubtedly influence many developmental and homeostatic pathways.

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92 DNA Damage induces targeted, genome-wide variation of poly(A) sites in budding yeast

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Systemic response to DNA damage and other stresses is a complex process that includes changes in the regulation and activity of nearly all stages of gene expression. One gene regulatory mechanism used by eukaryotes is selection among alternative transcript isoforms that differ in polyadenylation (poly(A)) sites, resulting in either changes to the coding sequence or portions of the 3'-UTR that govern translation, stability, and localization. To determine the extent to which this means of regulation is used in response to DNA damage, we conducted a global analysis of poly(A) site usage in *Saccharomyces cerevisiae* after exposure to the UV mimetic, 4-nitroquinoline 1-oxide (4NQO). 2031 genes were found to have significant variation in poly(A) site distributions following 4NQO treatment, with a strong bias towards loss of shortened transcripts, including many with poly(A) sites located within the protein coding sequence (CDS). The change in poly(A) site profile was associated with an inhibition of cleavage and polyadenylation in cell extract and a decrease in the levels of several subunits in the mRNA 3'-end processing complex. Sequence analysis identified differences in the cis-acting elements that flank putatively suppressed and enhanced poly(A) sites, as well as a common pattern that suggests a general model for differentiating variable from constitutive poly(A) sites. Our analysis suggests that variation in transcripts that differ in their poly(A) sites is an important part of the regulatory response to DNA damage.

93 Polyadenylated histone mRNAs accumulate upon PARN knock-down

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The poly(A)-specific ribonuclease (PARN) preferentially degrades poly(A). Although a 3' exonuclease, the enzyme is stimulated by a 5' cap, thus mRNAs are likely substrates. However, PARN is not involved in the degradation of bulk mRNA; instead it processes H/ACA box snoRNAs1 and degrades a small subset of specific mRNAs2 3. Using PARN knock-down we identified histone mRNAs as likely substrates by microarray and qRT-PCR analyses. Whereas histone mRNAs normally do not carry poly(A) tails, cDNA sequencing, Northern blot and ribonuclease protection assays show that polyadenylated histone mRNAs accumulate upon PARN knock-down. The poly(A) tails were up to ~200 nt long, and in many cases a canonical polyadenylation signal was present just upstream of the polyadenylation site, suggesting that the regular mRNA 3' end processing machinery is responsible for poly(A) addition. In cells transfected with a histone construct carrying a mutation in the histone downstream element, read-through transcripts appear in large amounts. Using this construct, we will address whether PARN plays a role in a "fail-safe mechanism" to produce correctly processed histone mRNAs lacking a poly(A) tail or whether the enzyme participates in the degradation of misprocessed histone mRNAs.

Preliminary data suggest that a PARN-knock-down may affect the cell cycle. This is interesting as improper histone stoichiometry leads to genomic instability4.

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94 Non-coding Y1/3 RNAs promote the 3'-processing of canonical histone pre-mRNAs

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The human Y RNAs constitute a family of four small (85-115nt) non-coding (nc) RNAs (Y1, Y3, Y4 and Y5). They are transcribed from individual genes by RNA-polymerase III and fold into characteristic stem-loop-structures. The depletion of Y RNAs in *Xenopus* and *Zebrafish* leads to developmental defects, resulting in embryonic lethality at the mid-blastula-stage. Despite their essential role during development the cellular role of Y RNAs remains largely elusive.

To characterize the function of Y RNAs in mammalian cells, we aimed at identifying proteins associated with these ncRNAs using Streptavidin-pulldown-assays. MS-analyses identified various Y RNA-associated proteins involved in the 3'-processing of pre-mRNAs, for instance subunits of CPSF (Cleavage and polyadenylation specificity factor). To test a potential role of Y RNAs in the 3'-processing of mRNAs, we used *chimericantisense-Oligonucleotide* (ASO)-mediated knockdown in HEK293 cells. The depletion of Y1 and more prominently Y3 but not Y4 impaired the processing of replication-dependent histone mRNAs. Notably, the depletion of Y3 also induced an up-regulation of histone mRNA polyadenylation supporting a role of this RNA in the processing of histone mRNAs. This was essentially mediated via the U-rich region within the Y3 loop that promotes association with the histone pre-mRNA cleavage complex (HCC). However, binding of the HCC to the Y3 RNA was facilitated via FIP1L1/CPSF30 that directly associates with this U-rich region. Both factors are essential components of the HCC and facilitate the RNA dependent anchorage of the HCC on Y3 as well as histone pre-mRNAs. Pre-formation of this complex on Y3 enhances the association of the HCC with Histone-reporter RNAs *in vitro* and promotes the processing of histone pre-mRNAs in cells. We propose that Y3 RNA serves as a binding scaffold required to activate the HCC in order to facilitate efficient histone pre-mRNA processing in *trans*. This provides a new concept suggesting a pivotal role of ncRNAs in the assembly of mRNA processing complexes.

95 Dicer-2 is involved in mRNA activation through cytoplasmic polyadenylation

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Cytoplasmic poly(A) tail elongation is a widespread mechanism to regulate mRNA translation. The biochemistry of cytoplasmic polyadenylation has been elucidated in vertebrates, where it requires two sequence elements in the 3' UTR of substrate mRNAs: the U-rich cytoplasmic polyadenylation element (CPE) and the AAUAAA hexanucleotide (Hex). We have recently found CPE- and Hex- independent cytoplasmic polyadenylation in*Drosophila* (1). This "non-canonical" polyadenylation mechanism operates on maternal *Toll* mRNA and requires a region distal to the 3' end of the mRNA which we term PR (Polyadenylation Region).

To identify the components of the non-canonical cytoplasmic polyadenylation machinery we have performed RNA affinity chromatography using PR as bait. We have found that the cytoplasmic poly(A) polymerase Wispy and the siRNA processing factor Dicer-2 specifically associate to PR. Depletion and co-immunoprecipitation studies indicate that Dicer-2 interacts with Wispy and is necessary for polyadenylation of *Toll* reporters *in vitro*. Furthermore, embryos derived from Wispy or Dicer-2 mutant mothers show defects in polyadenylation of endogenous *Toll* mRNA. These results uncover a novel function of Dicer-2 in activation of mRNA translation through cytoplasmic polyadenylation.

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96 Assembly and function of the NOT module of the CCR4-NOT complex

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The CCR4-NOT complex plays a crucial role in post-transcriptional mRNA regulation in eukaryotic cells. It catalyzes the removal of mRNA poly(A) tails, thereby repressing translation and committing mRNAs to degradation. The complex consists of a catalytic module comprising two deadenylases (POP2/CAF1 and CCR4) and the NOT module minimally containing the NOT1, NOT2 and NOT3 subunits. It is known that NOT1 acts as a scaffold protein for the assembly of the CCR4-NOT complex. However, the mechanism by which the NOT2 and NOT3 proteins interact with each other and dock onto the NOT1 scaffold remains unknown.NOT2 and NOT3 are related proteins that both contain a highly conserved C-terminal domain referred to as "NOT-box". Here we show that the NOT-box is a heterodimerization domain mediating the assembly of the NOT2-NOT3 subcomplex. We have solved the crystal structures of the human NOT2 and NOT3 NOT-boxes at 2.4Å and 2.5Å resolution, respectively. The NOT-box consists of a four-stranded C-terminal open b-barrel as well as N-terminally located a-helices, which are required for heterodimerization. We also defined the domains of NOT1, NOT2 and NOT3 required for the assembly of the NOT1-NOT2-NOT3 module. Functional studies in Drosophila melanogaster cells revealed that depletion of NOT1, NOT2 or NOT3 inhibits mRNA deadenylation with a stronger effect for the NOT1 depletion, followed by NOT3. Importantly, NOT3 depletion destabilizes both NOT1 and NOT2 indicating that one important function of NOT3 is the stabilization of the NOT1 scaffold. We used mutagenesis and functional studies to identify key residues in the NOT module required for mRNA deadenylation. These studies revealed that the interaction of NOT1 with NOT2-NOT3 heterodimers is required for deadenylation in D. melanogaster cells. Similarly, NOT3 mutants that do not interact with NOT1 cannot restore deadenylation in cells depleted of endogenous NOT3. Collectively, our data shed light on the assembly of the CCR4-NOT complex and provide the basis for dissecting the role of this complex in mRNA deadenylation.

97 Structural insights into the Not module of the Ccr4-Not complex

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Deadenylation is the first and rate-limiting step in mRNA turnover. Shortening of the poly-A tail is catalyzed by the processive deadenylation activity of the Ccr4-Not complex and the distributive deadenylation activity of the Pan2-Pan3 complex. Both complexes are conserved in all eukaryotes examined to date and have been recently shown to be involved in microRNA-mediated silencing in human cells. The Ccr4/Not complex is a multisubunit complex organized around a large scaffold protein, Not1. The complex has a modular architecture with at least two distinct modules: a "deadenylase module" and a "Not module". Previous studies have revealed the structure and have dissected the activities of the deadenylase module. However, very limited information on the structure and function of the Not module is currently available. We have mapped the interacting domains of yeast Not1, Not2 and Not5 proteins and have determined the 2.8 Å structure of this ternary complex that forms the core of the Not module. The structure shows how the Sm-like domains of Not2 and Not5 dock on to an extensive HEAT-repeat region in Not1. Based on the structural information, we are testing possible functions of the Not module using biochemical *in vitro* assays and *in vivo* experiments in yeast.

98 Roquin Promotes Constitutive mRNA Decay via a Conserved Class of Stem-Loop Recognition Motifs

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Tumor necrosis factor alpha (TNFα) is the most potent pro-inflammatory cytokine in mammals. Numerous posttranscriptional mechanisms control the expression of this potentially harmful cytokine, including an AU-rich element (ARE) and a constitutive decay element (CDE) in the 3'UTR. Whereas ARE-mediated mRNA decay is transiently blocked during macrophage activation, the CDE causes constitutive mRNA decay, thereby limiting the expression of this cytokine under pro-inflammatory conditions. We now demonstrate that the CDE is a 17 nt long sequence that does not serve as a microRNA binding site. Rather, structural probing and mutagenesis provide evidence that it forms a short RNA stem-loop in its active conformation.

Using an improved streptavidin-binding aptamer-tag for RNA affinity purification, we identified CDE-associated proteins by mass spectrometry. Thereby, we found that the CCCH-type zinc and RING finger proteins Roquin (Rc3h1) and Roquin2 (Rc3h2) are CDE-binding proteins. RNA-IP, crosslinking and EMSA experiments confirmed that the ROQ domain of Roquin specifically and directly binds to the CDE stem-loop in cells and *in vitro*. Overexpression and knockdown analyses further showed that Roquin suppresses TNFα mRNA and protein expression in macrophages. TNFα mRNA expression was also upregulated by introduction of a morpholino that interferes with the Roquin-CDE interaction. By affinity purification followed by mass spectrometry, we found that Roquin and Roquin2 recruit the Ccr4-Caf1-Not deadenylase complex and thereby promote mRNA degradation.

To identify CDEs on a genome-wide scale, we developed a bioinformatics approach based on experimentally determined sequence and structural requirements for an active CDE. Thereby, we identified more than 50 highly conserved CDEs in the mouse transcriptome, many of which encode regulators of development and inflammation. RNA-Seq of Roquin-associated mRNAs in macrophages confirmed that CDE-containing mRNAs are the primary targets of Roquin. Taken together, we demonstrate that Roquin proteins act as major mediators of mRNA deadenylation by recognizing CDEs, a novel class of conserved stem-loop RNA degradation motifs.

99 The crystal structure of the nucleolar exosome engaged with RNA

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The RNA exosome is an essential, multisubunit and multipurpose ribonuclease required for processing a large variety of coding and noncoding RNAs. The exosome core includes nine distinct subunits (Exo9) with a six-membered RNase PH-like ring on the bottom, and three S1/KH binding proteins on the top (Liu *et al*., 2006). The exosome core is catalytically inert but includes a prominent central channel that is lined with RNA binding surfaces. RNA exosome ribonuclease activity is catalyzed by two enzymes that associate with the exosome core, Rrp6 and Rrp44. Rrp6, a member of the RNase D family, is a distributive 3' to 5' exoribonuclease that associates with Exo9 in the nucleolus (Tomecki *et al*., 2010) to form a 10 subunit complex, $Exo10^6$. In the nucleus, Rrp6 associates with Exo9 and the processive 3' to 5' exoribonuclease and endoribonuclease, Rrp44 (Allmang et al., 1999). A longstanding question in the field of exosome biology is how RNA is directed to either the Rrp6 or Rrp44 active sites for processing or decay. A recent crystal structure has illustrated how an Rrp44-associated exosome uses the entire length of the Exo9 central channel to funnel RNA from the top of the S1/KH ring, through the PH-like ring, and into the Rrp44 active site (Makino et al., 2013). However, how Rrp6 engages RNA, and its position on the Exo9 core, has remained unclear. Using x-ray crystallography, we have obtained a preliminary atomic resolution structure of reconstituted $Exo10^6$ bound to an RNA substrate, the results of which will be discussed.

100 Nonsense mediated mRNA decay is inefficient on long ORF transcripts

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Nonsense mediated decay (NMD) is the most studied translation dependent mechanism for "quality control" of mRNAs, proposed to serve in removing aberrant mRNAs with premature termination codons from the cell. Conserved in eukaryotes and essential for mammals development, NMD was found to require the presence of a long 3' UTR region (Amrani et al., *Nature* 2004). Our results suggest that a long 3'UTR is required but not sufficient for efficient NMD.

We performed a large scale study of the importance of the region upstream the stop codon in triggering NMD. We have built *Saccharomyces cerevisiae* strains that allowed the testing of hundreds of natural transcripts expressed from their chromosomal location and modified to have the same long 3'UTR region, known to induce NMD. We have used our previously developed Genetic Interactions Mapping (GIM) method (Decourty et al., *PNAS* 2008) to test the effect of NMD inactivation on the steady state levels of 650 barcoded reporter mRNAs. We measured the barcoded transcript level changes in cells lacking Upf1 (Nam7) or Upf2 (Nmd2) in comparison with the corresponding wild-type strains. In contrast with short ORF containing RNAs, transcripts with long ORFs were only marginally affected by the addition of a long, 1.4 kb 3'UTR, a surprising result that was not predicted and could not be explained by current NMD models. The large scale results were confirmed on individual strains and mRNAs. Long ORF transcripts were only slightly destabilized by a long 3'UTR, with steady-state levels higher than 50% of those of the corresponding unmodified transcripts. Both long ORF and short ORF 3' extended mRNAs were highly enriched in a Upf1 (Nam7)-TAP purification to the same extent. Increasing the ORF length of a PGK1 NMD reporter gene, with an mRNA half-life of 3 minutes led to the stabilization of the corresponding mRNA despite the fact that the 3'UTR length, promoter, initiation and stop codon were identical in the two reporter mRNAs.

These data provide an essential piece of evidence for an "early termination" model in NMD, which proposes that translation termination efficiency is intrinsically dependent on the length of the translated ORF. According to this model, early termination is inefficient and, in this context only, NMD competes with "normal" termination to avoid ribosome stalling at stop codons. Thus, while a long 3' UTR is required for NMD, presumably through an increase in the local concentration of Upf1 (Hogg & Goff, *Cell* 2010; Kurosaki & Maquat *PNAS* 2013), our results show that only transcripts with short ORFs will be efficiently recognized and degraded through NMD. Our conclusions correlate with the fact that short ORFs are present in the vast majority of natural NMD substrates in yeast (He et al., *Mol. Cell*, 2003), are compatible with previous publications and give a new perspective on how a premature termination codon could be detected by the NMD process in eukaryotes.

101 eIF4E-bound mRNPs are substrates for nonsense-mediated mRNA decay in mammalian cells

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Eukaryotic mRNAs with premature translation-termination codons (PTCs) are recognized and degraded by a process referred to as nonsense-mediated mRNA decay (NMD). The evolutionary conservation of the core NMD factors UPF1, UPF2 and UPF3 would imply a similar basic mechanism of PTC recognition in all eukaryotes. However, unlike NMD in yeast, which targets PTC-containing mRNAs irrespectively of whether their 5' cap is bound by the cap-binding complex (CBC) or by the eukaryotic initiation factor 4E (eIF4E), mammalian NMD has been claimed to be restricted to CBC-bound mRNAs during the pioneer round of translation. In our recent study we compared decay kinetics of two NMD reporter systems in mRNA fractions bound to either CBC or eIF4E in human cells. Our findings reveal that NMD destabilizes eIF4E-bound transcripts as efficiently as those associated with CBC. These results corroborate an emerging unified model for NMD substrate recognition, according to which NMD can ensue at every aberrant translation termination event. Additionally, our results indicate that the closed loop structure of mRNA forms only after the replacement of CBC with eIF4E at the 5' cap.

102 PAXT-1 binds XRN-2 and promotes its activity

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XRN-2 is a conserved 5'-to-3' exoribonuclease in eukaryotes, which is predominantly localized in the nucleus. Although XRN-2 functions in turnover and processing of various classes of RNA such as rRNA, snoRNA, and microRNA, little is known about how XRN-2 is regulated in these processes. In order to address this issue, we screened for XRN-2 interacting partners in *C. elegans*. Using two independent approaches, immunoprecipitation of XRN-2 and screening for synthetic lethality of a newly engineered *xrn-2* temperature-sensitive allele, we have identified PAXT-1 (PArtner of Xrn-Two) as a cofactor of metazoan XRN-2. Endogenous XRN-2 and PAXT-1 co-immunoprecipitate in an RNase-insensitive manner, and depletion of one reduces levels of the other. Although wild-type animals are unaffected by RNAi-mediated depletion of PAXT-1, *paxt-1* null animals, generated by genome engineering using TALENs (Transcription Activator-Like Effector Nucleases), are inviable at elevated temperature. Consistent with XRN-2 stabilization being a major function of PAXT-1, overexpression of XRN-2 can suppress this lethality. Thus we identified PAXT-1 as a novel cofactor of XRN-2, which stabilizes XRN-2 to promote its activity. We are currently investigating whether PAXT-1 enhances exoribonuclease activity of XRN-2 beyond stabilization of the protein. We will also examine functional conservation for a mammalian counterpart of PAXT-1.

103 Molecular Clues to Tissue-Specific Control of MiRNA Biogenesis

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At present, around 2000 miRNAs are annotated in the human genome. In spite of the great effort to understand the various biological roles of individual miRNAs there is a huge void of knowledge about the regulation of their own biogenesis. Until now only a handful of miRNAs have been investigated individually for their features and trans-acting factors that play significant roles in their production.

We show the first report of tissue-specific control of brain-enriched miRNA biogenesis by defined tissue-specific factors. We demonstrate that the level of miR-7, which is processed from the ubiquitous hnRNP K pre-mRNA transcript, is shaped by the inhibition of its biogenesis in non-neural cells. Using SILAC combined with RNA pull down we reveal MSI2 and HuR to be factors that cooperatively inhibit miR-7 biogenesis by binding to its conserved terminal loop. We provide evidence that the role of HuR is to assist MSI2 binding, which is the actual factor regulating miR-7 maturation. Importantly, we present evidence that the MSI2/HuR complex increases the rigidity of the pri-miR-7 structure. Due to the fact that Microprocessor cleavage is dependent on DEAD-box RNA helicase activity, such stabilization provides the first mechanistic clue to the inhibitory effect of tissue-specific factors on the miRNA processing. Finally, we confirm the physiological relevance of MSI2/HuR-mediated inhibition of miR-7 biogenesis in human neuronal differentiation system and in MSI2 KO mouse.

Currently we are working on the mechanisms controlling biogenesis of other tissue-specific miRNAs. Importantly, our methodological approach provides a platform for future studies on miRNA biogenesis and on all other processes involving RNA-protein interactions.

104 Mouse Tudor domain containing 12 (Tdrd12) is essential for biogenesis of piRNAs associating with the nuclear Piwi protein Miwi2

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Transposons are selfish genetic elements that spread in the population by mobilizing themselves in the germline. A gonad-specific class of small RNAs (25-30 nucleotides long), called Piwi-interacting RNAs (piRNAs), provide protection against these elements, ensuring genome integrity and fertility. The initial pool of primary piRNAs is derived by processing single-stranded transcripts originating from discrete genomic loci called piRNA clusters, which then guide endonulceolytic cleavage of transposon transcripts. An epigenetic memory of this encounter is recorded in germ cells by generating a secondary piRNA from one of the transposon cleavage fragments. While flies use the newly generated secondary piRNA to initiate a piRNA amplification loop, mice integrate this information to effect DNA methylation of the loci containing active transposon elements. Here we identify a Tudor and helicase domain-containing protein, Tdrd12, as a novel component of the secondary piRNA biogenesis machinery in mice. Tdrd12 is detected in complexes containing the Piwi protein Mili and other factors already implicated in this process. While primary piRNAs are present in mice lacking Tdrd12, the nuclear Piwi protein Miwi2 is devoid of piRNAs. Consequently, unloaded Miwi2 remains in the cytoplasm, contributing to the reduced promoter DNA methylation and derepression of transposons observed in the mutant. We used the Bombyx mori (Silkworm) BmN4 cell culture model to molecularly dissect the formation of a complex between BmTdrd12 and Piwi. Additionally, our studies point to the importance of an intact ATPase motif in BmTdrd12 for its proper sub-cellular localization in the Nuage. We propose that Tdrd12 function as a molecular scaffold that dynamically bridges the association between Piwi proteins engaged in piRNA amplification.

105 Regulation of miRNAs and endo-siRNAs during oocyte-to-zygote transition in the mouse

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In the mouse, transcription ceases prior to ovulation and it is renewed during the major phase of the zygotic genome activation, which takes place at the 2-cell stage. Thus, mechanisms of post-transcriptional control of maternal transcripts play key roles during the oocyte-to-zygote transition. RNA silencing is a common name for a group of pathways utilizing small RNAs as sequence-specific guides regulating gene expression, usually post-transcriptionally. Previous research on small RNAs revealed that three different classes of small RNAs are present in the mouse oocyte: piRNAs, miRNAs, and endo-siRNAs. Mutation studies however, suggest that only the endogenous RNAi is essential for the oocyte. Furthermore, while miRNA biogenesis appears intact in the oocyte, biological activity of miRNAs is suppressed. This is apparently because the effector complex (miRISC) does not efficiently form in the oocyte. Our new data suggest that GW182 is the limiting factor for GW182-AGO2 interaction.

Our earlier analysis of dsRNA processing in transgenic animals revealed preferential production of siRNAs in the oocyte while siRNA production in somatic tissues was negligible. Therefore, we investigated control of small RNA biogenesis in the female germline. We have found that growing mouse oocytes express a unique isoform of Dicer, which is truncated at its N-terminus. Several lines of evidence suggest that this Dicer isoform is the factor responsible for increased production of endogenous siRNAs in the oocyte. The oocyte-specific Dicer isoform is the first evidence for molecular divergence of mammalian miRNA and RNAi mechanisms. Notably, poduction of oocyte-specific Dicer is controlled by a rodent-specific retrotransposon. This fact has interesting implications regarding evolution and conservation of RNA silencing pathways in animals.

106 Kinetic and biophysical models improve identification of miRNA targets

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MicroRNAs (miRNAs) are small RNA regulators that are involved in the establishment of gene expression patterns, frequently at transitions between cellular states or developmental stages. Many types of methods have been developed to uncover the mRNA targets of miRNAs. Experimental methods frequently employ transfection or knockdown of individual miRNAs followed by the profiling (with microarrays or deep sequencing) of mRNAs, 1-2 days after the miRNA expression was manipulated. Based on previously published data as well as new experimental measurements we inferred the parameters that determine the kinetics of miRNA-dependent responses, including the rate of loading and dissociation of miRNAs to/from Argonaute proteins, their decay rate and the relative magnitude of the miRNA effect on protein translation and mRNA decay rates. We show that the inferred computational models predict with good accuracy the mRNA, protein and ribosome footprinting response of mIRNA targets following miRNA transfection. The model further predicts that protein decay rates introduce substantial bottlenecks in miRNA-dependent regulation of protein levels and that active miRNA turnover and increased protein decay are necessary to achieve appreciable protein changes over the time scale of a cell cycle. We further developed a biophysical model of miRNA-target interaction that enables accurate identification of non only canonical but also non-canonical targets. We found that the latter are particularly frequent when the level of miRNA expression is high. These results provide new insights into the dynamics of miRNA-dependent regulation of mRNA decay and translation rates.

107 Insights into the recruitment of the PAN2-PAN3 deadenylase complex to miRNA targets by the GW182/TNRC6 proteins

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The PAN2-PAN3 deadenylase complex functions in general and miRNA-mediated mRNA degradation and is specifically recruited to miRNA targets by the TNRC6 proteins. PAN3 is an adaptor protein that recruits the PAN2 deadenylase to mRNA targets. PAN3 contains three prominent regions: an N-terminal region that is predicted to be unstructured, a central pseudokinase (PK) domain and a highly conserved C-terminal domain (C-term), which is unique to the PAN3 protein family. While the N-terminal region of PAN3 contains a well-characterized PAM2 motif, studies on the roles of the PK and C-term domains have been restricted to the yeast protein. In particular, yeast two-hybrid assays have demonstrated that a C-term domain of yeast PAN3 is required for PAN2 binding, while both the PK+C-term domains mediated self-interaction. However, the stoichiometry and significance of PAN3 oligomerization has remained unclear. Here we show that PAN3 forms dimers *in vitro* and *in vivo*. Surprisingly, despite containing nonconservative substitutions in all the sequence motifs required for kinase activity, the PAN3 pseudokinase domain is capable of binding ATP and the integrity of the nucleotide binding pocket is required for deadenylation *in vivo*. Through a combined mutational and functional analysis, we have identified critical residues that mediate PAN3 interaction with PAN2 and TNRC6 proteins, as well as additional residues required for deadenylation *in vivo*. Collectively, our data provides a framework for understanding the role of PAN3 in recruiting PAN2 to mRNA targets to initiate their deadenylation and reveals a role for ATP-binding in mRNA deadenylation.

108 Translational repression and eIF4A2 activity are critical for microRNA-mediated gene regulation.

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In the cytoplasm, miRNAs play a major role in the control of gene expression by down-regulating the protein product of their target mRNAs. The binding of miRISC has been shown to exert both translational repression and degradation of target mRNA. However, the interplay between these processes and the precise molecular mechanisms involved remain unclear. We show that translational inhibition is the primary event required for mRNA degradation. Moreover, we demonstrate that miRNA-mediated repression is only enhanced by the poly(A) tail nor does it require deadenylation. The use of IRES-containing mRNAs allowed us to determine that translational inhibition depends on miRNAs impairing the function of the eIF4F initiation complex. We further define the RNA helicase eIF4A2 as the critical factor involved in this process. We uncover a correlation between the presence of miRNA target sites in the 3'UTR of mRNAs and secondary structure in the 5'UTR, and show that mRNAs with unstructured 5'UTRs are refractory to miRNA repression. Finally, immunoprecipitation studies reveal that *in vivo* eIF4A2 associates with the Ccr4-NOT complex while eIF4A1 is predominantly found to bind eIF4G. eIF4A2 may interact with CNOT1 through its MIF4G domain and impede the helicase activity of the eIF4F complex by clamping onto the structure in the 5'UTR of target mRNAs. These data support a linear model for miRNA-mediated gene regulation in which translational repression via eIF4A2 is required first, followed by mRNA destabilization, and suggest a mechanism by which the repression machinery at the 3' end of the mRNA inhibits translational initiation on the 5' end.

109 The conserved concave surface of the MIF4G domain of CNOT1 is involved in miRNA-mediated translational repression

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MiRNAs are endogenous ~21-nt non-coding RNAs that act as post-transcriptional regulators of gene expression by base pairing to target mRNAs. Mature miRNAs form part of ribonucleoprotein complexes, miRISCs, that contain Argonaute (AGO) and GW182 as core proteins. Recently, we have shown that GW182 proteins recruit the CCR4-NOT deadenylase complex via tryptophan-containing motifs to repress both poly(A)-containing and poly(A)-free mRNAs, indicating that recruitment of the CCR4-NOT complex, in addition to catalyzing deadenylation, also mediates translational repression.

We have now investigated the functional role of the CCR4-NOT complex in miRNA-mediated translational repression. Pull-down experiments revealed that the C-terminal effector domain (CED) of the GW182 protein TNRC6C interacts, in a W-motifs-dependent manner, with two different non-overlapping regions of CNOT1, the large scaffold subunit of the CCR4-NOT complex. Deletion analysis of CNOT1 also identified a central part of CNOT1 (named CNOT1-R) as a region mediating translational repression of mRNA. A highly conserved region of CNOT1-R adopts a MIF4G-like fold and its convex surface interacts with the deadenylase CAF1 (Basquin et al., Mol Cell 2012, Petit et al., NAR 2012). Mutations in the convex surface of MIF4G abrogating the interaction of CNOT1 with CAF1 alleviated the CNOT1R-mediated repression of a poly(A)$^+$mRNA reporter but had no effect on repression of a poly(A)$^-$ mRNA. Furthermore, the repression of a poly(A)$^-$ reporter mRNA by tethered CAF1 was largely dependent on the ability of CAF1 to interact with CNOT1, strengthening the conclusion that it is CNOT1 that acts, via its CNOT1-R region, as an inhibitor of translation.

The concave surface of the MIF4G domain of the initiation factor eIF4G serves as a binding platform for the initiation factor eIF4A (Schutz et al., PNAS 2008). To test whether the CNOT1 MIF4G competes with eIF4G for binding to eIF4A and thereby interferes with translation initiation, we introduced mutations to the CNOT1 MIF4G expected to interfere with the eIF4A binding. Indeed, the mutations alleviated the CNOT1R-mediated repression of poly(A)+ and poly(A)– mRNAs. However, this effect appears not to be due to the compromised interaction with eIF4A since we were unable to detect interaction with either its eIF4AI or eIF4AII isoform. We will present our progress towards identification of the factor acting downstream of the CNOT1 MIF4G in mediating translational repression.

110 Single-molecule observation of DNA targeting and cleavage by the RNA-guided Cas9 endonuclease

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Bacteria and archaea maintain a history of viral infections by integrating small fragments of foreign DNA into specialized genomic loci called clustered regularly interspaced short palindromic repeats (CRISPRs). Subsequent infections trigger an adaptive immune response that relies on CRISPR-derived RNAs (crRNAs) functioning together with CRISPR-associated (Cas) proteins to identify and destroy complementary viral DNA sequences known as protospacers. In type II CRISPR/Cas immune systems, the Cas:crRNA ribonucleoprotein surveillance complex comprises two RNA molecules and the Cas9 endonuclease, which cleaves both strands of the protospacer following initial recognition. Prior studies have used oligonucleotide substrates to show that DNA targeting proceeds via RNA–DNA base-pairing interactions and requires melting of the double-stranded substrate, as well as protein-mediated recognition of a protospacer adjacent motif (PAM) to discriminate self from non-self. Nevertheless, the mechanism by which Cas9:RNA locates and recognizes its target sequence within the larger context of genomic DNA is unknown.

To gain deeper insights into this critical step of CRISPR/Cas-based immunity, we used single-molecule fluorescence microscopy to visualize Cas9:RNA as it searches for DNA targets in real-time. We demonstrate that Cas9:RNA exhibits specific DNA binding in a highly programmable fashion. Both bulk experiments and single-molecule observation of off-target interactions reveal that the search process relies in part on facilitated diffusion, and that PAM sites recruit Cas9:RNA to potential targets. Surprisingly, the Cas9:RNA complex stays tightly bound to DNA protospacers after the cleavage reaction, suggesting the possibility that product association is an important component of CRISPR interference *in vivo*. Collectively, these experiments have elucidated mechanistic details of DNA targeting and cleavage by Cas9:RNA and will facilitate future efforts to develop this system for genome engineering.

111 Processing-Independent CRISPR RNAs Limit Natural Transformation in Neisseria meningitidis

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CRISPR interference confers adaptive, sequence-based immunity against viruses and plasmids in bacteria and archaea. Interference is specified by CRISPR RNAs (crRNAs) that are transcribed and processed from spacer-repeat units. Most forms of CRISPR interference involve RNA-directed DNA targeting, and this mechanism is being exploited for facile, RNA-guided genome engineering applications. Processing of monomeric crRNAs (i.e., each containing only a single spacer sequence) from a multimeric precursor is essential for CRISPR interference in all natural systems studied thus far.

Several bacterial strains from the genus *Neisseria* have "Type II" CRISPR/Cas systems in which the Cas9 protein provides the catalytic activities required for crRNA-directed DNA targeting. Type II systems are those that have proven most effective for eukaryotic genome engineering. Our analyses of crRNA biogenesis and CRISPR interference in *Neisseria* have revealed a unique crRNA biogenesis pathway in which crRNAs are transcribed from promoters that are embedded within each CRISPR repeat, yielding crRNA 5' ends that are formed by transcription and not by processing. This is in contrast with previously studied systems, where pre-crRNA transcription is driven by promoter(s) within a "leader" sequence outside of the CRISPR array. Although *Neisseria* crRNA 3' end formation involves RNase III-catalyzed cleavage of duplexes formed by pre-crRNA and a *trans*-encoded tracrRNA (as in other Type II CRISPR systems), this processing is dispensable for interference, and unprocessed, multimeric crRNAs are functional. Even though pre-crRNA processing is dispensable for interference, the tracrRNA is still required, indicating that the tracrRNA likely contributes to DNA binding and cleavage steps in bacterial cells, as previously established by others *in vitro*. The pathway from the deadly human pathogen *Neisseria meningitidis* exemplifies a newly defined CRISPR/Cas subtype (Type II-C) and is the most streamlined CRISPR/*cas* system characterized to date, as indicated by the number of required components and steps. Its simplicity, as well as its ability to function via unprocessed, multimeric crRNAs, suggests the possibility of unique practical applications for engineered systems in eukaryotes. Endogenous CRISPR spacers frequently target genomic sequences of other *Neisseria* strains and so limit natural transformation, which is the primary source of genetic variation that contributes to immune evasion, antibiotic resistance, and virulence in *N. meningitidis*. We conclude that native CRISPR/Cas systems can limit all three primary routes of horizontal gene transfer (phage transduction, conjugation, and natural transformation) in bacteria and archaea.

112 T box riboswitch decodes both the information content and geometry of tRNA to affect gene expression

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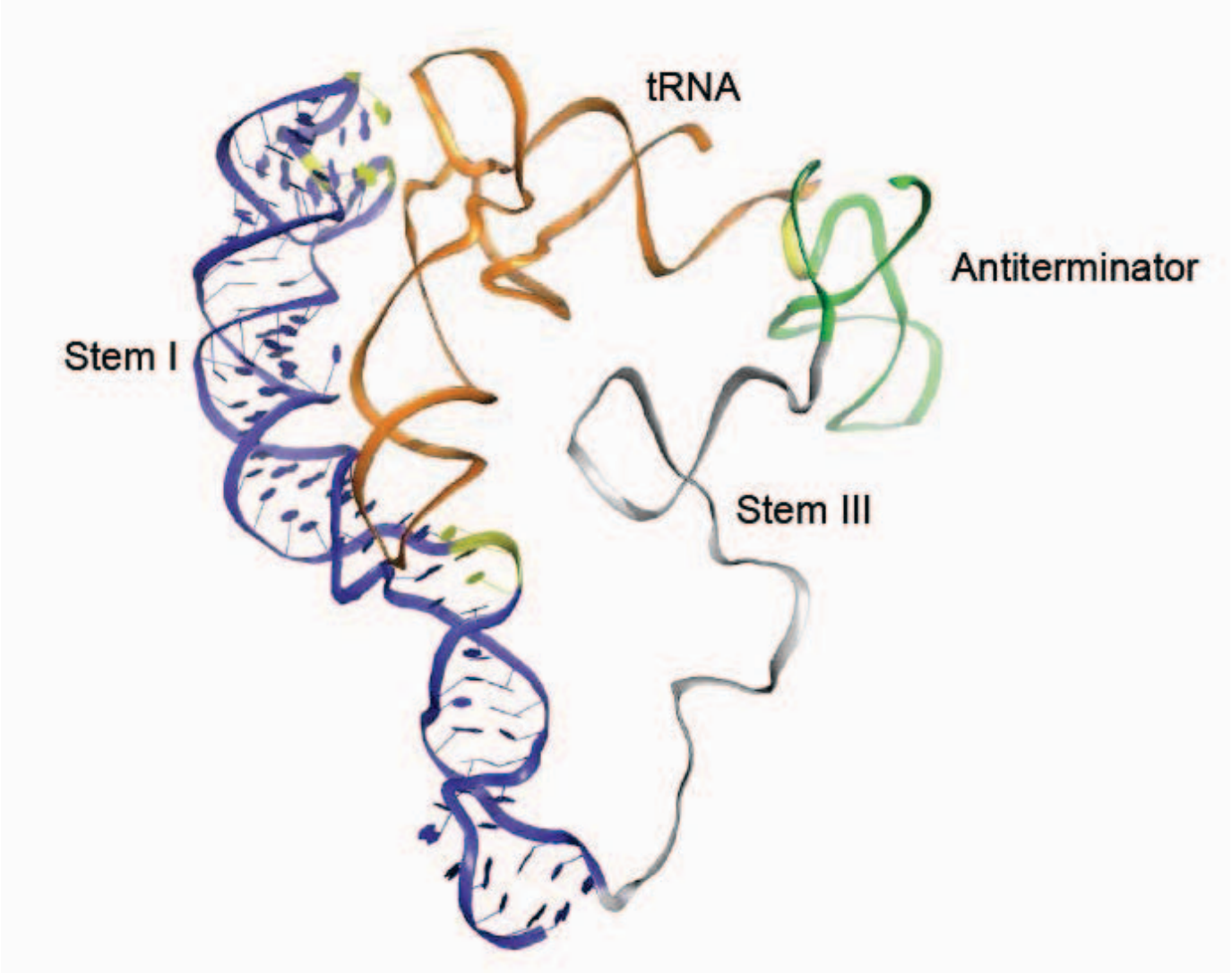
The T box riboswitch is an RNA element that controls gene expression by directly binding to a specific tRNA and sensing its aminoacylation state. This interaction controls expression of amino acid-related genes in a negative feedback loop. The T box RNA structure is highly conserved, but its tRNA binding mechanism is only partially understood. Known sequence elements are the Specifier Sequence, which base-pairs with the tRNA anticodon, and the antiterminator loop, which base-pairs with the tRNA acceptor end. Here, we reveal the crucial function of the highly conserved Stem I distal region in tRNA recognition and report its 2.65 Å crystal structure. The apex of this region contains an intricately woven loop-loop interaction between two conserved motifs, the AG Bulge and the Distal Loop. This loop-loop structure presents a base triple on its surface that is optimally positioned for base-stacking interactions. Mutagenesis, cross-linking and small-angle X-ray scattering data demonstrate that the apical base triple serves as a binding platform to dock against the tRNA D- and T-loops. Strikingly, the binding platform strongly resembles the D- and T-loop binding elements from RNase P and the ribosome P/E sites, suggesting that this loop-loop structure may represent a widespread tRNA recognition platform. We propose a new double checkpoint model for decoding tRNA, in which the information content of tRNA is first examined through the Specifier Sequence-anticodon interaction, the length of the tRNA anticodon arm is then measured by the distal loop-loop platform. When both conditions are met, tRNA is secured and its aminoacylation state is sensed.



113 Structural basis of specific tRNA recognition by the T-box riboswitch

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The T-box riboswitches regulate tRNA aminoacylation, amino acid synthesis and transport in Gram-positive bacteria in response to cellular tRNA aminoacylation levels. They couple direct binding of non-aminoacylated tRNAs with formation of a transcription antiterminator allowing expression of downstream genes. The T-box consists of two phylogenetically conserved domains connected by a flexible, highly variable linker. These domains are an elongated Stem I that has extensive sequence conservation beyond the "specifier" loop that base pairs with the tRNA anticodon, and an antiterminator that is stabilized by base pairing with the non-aminoacylated tRNA 3' end [1]. We employed isothermal titration calorimetry to monitor tRNA binding to a T-box truncation series and found that the Stem I is necessary and sufficient for robust and specific tRNA binding (K_D~120 nM). Indeed, Stem I-tRNA association accounts for the majority of the binding free energy of full-length T-boxes. To elucidate the molecular basis of tRNA recognition by T-boxes, we have now determined the crystal structure of a representative T-box Stem I (102 nt) in complex with its cognate tRNA (75 nt) and a bacterial protein that binds the conserved Stem I K-turn. Stem I adopts an extended "C" shape that cradles the tRNA. As predicted genetically [2], the anticodon is recognized by the specifier element through base pairing. The specifier is in the middle of the C, and is flanked by loop E and K-turn motifs. The distal end of Stem I comprises two T-loop motifs that interdigitate to form a compact module with a flat molecular surface that recognizes the elbow of tRNA (where its D- and T-loops come together) through shape complementarity. Sequence conservation indicates that this bipartite mechanism of tRNA recognition, in which the specifier binds the anticodon, and the interdigitated T-loops reach out to recognize the tRNA elbow, is near-universal in T-box riboswitches. Remarkably, recognition of the tRNA elbow by two interdigitated T-loops has been also demonstrated for RNase P [3], even though the T-box and RNase P recognize the opposite arms of tRNA. Thus, it appears that these two RNAs evolved independently to converge on using the same interdigitated T-loop module for recognizing tRNA.

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2. Grundy et al. 1993 Cell 74:475.
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114 Crystal Structure and Biophysical Analysis of a Class 2 PreQ1 Riboswitch

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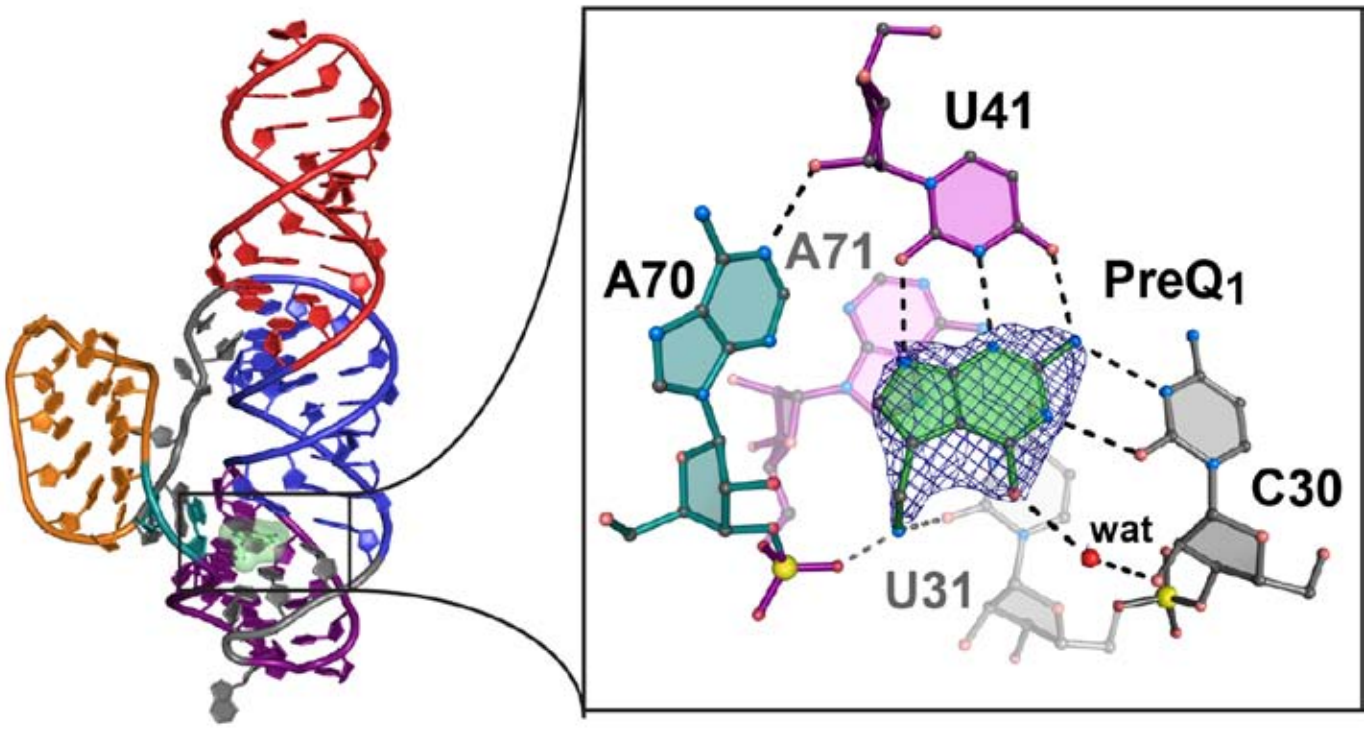
Riboswitches are a class of *cis*-acting functional RNAs that regulate gene expression by directly binding ligands. Typically found in the 5′-leader sequences of bacterial genes, riboswitches have great potential as novel antimicrobial targets. Of special interest are riboswitches that bind to ligands that are unique to the bacterial metabolome. One such molecule is $preQ_1$, a hypermodified guanine base that interacts with a cognate riboswitch in over 29 bacterial species including pathogenic organisms. Two classes of $preQ_1$ riboswitches, termed $preQ_1$-I and $preQ_1$-II, have been discovered, but thus far structural information has been described only for class 1.

Here we present the crystallographic analysis and structure determination of a class 2 $preQ_1$ riboswitch in the ligand bound state. The overall structure comprises 77 nucleotides, and reveals the mode of ribosome-binding site sequestration by an H-type pseudoknot whose formation is fortified by the adjacent $preQ_1$-binding pocket. The ligand binds at the confluence of a three-way helical junction (figure 1) and is recognized by a readout mechanism that does not entail canonical Watson-Crick base pairing (figure 1). Thermodynamic parameters of ligand binding have been determined by isothermal titration calorimetry to both wild type and binding site mutants, which confirm the mode of interaction between the riboswitch and ligand observed in the crystal structure.

In-line probing analysis has been conducted and confirms key structural changes that occur upon ligand binding. Combined with the crystal structure, thermodynamic analysis, and comparison with similar structures such as human telomerase RNA, we propose a mechanism for gene regulation whereby binding of $preQ_1$ stabilizes formation of three base triples at the base of the pseudoknot that sequesters the RBS. The model for translational regulation will be presented as well as a comparison to other riboswitches that bind purine-like effectors.



115 A novel class of self-cleaving ribozymes is prevalent in many species of bacteria and eukarya

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Ribozymes are noncoding RNAs that promote chemical transformations with rate enhancements approaching those of protein enzymes[1]. Although ancient RNA World organisms[2] likely used many types of ribozymes[3], only ten classes have been previously verified in nature. We report the discovery and analysis of an additional self-cleaving ribozyme class, called twister, which is present in many species of bacteria and eukarya. More than 2690 twister ribozymes were identified that conform to a secondary structure consensus that is small yet complex, with three stems conjoined by internal and terminal loops (Fig 1a). Two pseudoknots provide tertiary structure contacts that are critical for catalytic activity. Many examples of these ribozymes are circularly permuted, bearing striking similarities to hammerhead ribozymes (Fig 1b,c). Twister ribozymes cleave RNA via an internal phosphoester transfer reaction, which is identical to the general mechanism used by other five classes of self-cleaving RNAs discovered previously[4]. The discovery of twister ribozymes deepens the mystery regarding the biological roles of widespread classes of self-cleaving RNAs.

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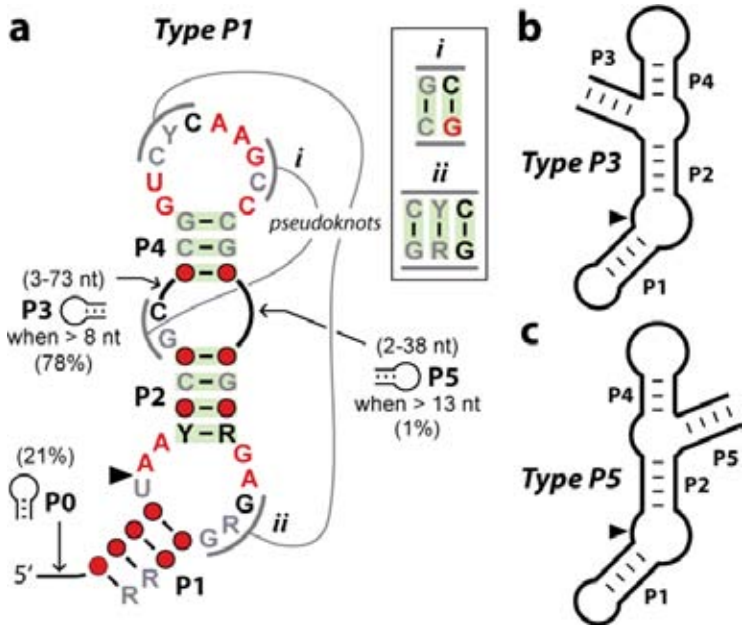

Figure 1 | Consensus sequence and secondary-structure model for twister self-cleaving ribozymes. (**a**) Consensus model based on 2690 twister ribozymes in its type P1 configuration, wherein the RNA chain begins and ends at the base of the P1 stem. The arrowhead identifies the cleavage site. Gray, black and red nucleotides designate conservation of at least 75, 90, and 97%, respectively; positions in which nucleotide identity is less conserved are represented by circles. Green shading denotes predicted base pairs supported by natural covariation. Notations *i* and *ii* identify predicted pseudoknots. Numbers in parentheses are the variable lengths of linker sequences that sometimes form stem structures as indicated. (**b**,**c**) The RNA chains of naturally occurring type P3 and type P5 configurations enter and depart the motif at the optional P3 or P5 stems, respectively.

116 Spliceosomal Prp24 unwinds a minimal U2/U6 complex from yeast

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Splicing plays a major role in eukaryotic gene expression by processing pre-mRNA to form mature mRNA. Pre-mRNAs undergo splicing to remove introns, non-protein coding regions, and religate exons, protein coding regions. This process is catalyzed by the spliceosome, which consists of five small nuclear ribonucleoprotein particles (snRNPs: U1, U2, U4, U5 and U6) and numerous protein factors. Proper assembly of spliceosomal components is critical for function, and thus, defects in assembly can be lethal. Several spliceosomal proteins facilitate structural rearrangements important for spliceosomal assembly and function. Prp24 is an essential factor in U6 snRNP assembly, and it has been proposed to assist in U4/U6 formation and unwinding. Here, we address the question whether Prp24 affects the U2/U6 complex dynamics. Using single-molecule Fluorescence Resonance Energy Transfer (smFRET), we have previously shown that a minimal U2/U6 complex from yeast can adopt at least three distinct conformations in dynamic equilibrium. Our new single molecule data show that Prp24 unwinds U2 from U2/U6 complex and stabilizes U6 in a low FRET conformation. We also show that the RNA Recognition Motifs of Prp24 affect the binding affinity of Prp24 for U6 and unwinding activity. We propose that Prp24 plays an important role in U2 and U6 snRNP recycling by dissociating the U2/U6 complex.

117 Conformational Heterogeneity of the Protein-Free Human Spliceosomal U2-U6 snRNA Complex

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The complex formed between the U2 and U6 small nuclear (sn)RNA molecules of the eukaryotic spliceosome plays a critical role in the catalysis of precursor mRNA splicing. We used ^{19}F NMR and enzymatic structure probing to characterize the fold of a protein-free paired construct representing the human U2-U6 snRNA complex. For the NMR studies, we included a single 5-^{19}F-cytidine residue in a region of U2 that would be part of Stem I (*i.e*. double-stranded) in a four-helix model or single-stranded in the three-helix model. Results for both enzymatic probing and NMR of the complex in the absence of Mg^{2+} are consistent with formation of a four-helix structure as a predominant conformation. However, ^{19}F NMR data also identify a lesser fraction (up to 14% at 25°C) of an alternative conformation presumed to be the three-helix conformer. In the presence of 5 mM Mg^{2+}, the fraction of the three-helix conformation increased to ~17%, suggesting a slight shift to the three-helix conformation. A mutant complex designed to favor the four-helix conformer displayed almost complete formation of Stem I, and a complex designed to favor a three-helix formation showed enhanced formation of Helix 1b, a hallmark of that conformer (Zhao C, Bachu R, Popovic M, Devany M, Brenowitz M, Schlatterer JC, Greenbaum NL. *RNA,* advance publication February 20, 2013, doi:10.1261/rna.038265.1130). Based upon this distribution, the calculated DG for interconversion to the four-helix structure from the three-helix structure is approximately -4.6 kJ/mol. To evaluate the possibility of interconversion between the two conformations and the time scale, we acquired two-dimensional ^{19}F-^{19}F NOESY spectra of the complex labeled with ^{19}F-cytidine in the Stem I site of the U2 snRNA strand. We observed a NOE between the two peaks in a spectrum with a mixing time of 400 ms. This finding provides compelling evidence of exchange between the alternative conformations on a slow exchange time scale. These observations indicate conformational heterogeneity in the protein-free human U2-U6 snRNA complex consistent with a model in which the RNA has sufficient conformational flexibility to facilitate interconversion between steps of splicing *in situ*.

118 A new class of minimal Hammerhead ribozymes conserved in the eukaryotic family of Penelope-like retroelements

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Small self-cleaving RNAs like the paradigmatic Hammerhead ribozyme (HHR) have been recently found widespread in DNA genomes from bacteria to humans[1,2,3]. Their patchy distribution and common connection with retrotranscriptases and repetitive DNA suggest that most of these ribozymes would have a role in retrotransposition. Following bioinformatic and phylogenetic analysis, we have now discovered a new class of HHR motif conserved in the superfamily of Penelope-Like retrotransposable Elements (PLEs), a peculiar group of intron-containing retroelements different from the LTR and non-LTR retrotransposons of eukaryotes[4]. These new PLE-HHRs are minimalist variants of the canonical HHR that lack one of the three helixes and show some characteristic variations within their catalytic core. The PLE-HHR motifs consistently map in the 5' and 3' flanking repeats of PLEs from plants, invertebrates and lower vertebrates, although for some organisms, conserved PLE-HHRs can be also found in their genomes without any connection to a recognizable retrotransposon. *In vitro* analysis confirmed that these ribozymes may only work as dimeric forms during transcription of the retrotransposon. Our observations, together with those reported for HDV-like ribozymes in non-LTR retrotransposons[5,6], expand the role of RNA self-cleavage to the biology of diverse eukaryotic retroelements and leave open the question of how many more forms of RNA self-cleaving motifs can be possible.

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119 Probing EF-G Power Stoke During Ribosome Translocation

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The mechanism of error control for the ribosome to move exactly three nucleotides during the translocation process and the role of EF-G remain elusive. The two possible mechanisms are the "Brownian motor" model and the "power stroke" hypothesis. Many recent studies support the former mechanism, implying that the ribosome moves by intrinsic Brownian motions and EF-G biases the random motion into the forward direction. However, the spontaneous translocation rate is several orders slower than the translocation with EF-G catalysis. This is contradictory to the "Brownian motor" hypothesis in which the maximum rate is limited by the intrinsic fluctuation. Because the EF-G can dramatically accelerate translocation comparing to the intrinsic rate, one intriguing question is whether EF-G exerts a mechanical force, i.e., a power stroke, to push the mRNA movement. At present, there has been no direct experimental evidence supporting such power stroke.

We have obtained direct force measurements in ribosome translocation using a combination of single-molecule fluorescence resonance energy transfer (FRET) and a new force-induced remnant magnetization spectroscopy (FIRMS) technique. The latter probes different noncovalent interactions and resolves them based on the binding forces. Single-basepair force resolution has been achieved. The ribosome pre-translocation complex and post-translocation complex are well resolved, and the translocation yield can be obtained. The EF-G power stroke is directly obtained by force-induced dissociation of a series of mRNA-DNA duplexes. A clear transition in magnetic signal by FIRMS indicates a substantial mechanical force produced by the EF-G. Our new results provide direct evidence, for the first time, that EF-G is the mechanical motor that generates power stroke to promote mRNA movement during its GTP hydrolysis cycle. The amplitude of the force may play an important role in determining the translocation fidelity.

120 Understanding RNA Interference One Molecule at a Time

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In mammals, small RNAs direct Argonaute2 (Ago2) to cleave complementary target mRNAs. For *Drosophila*, the development of efficient, cell-free systems that recapitulated RNAi led to a detailed mechanistic understanding of the RNAi pathway. Until recently, no such system existed for mammals because cell extracts that recapitulate RNAi are surprisingly inefficient. We find that cytoplasmic S100 extracts from *Ago2–/–*immortalized mouse embryonic fibroblast (MEF) cells that stably over express wild-type mouse AGO2 support highly efficient RNAi in vitro. Unlike extracts from HeLa or wild-type MEFs, extracts from the AGO2 over-expressing cells allow efficient assembly of a synthetic small RNA guide into AGO2 to form RISC, the RNA-induced silencing complex. Using a novel affinity purification strategy, we can readily purify AGO2-RISC assembled in these extracts with a specific guide sequence (1). We will present our recent studies analyzing the biochemical properties of this purified AGO2-RISC using the "CoSMoS" (co-localization single molecule spectroscopy) single-molecule technique.

CoSMoS can measure the kinetic properties of Ago2-RISC, including *kon*, *koff*, and *kcat*. Our single-molecule data agrees well with recently published biochemical measurements of AGO2-RISC using population-based (ensemble) methods (2). Consistent with ensemble measurements, we find that AGO2-RISC binds to an mRNA target at the speed of macromolecular diffusion. This stands in contrast to the annealing of nucleic acids in the absence of protein, a process whose speed is limited by its on-rate. Intriguingly we find that target cleavage by AGO2-RISC is highly temperature-dependent, suggesting that a conformational rearrangement is required for RISC activity in mammals. Using both ensemble and single molecule experiments, we are now measuring the departure from RISC of the cleaved target fragments, a step proposed to limit the turnover rate of AGO2-RISC. In the future, we plan to use the CoSMoS system to determine the evolutionarily conserved and the specialized properties of Argonaute family proteins from diverse species.

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121 Promoter directionality is controlled by U1 splicing and polyadenylation signals

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Transcription of the mammalian genome is pervasive but productive transcription outside protein-coding genes is limited by unknown mechanisms. In particular, we and others recently discovered that although RNAPII initiates divergently from most active gene promoters, productive elongation occurs primarily in the sense coding direction. The data presented here reveal that asymmetric sequence determinants flanking gene transcription start sites (TSS) control promoter directionality by regulating promoter-proximal cleavage and polyadenylation. We demonstrate in various tissues of mouse and human that upstream antisense RNAs (uaRNAs) are cleaved and polyadenylated at a poly (A) site (PAS) shortly after their initiation. De novo motif analysis reveal PAS signals and U1 snRNP binding sites (U1) as the most depleted and enriched sequences, respectively, in the sense direction relative to the upstream antisense direction. In agreement with the recent finding that U1 snRNP suppresses cleavage and polyadenylation, our data suggests that a U1-PAS axis characterized by low U1 recognition and high density of PAS in the upstream antisense region results in early termination of uaRNAs whereas proximal sense PAS signals are primarily suppressed by U1. Experiments to modulate U1 snRNP levels at the upstream antisense region of divergent promoters will be discussed. We propose that the U1-PAS axis may limit pervasive transcription throughout the genome.

122 Suppression of promoter upstream transcripts (PROMPTs) by polyadenylation site-induced RNA decay provides directionality to transcription of human promoters

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Active eukaryotic promoters are associated with unorthodox transcription. In human cells, this leads to the production of PROMoter uPstream Transcripts (PROMPTs), which are rapidly cleared by the ribonucleolytic RNA exosome. How PROMPT transcription is terminated and coupled to RNA decay, while its neighboring promoter-downstream mRNA production is not, is unknown. Here, we use exosome depletion to create genome-wide maps characterizing PROMPT RNAs and their termini in unprecedented detail. The clear majority of PROMPTs initiate in the antisense direction from 150-200bp upstream of the transcription start site (TSS) of the associated gene. PROMPT TSSs share features with mRNA-producing TSSs, including stalled RNA polymerase II (RNAPII) and the production of small TSS-associated (TSSa) RNAs. Thus, PROMPTs are unstable, capped full-length products of the previously reported bi-directional antisense transcription. Importantly, motif analyses around PROMPT 3'ends reveal polyadenylation (pA)-like signals, well-positioned to elicit PROMPT 3'end formation. However, contrary to their promoter-distal counterparts at mRNA 3'ends, RNA derived from TSS-proximal pA signals is highly exosome-sensitive. Interestingly, promoter-downstream regions contain significantly less pA signal information, but are instead enriched, relative to their promoter upstream counterparts, for U1 snRNP binding sites, which are known to suppress pA site utility. We conclude that asymmetric sequence distribution around human gene promoters serves to provide an overall directionality to the transcription process by rapidly terminating antisense transcription and degrading its RNA product.

123 Pathway of histone mRNA decay determined by high-throughput sequencing (HTS)

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Histone mRNAs are the only mRNAs in animal cells that are not polyadenylated ending instead in a conserved stemloop. Inhibition of DNA replication results in rapid histone mRNA decay directed by the stemloop. Decay of histone mRNA is initiated by addition of a non-templated oligouridine tail of ~10 nts, which binds Lsm 1-7. Previous studies used low throughput approaches for detecting non-templated additions. The low number of sequences generated limits the detailed analysis of mRNA decay. We have developed a high throughput sequencing strategy that allows detection of all intermediates in histone mRNA decay, and can specifically target mRNAs with non-templated 3' tails. This approach provides the depth of coverage necessary to detect patterns of decay intermediates for individual histone mRNAs. We find multiple oligouridylated intermediates resulting from partial decay located in the 3' side of the stem and in the coding region near the stop codon. These intermediates contain oligo(U) tails of 4-10 nts, and many are capped indicating they result from 3'-5' decay. Analysis of polyribosome gradients using HTS, show oligouridylated mRNAs are polyribosome associated, including decay intermediates degraded into the coding region. Blocking translation with pactamycin results in run-off of ribosomes and accumulation of monosomes with histone mRNAs, including capped oligouridylated decay intermediates. Thus a major pathway for histone mRNA decay after HU treatment is 3' to 5', with repeated exosome stalling, possibly when it contacts translating ribosomes. The degradation intermediate is then uridylated to re-prime 3'-5' decay. Exosome knockdown results in accumulation of the initial intermediates in the stemloop and reduction in intermediates stalled in the coding region. This is consistent with initial decay stalling in the stemloop catalyzed by 3'hExo (eri-1) and subsequent decay carried out by the exosome. To assess the simultaneous 5' and 3' pattern of decay on individual transcripts we used an anti-biotin oligonucleotide designed to isolate both capped and uncapped intermediates from the H2a mRNAs. A capped luciferase mRNA ending in a stemloop transfected into cells is rapidly degraded after treatment of cells with HU with a similar set of decay intermediates. This technique should be generally applicable to determining the degradation pathway of any set of mRNAs.

124 Global poly(A)-tail length measurements reveal that the relationship between tail length and translational efficiency varies between biological contexts

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Poly(A) tails are found at the 3′ ends of nearly all eukaryotic messenger RNAs (mRNAs) and long non-coding RNAs. The presence of a poly(A) tail promotes efficient mRNA translation, an effect mediated through poly(A)-binding protein. However, an understanding of the relationship between the length of a poly(A)-tail and translational efficiency, as well as other aspects of mRNA metabolism, has been limited, primarily because of the lack of a technology that provides high-resolution poly(A)-tail length measurements in a global manner. Here, we describe a high-throughput-sequencing-based method that measures the tails of individual mRNA molecules by coupling a fluorescence-based readout of poly(A)-tail length with sequencing of the poly(A)-proximal region. We have applied this method to diverse eukaryotic organisms and cellular contexts, including budding and fission yeasts, insect and mammalian cell lines, mouse liver, and zebrafish embryos. Poly(A)-tail lengths vary across mRNAs and show some conservation between orthologous genes. In particular, transcripts corresponding to ribosomal protein genes and certain other classes of "housekeeping" genes tend to have short tails. Poly(A)-tail lengths exhibit a notably poor correlation with translational efficiency (as measured by ribosome profiling) across genes in nearly all systems we have examined. This lack of correlation also applies to different translational states of mRNAs from the same gene, as indicated by poly(A)-tail lengths across a polysome gradient. Unlike all other systems we have studied, the early zebrafish embryo exhibits a striking correlation between poly(A)-tail length and translational efficiency (Spearman $R > 0.6$). Remarkably, this correlation disappears later, indicating a dramatic and rapid developmental switch in the nature of translational control. Our results clarify the relationship between poly(A)-tail length and translational efficiency, and enable further investigations of the determinants and consequences of poly(A)-tail length.

125 Capture of a microRNA targetome using chemically modified miRNA mimics ("miR-CLIP")

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Identification of the targets of microRNAs (miRNAs) remains a significant barrier to understanding their function. Recently, several methods including PAR-CLIP and affinity purification by biotinylated miRNAs have been developed to achieve this goal, either in a global fashion, or for selected miRNAs. All of these methods have their strengths and weaknesses. We have developed a two-step approach to screen in straightforward fashion for targets using miRNAs chemically modified with psoralen and biotin. Prior to Ago-2 immunoprecipitation as the first step *in vivo* photo-crosslinking is performed and followed by a second step by stringent affinity purification of bound target RNAs. We have termed this method "miR-CLIP".

We applied miR-CLIP to isolate targets of miR-106a in HeLa cells by using functionally modified mimics of pre- and mature miR-106a. Deep sequencing of isolated RNAs yielded a large number of significantly enriched mRNAs compared to "mock"-treated controls. Among these were several hundred potential new targets. No enrichment was observed for predicted targets of unrelated miR-let-7a. Mostly, target recognition followed a canonical seed-binding mode however shorter non-canonical seed matches were also detected in enriched RNAs. In follow-up assays, overexpression of miR-106a significantly repressed 8 from 10 of the top-ranked potential mRNA targets detected in the analysis validating the approach. Surprisingly, the sequencing also showed enrichment of the long non-coding RNA (lncRNA) H19 as an interacting partner of miR-106a. H19 is expressed from a paternally imprinted gene which is strongly induced during muscle development. Indeed, H19 was strongly and unexpectedly elevated by overexpression of miR-106a in HeLa and in myoblasts, and conversely, its inhibition by an anti-miR showed the opposite effect. RNAi-mediated suppression of H19 led to reduced levels of known miR-106a targets in myocytes leading us to speculate a mechanism in which H19 buffers the activity of miR-106a during muscle cell differentiation.

We are currently testing the functional consequences of this new interaction. In summary, the miR-CLIP method appears to be a valuable technique to uncover the "targetome" of specific miRNAs.

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126 Identification of multiple regulatory microRNAs by miTRAP

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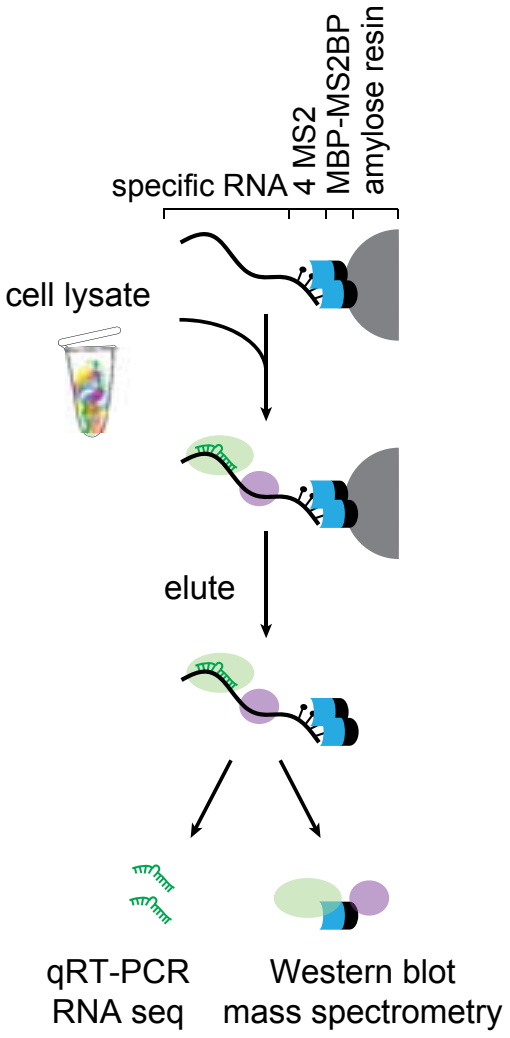
MicroRNAs (miRNAs) are endogenous, small non-coding RNAs which serve essential functions in post-transcriptional control of gene expression. Animal miRNA target sites (MTS) are typically characterized by imperfect complementarity and are mainly located in the 3'UTR of mRNAs. The length of such 3'UTRs and the number of putative MTSs could be correlated with the morphological complexity of organisms. However, for only a few human targets multiple miRNA regulation has been verified as experimental identification and *in silico* prediction remains challenging and limited by various means. Aiming to provide a procedure allowing the comprehensive identification of regulatory miRNAs, we established miTRAP (microRNA Trapping by RNA Affinity Purification). MiTRAP co-purifies miRNAs and RNA-binding proteins (RBPs) with *in vitro*transcribed bait RNAs from cell lysates by adapting MS2-tethering (Figure 1). Therefore, miTRAP is independent of genetic manipulation of cellular systems and *in silico* predictions.

Applying MiTRAP allowed the validation of regulatory miRNAs and RBPs controlling the expression of the transcriptional regulators MYC and ZEB2. Moreover, miTRAP and subsequent deep sequencing identified 13 novel MYC-regulatory miRNAs all of which could be validated by gain- as well as loss-of-function studies. Four of these novel miRNAs target 'non-canonical' sites and thus could not have been identified by *in silico*prediction. We consider miTRAP a reliable and easy to handle technology allowing the rapid and comprehensive identification of miRNAs targeting RNAs of interest in a given cellular context. MiTRAP thus provides a powerful tool, which will significantly expedite analyses aiming the characterization of miRNA-facilitated regulation of gene expression in development, metabolic control and diseases.



127 Neurodegenerative diseases: Quantitative predictions of protein-RNA interactions

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Increasing evidence indicates that RNA plays an active role in a number of neurodegenerative diseases. We recently introduced a theoretical framework, to predict the binding ability of protein and RNA molecules. We use our approach to investigate ribonucleoprotein interactions linked to inherited intellectual disability, amyotrophic lateral sclerosis, Creutzfeuld-Jakob, Alzheimer's, and Parkinson's diseases. In my presentation, I will focus on (1) RNA interactions with fragile X mental retardation protein FMRP; (2) protein sequestration caused by CGG repeats; (3) noncoding transcripts regulated by TAR DNA-binding protein 43 TDP-43; (4) autogenous regulation of TDP-43 and FMRP; (5) iron-mediated expression of amyloid precursor protein APP and α-synuclein; (6) interactions between prions and RNA aptamers. Our results are in striking agreement with experimental evidence and provide new insights in processes associated with neuronal function and misfunction.

128 New in vivo RNA-binding architectures discovered by RBDmap

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The RNA interactomes of HeLa and HEK293 cells jointly comprise 1106 RNA-binding proteins (RBPs) (1, 2), with almost half of these lacking well-defined RNA-binding domains (RBDs), suggesting the existence of numerous unknown RNA-binding architectures. Here, we report RBDmap, a new method built on interactome capture (3), to comprehensively identify the RBDs of native RBPs in proliferative HeLa cells. Making use of in vivo UV-crosslinking of RBPs to polyadenylated RNAs, capture on oligo(dT) magnetic beads, proteolytic mapping and mass spectrometry combined with a sophisticated scoring algorithm, RBDmap "re-discovered" the known RNA-binding sites (e.g. RRM, KH) of numerous well characterized RBPs, validating the approach. Strikingly, RBDmap identified dozens of additional RNA-binding architectures (e.g. thioredoxin, SSB, RAP, WD40) in multiple non-homologous proteins, also including disordered motifs such as basic patches. RBDmap thus instructs on the modes of RNA-binding of hundreds of proteins in their native cellular states, providing valuable structural and functional insights into RNA biology. For example, the identification of the thioredoxin domain as a high-confidence RBD reveals an intriguing link between the redox state of cells and RNA metabolism.

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129 Development of Dynamic Polyconjugates for tissue-targeted delivery of siRNA

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Dynamic Polyconjugates (DPCs) are siRNA delivery vehicles composed of reversibly-masked, membrane-active polymers whose activity is revealed only within endosomes. A new generation of hepatocyte-targeted DPC consists of a fully biodegradable peptide with membrane lytic properties (MLP) as the membrane-active polymer that is conjugated to N-acetylgalactosamine (NAG) for targeting. This NAG-MLP is co-injected with a liver-tropic, cholesterol-conjugated siRNA (chol-siRNA). Effective target gene knockdown is accomplished without complex formation or interaction of the NAG-MLP and the chol-siRNA prior to reaching the target cell.

We are utilizing this separately-targeted siRNA delivery approach for the treatment of chronic hepatitis B virus (HBV) infection. The use of RNAi therapeutics for the treatment of HBV infection enables knockdown of viral RNAs, including the pre-genomic RNA from which the replicative intermediates are derived, thus reducing both viral load and viral proteins. A single i.v. co-injection of NAG-MLP with chol-siRNAs targeting conserved human HBV sequences resulted in profound reduction of viral RNA and multi-log knockdown of viral proteins and DNA with long duration of effect in mouse models of chronic HBV infection. Using a hybridization/HPLC-based method of detection, we are able to correlate the degree of viral repression and duration of effect with the amount of the active form of the siRNA guide strand in the liver, the 5' phosphorylated guide strand, which can be detected up to one month after a single administration.

In addition to our existing pH-labile masking reagents, we are developing DPC formulations with masking chemistry that is reversed by the action of proteases. These delivery vehicles are stable in the absence of lysosomal proteases, allowing for functional delivery of subcutaneously-administered DPC (scDPC). The protease-sensitive DPCs display long circulation times, indicating that the masking chemistry is stable in serum. Attachment of the NAG ligand to scDPCs results in rapid liver uptake and >90% knockdown at sub-mg/kg siRNA doses in mice and non-human primates. The remarkable stability and efficacy of protease-sensitive DPCs make them amenable for use in liver indications for which subcutaneous dosing is desirable as well as in targeting tumor and other extra-hepatic tissues.

130 Specific Gene Activation by Disruption of PRC2-lncRNA Interactions

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A wide variety of nucleic acid compounds are used in both the laboratory and the clinic to inhibit specific RNA expression and subsequent protein production. We are developing nucleic-acid-based therapeutics that activate genes—rather than inhibiting them—by blocking the interaction of Polycomb repressive complex 2 (PRC2) with its target long noncoding RNA (lncRNA).

Histone H3K27 trimethylation is generated by PRC2 and results in inhibition of transcription. Our platform is based on the discovery that PRC2 is recruited by non-coding RNAs to methylate histones local to the site of the lncRNA. The EZH2-containing PRC2 complex represses gene expression at thousands of sites across the genome. During its transcription, an lncRNA that contains a PRC2-recognition sequence is tethered to the site of transcription. This tethering leads to very localized repression of neighboring genes. Since each PRC2-associated lncRNA interacts with PRC2 through distinct sequences, we are able to design single-stranded oligonucleotides that specifically block the binding of PRC2 to an individual lncRNA, thus de-repressing the expression of a single mRNA to elevate protein levels. Starting with a genome-wide pool of RNA sequences that interact with PRC2, we are now developing nucleic acid compounds that selectively upregulate gene expression for therapeutic benefit. We are applying this technology to develop new treatments in the therapeutic areas of rare genetic disorders, oncology, metabolic diseases and neurodegenerative diseases.

131 RNA-based immunotherapeutics against cancer

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RNAs with optimized properties are increasingly investigated as a tool to deliver the genetic information of complete antigens into professional antigen-presenting dendritic cells for HLA haplotype-independent immunotherapies against cancer. Upon entering dendritic cells, the encoded antigen is translated from the RNA, and then processed into epitopes that are presented on MHC class I and II complexes triggering an antigen-specific T cell response (1).

The dose of the antigen and the duration of its presentation are critical factors for generating strong and sustained immune responses. Substantial increase of the amount of antigen produced from RNA can be achieved by optimizing RNA stability and translational efficiency. Both features are determined by *cis*-acting elements in the RNA, i.e. the 5' cap, the poly(A)-tail, and the sequence of the non-coding and coding regions. Continuous improvement of RNA by modifying the different elements for increased expression of foreign proteins in dendritic cells has been a main goal of our studies (2).

We have developed plasmid templates for *in vitro* transcription of RNAs with modified 3' structures (3' UTR and poly(A)-tail) stabilizing the RNA and optimizing its translational performance in dendritic cells. Moreover, we observed that RNAs capped with the D1 diastereoisomer of $m_2^{7,2'\text{-O}}\text{Gpp}_\text{S}\text{pG}$ (beta-S-ARCA) have increased stability and translational efficiency in dendritic cells. Recently, we have analyzed the effect of the codon composition on the level of antigen expression by testing up to ten different constructs for several antigens. Significant differences can be observed, and the expression of the antigen can be enhanced up to ten-fold compared to the wild-type codon composition. By combining these measures we were able to profoundly improve properties of RNA-encoded immunotherapeutics.

To initiate human clinical testing of our RNA technology, a suitable manufacturing process to produce gram amounts of RNA using the modified cap analog and optimized sequence elements as given above has been established under "Good Manufacturing Practice". The first patients have been treated, demonstrating safety of the treatment. Immunological assessment of the efficacy to induce antigen-specific immune responses in humans is in progress.

In principle, our optimized RNA technology can be generally used for exogenous protein expression in target cells (3). Importantly, cell-type dependent differences have been observed for the effects of the *cis*-acting RNA elements. Therefore we have started a screening program to identify the optimal combination of cap structures and UTR sequences for multiple cell lines and primary cells.

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132 Antisense Oligonucleotide Therapies for the Treatment of Neurodegenerative and Muscular Diseases

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Antisense oligonucleotides (ASOs) are chemically modified oligonucleotides that are designed to specifically bind to a targeted RNA through Watson-Crick base pairing. There are a variety of mechanisms by which ASOs modulate RNA function after hybridization, including promoting the degradation of the RNA and modulating RNA processing. Neurodegenerative and muscular diseases are an attractive area for antisense technology in that there a number of diseases in which the genetic basis is known. Because ASOs target RNA, rather than protein, they can be used to directly modulate a broad range of genetic defects that cannot be targeted easily by conventional small molecule or protein based drugs. Isis Pharmaceuticals is developing antisense drugs for several inherited neurodegenerative and muscular diseases including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), Huntington's disease (HD), tauopathies and myotonic dystrophy (DM1). Preclinical studies in rodents and non-human primates demonstrate broad distribution of single stranded ASOs in CNS tissues following central administration, directly into the CSF, with commensurate modulation of gene expression in spinal cord and different brain regions. In addition, we have demonstrated that ASOs can also modulate gene expression in skeletal muscle when administered peripherally. The tissue half lives of the drugs in the CNS and muscle of mice and non-human primates is in the order of months. We have also used ASOs in mouse models of disease and will present out work in mouse models of SMA and DM1. We recently completed an early stage clinical study in SMA demonstrating safety and tolerability of antisense drugs administered by intrathecal injection. This early clinical experience supports the continued development of antisense drugs for severe neurodegenerative and muscular diseases.

133 Extracellular RNAs are markers of muscle myogenesis following splice switching oligonucleotide therapy in a mouse model of Duchenne muscular dystrophy

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Extracellular RNAs are promising disease biomarkers for a number of disease conditions. Here we report that in the mdx dystrophin-deficient mouse model of Duchenne muscular dystrophy (DMD), extracellular dystromiRs (miR-1, miR-133a and miR-206) show dynamic patterns of expression in serum throughout disease progression and in response to exon skipping-mediated dystrophin restoration utilizing potent peptide-morpholino oligonucleotide conjugates. This dynamicDystromir expression pattern is found to be distinct from the expression of the corrected dystrophin protein, while the extracellular RNAs show a positive correlation with the expression of myogenic transcription factors suggesting that these miRNAs are indices of muscle myogenic i.e. regenerative / degenerative status and probably reflect activity of the myogenic stem cell niche within the diseased muscles. Further data obtained following experimentally-induced skeletal muscle regeneration in non-dystrophic mice support this hypothesis. Moreover the release of these extracellular miRNAs is likely to be a regulated, specific process (i.e. not simply a result of passive release from degenerating muscle) given that the extracellular RNA expression pattern is significantly different from that found within the muscle tissue. Further, the majority of the extracellular dystromiRs are bound in protein/lipoprotein complexes and only a minority are found in extracellular vesicles. These data therefore have important implications for understanding the biological and clinical significance of extracellular RNAs in DMD and other neuromuscular diseases, and in particular following splice switching oligonucleotide therapy.

134 Silencing gene expression by recruiting RISC

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The capacity for small RNAs to repress gene expression depends on nucleic acid base-pairing between the target mRNA and a small RNA guide bound to an Argonaute protein. A challenge to the successful use of small interfering RNA (siRNA) in mammals has been effectively triggering RNAi in vivo while simultaneously protecting the siRNA from nucleolytic degradation and delivering it to the correct tissue and cell type. We are exploring a new approach to gene silencing that takes advantage of the well understood stability and delivery properties of antisense oligonucleotides and the cell-type specific expression of endogenous microRNAs (miRNA). Our strategy employs a stablized, synthetic oligonucleotide tether that recruits endogenous microRNAs to a specific target mRNA. The oligonucleotide tether contains one region complementary to the target mRNA and another to an abundant endogenous miRNA. The tether binds endogenous miRNA-loaded Argonaute complexes and links them to the mRNA. A tether designed to recruit *let*-7 directed a >90% reduction in luciferase reporter expression, compared to a control tether directed towards a miRNA not present in animal cells. Tethers designed to recruit miR-21 or *let*-7a to the 3′ UTR of an endogenous mRNA reduced mRNA abundance by ~70%. Our results suggest that it is possible to (1) redirect an endogenous miRNA to silence an mRNA with no binding sites for that miRNA and (2) design the tether to silence only in specific tissues by choosing a miRNA with an appropriate expression pattern.

135 What can you know about noncoding RNAs without doing any experiments?

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This tutorial will address computational and other non-experimental means to improve the understanding of the structure and function of non-coding RNAs whose secondary structure is conserved. The session is aimed primarily at experimental biologists, and will not assume any computational background. Concepts to be addressed include: analysis of covariation to detect and understand a family of RNAs sharing a common structure, and using flanking genetic elements as clues to RNA function. These approaches are often useful both for finding novel noncoding RNAs and for developing knowledge and hypotheses about poorly understood RNAs.

136 Methods of Predicting RNA Structure Change Due to Mutation

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In determining the functional effect of a mutation in RNA, it is important to consider potential effects of the mutation on RNA structure. Alteration of the structural ensemble of an RNA due to mutation can influence the very network of regulatory interactions an RNA is involved with in its lifetime by altering the binding affinity of the transcript for various interacting RNAs and proteins, and in some cases where an RNA is enzymatically active, influence its very catalytic activity. We present various means of predicting the degree and nature of RNA secondary structure change in a given transcript from mutation. We first compare the traditional minimum free energy model of RNA structure to a probabilistic model that we utilize where a full ensemble of possible structures for a given RNA is represented. We then review the SNPfold algorithm as a means of quantifying the global RNA structure change brought about by given mutations using commonly available RNA structure software. Various flavors of the SNPfold algorithm, as well as a method of non-parametrically calculating the significance of global structure change brought about by a point mutation when compared to all other possible mutations in a transcript, are discussed. We review the use of Principal Component Analysis (PCA) as a method of visualizing structure ensembles and their disruption upon introduction of a mutation. We take measurements of structure change from Selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE) experiments, and compare the experimental data to predictions made using the described methodology in order to determine the sensitivity and specificity of our predictions.

137 RNAsnp: Predicting SNP Effects on Local RNA Secondary Structure

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The function of many noncoding RNAs and cis-regulatory elements of mRNAs largely depends on the structure, which is in turn determined by their sequence. Single Nucleotide Polymorphisms (SNPs) and other mutations may disrupt the RNA structure, interfere with the molecular function, and hence cause a phenotypic effect. Thus it is of relevance to search for structural changes induced by SNPs. Existing methods have addressed this problem by considering global folding of the wild-type and mutant sequences respectively, and then compared them in various ways. However, if the structural change is only local, it can be hard to detect on global scale. Therefore, we have developed RNAsnp (based on RNAfold from the Vienna package), which is an efficient method for prediction of SNPs effects on local RNA secondary structure. RNAsnp searches efficiently all subsequences (local regions) for structural effects and selects the one where structural difference is largest based on some distance measure (of which a number is benchmarked). To determine the significance of the SNP effects empirical p-values are computed from the background distribution generated using random sequences, for which computational efficiency is otained by using extensive precomputed tables describing the score distributions as a function of sequence length, G+C content, and SNP position.

Only some 30 examples are reported in the literature where the RNA structure could be the cause of dysfunction in the presence of the SNP. In four of these cases probing analysis was carried out and confirmed an actual structural change (on top of in silico predicted methods). Additionally, on a data set comprising 501 SNPs associated with human inherited diseases, we predicted 54 to have significant local structural effect in the UTR region of mRNAs. RNAsnp is available both as standalone software and as a web server. The RNAsnp Web server provide a convenient interface to provide input data to RNAsnp and to select different modes of operation. It helps to visualize the output using informative graphical representation, such as dot plot matrices comparing pair probabilities for wild-type and mutant. In addition, the web server is connected to a local mirror of the UCSC genome browser database that enables the users to select the genomic sequences of interest for analysis and to visualize the results in the UCSC genome browser. Both software and web server are available at http://rth.dk/resources/rnasnp.

138 Navigating through the MC-Flashfold 2D suboptimal solution maze using simple structural transformation rules

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RNA 2D structure prediction from a single sequence often requires the inspection of large numbers of suboptimal solutions proposed by the software. This exercise is unproductive and the modeler is left with little insights about the native structure. However, many of the proposed structures are very similar and networks can be build, where each connected pair of structures in a network differs from each others by a simple transformation. Structures that cannot be linked by a series of simple transformations are found in other networks. We consider simple a transformation between two structures when they differ by a bulge loop migration, or the formation of a base pair. Even with only these two simple transformations, we can transform long lists of suboptimal predictions into a tractable number of independent networks that can help to: 1) Interpret the RNA 2D proposed structures; and, 2) Compare the networks of two different sequences, for instance to evaluate the structural impact of mutations. Besides, these networks are particularly informative when non-canonical base pairs are considered. We generated such networks under the statistical framework of nucleotide cyclic motifs (NCM) using a faster and leaner version of the MC-Fold program, MC-Flashfold. For instance, MC-Flashfold with a threshold of 8 kcal/mol produces a quarter million suboptimal structures, within which the native cloverleaf is found in the few first hundreds. This is a problem for the end-user who has no clue about selecting the right one. Among more than 7,000 networks build from the suboptimal structures, the native structure was found in the second one, where the networks are ranked by the free energy of its most stable member. Other concrete examples, benchmarks, as well as MC-Flashfold and network applications will be discussed.

139 How to determine binding affinities and binding motifs for RNA-bindingproteins from CLIP-seq data

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The analysis of binding sites of RNA-binding proteins is becoming an increasingly important topic. The standard experimental approach for determining binding sites is CLIP-seq. Since not all possible binding sites are covered due to differential expression in tissues and developmental states, the main problem is to come up with good motif descriptions to find missing binding sites and to evaluate the binding strength.

It is well know that binding motifs for RNA-binding proteins have to contain both sequential and structural information [1,2]. Previous approaches used only structural profiles. Our new approach GraphProt uses an advanced machine learning approach based on a graph-kernel [3], and is able to use both structural profiles as well as detailed 2D-structures, without the need to decide a priori about the weight of the different structural components. In addition, we are able to integrate also context information. Furthermore, we provide support for mutagenesis analysis, which is a non-trivial task, provided that structural information is used.

We have compared our approach with the current state of the art, RNAcontext, on the in-vitro data that where used for evaluation in the RNAcontext publication. We outperform RNAcompete for all 9 proteins used in this evaluation (see Figure1 below). Furthermore, we will report on our results of applying GraphProt on CLIP-seq data for PTB. Our predictions highly correlate with measured affinities. We have used the discovered motifs to search for new binding sites within a mini-gene construct, which could then be verified experimentally by our cooperation partner.

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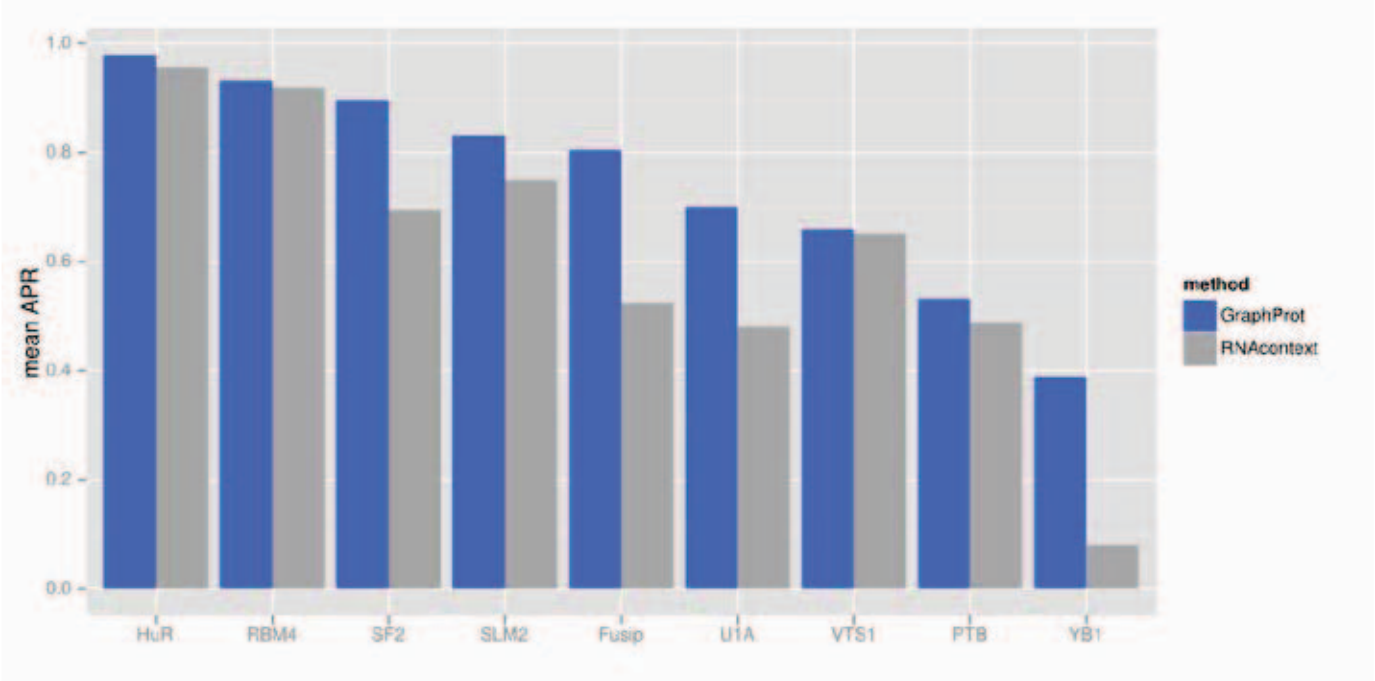

140 The HIV-2 Rev-Response Element: Determining Secondary Structure and Defining Folding Intermediates

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Similar to its' HIV-1 counterpart, the Rev response element of HIV-2 (RRE2) is responsible for transporting unspliced and partially spliced viral RNA from the nucleus to the cytoplasm. We applied complementary chemical and biochemical probing techniques to determine the structure of the RRE2 RNA. SHAPE analysis indicates that the low-energy form of RRE2 contains five peripheral stem loops linked by adjacent 4-way and 3-way junctions. Interestingly, electrophoretic fractionation of RRE2 RNA under non-denaturing conditions revealed additional conformers (open and intermediate) that undergo conversion to the more stable, low energy (closed) form in a time-dependent manner. This transition was verified by kinetic analysis, and the structures of the three forms were determined using a novel method for mathematically dissecting an ensemble of SHAPE profiles. Site-directed hydroxyl radical footprinting was used to examine the relative positioning of select motifs within these structures in three-dimensional space. Overall, our study provided detailed insight into the RRE2 conformation, and as such will aid in the development and application of nanoparticle-based antiviral drugs targeting *cis*-acting RNA regulatory signals.

For the workshop "Tutorial on prediction of RNA secondary structure"

141 Structural spectrum of long non-coding RNAs revealed by experiment

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Thousands of long non-coding RNAs (lncRNAs) have been identified in the past few years. These RNAs have emerged as key players in development, cancer and brain function in mammalian systems. As many have been discovered only recently, very few structural studies have been performed. Fundamental questions regarding the structure of lncRNAs have yet to be addressed, including, (1) do lncRNAs have structure? and (2) are lncRNAs organized into modular sub-domains or linear chains of stem loops [1,2]? We have previously produced the first experimentally-derived secondary structure of an intact lncRNA by performing extensive biochemical studies in our wet lab using SHAPE probing, in-line probing, DMS probing and RNase V1 digestion [3]. Recently we have developed a new divide-and-conquer experimental strategy, called Shotgun Secondary Structure (3S) determination. The 3S experimental strategy allows us to produce the secondary fold with little need for computational predictions. First, we perform SHAPE probing on the entire lncRNA transcript. We repeat the probing on several overlapping fragments of the transcript. If the SHAPE profile for the fragment matches the profile for the same region in the context of the full transcript, this strongly suggests the fragment folds into a modular sub-domain or secondary motif, eliminating a large number of possible folds. We have applied this technique to the steroid receptor RNA activator (SRA1), the growth arrest-specific 5 transcript (Gas5), portions of MALAT-1 and other lncRNA systems. We find a variety of architectures, which may each be commensurate to the needs of the lncRNA system. The secondary structure of SRA1 resembles the 16S rRNA in terms of the modular organization into sub-domains and the relative number of helices, stem loops, internal loops and junctions. The Gas5 RNA consists of a roughly linear chain of stem loops separated by unstructured regions of RNA. Using SHAPE probing, we validated the triplex formation of the 3'-end of human MALAT-1, demonstrating that this motif is also structurally conserved in other species. Other systems show either a compact fold or a linear chain-like architecture. Numerous studies over the past few years have displayed a tremendous range of functional roles and disease applications for lncRNAs. Our results demonstrate that lncRNAs also have a diverse range of structural architectures.

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142 The transcription factors ILF2 and ILF3 are trans-acting factors for 60S ribosomal biogenesis

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The interleukin enhancer binding factors ILF2 (NF45) and ILF3 (NF90/NF110) were originally identified as a heterodimeric transcription factor complex required for Interleukin 2 expression in T-cells. Since then, the nuclear proteins ILF2 and ILF3 have been shown to be involved in various cellular pathways such as miRNA processing, DNA double-strand break repair and translation. Interestingly, ILF3 possesses two double-stranded-RNA binding domains (dsRBDs), allowing it to interact with different RNA species.

Using tandem affinity purification followed by mass-spectrometry analysis, we identified human ILF2 and ILF3 as novel components of premature particles of the large ribosomal subunit (pre-60S). This was supported by sucrose gradient analysis of HeLa cell extract where a fraction of NF45 and NF90, but not NF110, co-sedimented with pre-60S particles. Moreover, depletion of ILF2 or ILF3 by RNAi leads to a defect in 60S biogenesis as observed in a tetracycline-inducible RPL29-GFP cell line. Further, ILF2 or ILF3 downregulation results in a change in nucleolar size and number. These defects are not due to impaired transcription or processing of pre-28S or pre-18S ribosomal RNA (rRNA) as shown by fluorescence in situ hybridization and Northern blotting of different pre-rRNA species.

Notably, whereas most eukaryotic ribosomal trans-acting factors are conserved from yeast to higher eukaryotes, ILF2 is metazoan- and ILF3 vertebrate-specific. This suggests that the added layer of complexity in higher eukaryotes calls for additional regulatory factors in ribosomal biogenesis. Taken together, this indicates that ILF2 and ILF3 are novel, higher eukaryote-specific biogenesis factors required for the maturation of the 60S ribosomal subunit.

143 Novel function for human Argonaute 2 in gene regulation at the tRNA genes

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Enhancer sequences are critical for controlling cell-type specific gene expression. These can function at great distance from their target genes, and in some cases can be closer to non-target genes. The maintenance of specificity in enhancer-promoter interactions remains poorly understood. However, insulator sequences are critical in ensuring that only proper enhancer-promoter interactions are formed. Insulators block productive transcription when placed in between an enhancer and its promoter. In humans, the zinc-finger protein, CTCF is largely responsible for insulator function. It has recently been shown in *Drosophila* that Ago2 is essential for CTCF/CP190-dependent *Fab-8* insulator activity. Additionally, recent work in *S.pombe*, *D. melanogaster* and *H. sapiens* has shown that TFIIIC bound tRNA genes can function as insulator sequences. The mechanism of how these tDNAs can function as insulators remains unknown. An identification of the genome-wide binding sites for hAgo2 via ChIP-seq indicates an interaction with ~60% of tDNAs. We assessed TFIIIC-occupancy, which is a requirement for tDNA insulator activity in humans, and found that greater than 90% of Ago2-bound tDNAs are TFIIIC-occupied. This binding is cell cycle-dependent; binding is abolished when cells are halted in M-phase. We have further characterized the role of hAgo2 at human tDNAs by measuring changes in gene expression, histone modifications patterns, and long-range chromatin interactions in the absence of hAgo2. Furthermore, we have utilized a well-studied heterologous assay for measuring insulator activity to accurately quantify the impact the RNAi machinery has on insulator function in human cells. Our data suggests a novel function for the human RNAi machinery in regulating gene expression and large scale genome architecture.

144 The human cap-binding complex is functionally connected to the nuclear RNA exosome

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Nuclear processing and quality control of eukaryotic RNA is mediated by the multi-subunit RNA exosome, which utilizes accessory factors to regulate its enzymatic activity. However, the mechanism of exosome recruitment to its ribonucleoprotein (RNP) targets remains poorly understood. We now disclose a physical link between the human nuclear RNA exosome and the cap-binding complex (CBC). The CBC associates with the ARS2 protein to form CBC-ARS2 (CBCA), and then further connects together with the uncharacterized ZC3H18/NHN1 protein to the nuclear exosome targeting (NEXT) complex, forming CBC-NEXT (CBCN). RNA immunoprecipitation analysis using CBCN factors as baits as well as the combinatorial depletion of CBCN and exosome components underscore the functional relevance of CBC-exosome bridging at the level of target RNA. Furthermore, we reveal that CBCN and the RNA Exosome together enforce a dual-level suppression of spurious transcripts in human cells. Specifically, CBCA suppresses U2 snRNA read-through- and promoter upstream-transcription by promoting transcriptional termination in these regions. We suggest that the RNP 5'cap links transcription termination to exosomal RNA degradation via CBCN.

145 A splicing-dependent transcriptional checkpoint

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It is well established that pre-mRNA splicing and other RNA processing events often occur co-transcriptionally. Furthermore, high resolution kinetic studies performed in our lab showed splicing-dependent RNAPII pausing near the 3' splice site of a reporter gene[1]. Pausing requires splicing, as mutations that block splicing lead to loss of pausing, and restoring splicing restores pausing. We have proposed that RNAPII pausing may occur at splicing-dependent transcriptional checkpoints[1].

In a search for factors that are involved in coupling these processes, we identified several splicing factor mutations that cause transcription elongation defects. Prp5p is an RNA helicase that has been reported to act as a fidelity factor during pre-spliceosome formation[2]. We will present *in vivo* RNA labelling and RT-qPCR data demonstrating reduced transcription in *PRP5* mutant at the non-permissive temperature. ChIP analysis shows polymerase accumulating over intron-containing genes in the mutant strain, supporting a transcription elongation defect. We also analysed the phosphorylation status of the CTD of the large subunit of RNAPII, revealing that the apparently stalled polymerase is hyper-phosphorylated at Ser5. Furthermore, ChIP-Seq analysis in the mutant strain revealed that polymerase accumulated on 40% of the intron-containing genes genome-wide. We then identified that this transcription elongation defect in *prp5-1* is abolished upon removal of Cus2p, a U2 snRNP-associated protein. Our observations suggest that Cus2p could be a checkpoint factor in transcription prior to pre-spliceosome formation. We speculate that splicing fidelity factors may impose transcriptional checkpoints at different stages of splicing.

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146 The Exon Junction Complex core component MLN51 interacts with eIF3 and activates translation

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The multiprotein Exon Junction Complex (EJC) that is deposited onto mRNAs by the splicing machinery nicely exemplifies the impact of nuclear history on mRNP lifecycle. This processing mark influences numerous post-transcriptional steps including splicing, transport, translation and surveillance via the nonsense-mediated mRNA decay pathway (NMD). The EJC notably offers a selective advantage to newly synthesized mRNAs by stimulating their translation efficiency. However, the exact mechanism by which the EJC functionally communicates with the translation machinery remains poorly understood.

The EJC is organized around a tetrameric core complex that accompanies spliced mRNAs to the cytoplasm. The core is composed of the DEAD-box RNA helicase eIF4AIII, MAGOH, Y14 and MLN51 (also known as Barentsz). Here, we show that among the EJC core components, the RNA-binding protein MLN51 is a translation enhancer. Overexpression of MLN51 in human cells preferentially increased the protein expression of intron-containing reporters through its incorporation within EJC cores. In contrast, its silencing decreased the translation of both spliced and unspliced reporters. In addition, modulation of MLN51 level in cell-free translational extracts confirmed its direct role in protein synthesis while metabolic labeling experiments demonstrated that it is a general translation activator. Polysome fractionation and immunoprecipitations indicated that MLN51 associates with translation initiation factors and ribosomal subunits in an RNase-insensitive manner. Finally, *in vitro* binding assays with purified factors revealed that MLN51 directly interacts with the central initiation factor eIF3 and that this interaction is compatible with EJC core assembly.

Altogether, our data define MLN51 as a new translation activator that constitutes a positive link between nuclear splicing and cytoplasmic translation machineries via the EJC.

147 SRSF1-mediated translational regulation and its role in cellular transformation

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The serine and arginine-rich protein family (SR proteins) are highly conserved regulators of pre-mRNA splicing. The SR protein prototype, SRSF1 (also known as SF2/ASF), has been initially characterized as a splicing factor but also shown to mediate post-splicing activities such as mRNA export and translation. SRSF1 has also been proposed to act as an oncogene (1). We have previously shown that SRSF1 promotes translation initiation of bound mRNAs by suppressing the activity of 4E-BP, a competitive inhibitor of cap-dependent translation. This activity is mediated by interactions of SRSF1 with components of the mTOR signaling pathway. These findings suggest the model whereby SRSF1 functions as an adaptor protein to recruit the signaling molecules responsible for regulation of cap-dependent translation of specific mRNAs (2).

In order to dissect the importance of SRSF1 in translational control, we have performed a high-throughput deep sequencing analysis of polysomal fractions in cells overexpressing SRSF1. A group of more than one thousand mRNAs shifts from the subpolysomal fraction to the heavier polysomal fractions upon SRSF1 overexpression. Interestingly, one third of these mRNAs were previously identified as bona fide RNA targets of this SR protein by CLIP-seq. Bioinformatics analyses showed that these mRNAs encode proteins involved in cell cycle regulation, such as spindle, kinetochore and M phase proteins, which are essential for accurate chromosome segregation. In parallel, protein quantification using SILAC (Stable isotope labelling by amino acids in cell culture) confirmed that this subset of proteins was upregulated upon SRSF1 overexpression. Notably, SILAC analysis also revealed an increased expression of key glycolytic enzymes upon SRSF1 overexpression and several experiments confirmed a role for SRSF1 in enhancing a glycolytic switch (Warburg effect), a hallmark of proliferating cancer cells. Finally, we have analyzed changes in alternative splicing in response to different levels of SRSF1 protein. Interestingly, a significant proportion of those mRNAs that display alternative splicing changes are also translationally regulated by SRSF1. This suggests that SRSF1 influences several steps of an mRNA life.

Altogether, the finding that SRSF1 promotes the increased translation of genes associated with cell division and also regulates metabolic reprogramming in cancer cells could partially explain the oncogenic role of SRSF1. In summary, these data provide insights on the complex role of SRSF1 in the control of gene expression and its implications in cancer.

1. Karni et al. (2007) NatStruct Mol Biol., 14, 185-93.
2. Michlewski et al.(2008)Mol Cell, 30, 179-189

148 The RNA kinase CLP1 is required for efficient tRNA splicing and regulates p53 activation in response to oxidative stress

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Both in mammals and archaeabacteria CLP1 proteins have been identified as kinases that phosphorylate 5'-hydroxyl ends of RNA molecules. Human CLP1 is a component of the mRNA 3'-end cleavage and polyadenylation machinery and associates in mammals with the tRNA splicing endonuclease (TSEN) complex. TSEN proteins remove an intronic region present within the anticodon loop of numerous pre-tRNAs generating 5' and 3' tRNA exon halves. Within the TSEN complex, CLP1 has been shown to phosphorylate the 5'-hydroxyl group of 3'tRNA exons *in vitro*, potentially contributing to tRNA splicing in mammals. Here we characterize tRNA metabolism in kinase-dead *Clp1* (*Clp1K/K*) mutant mice that contain a point mutation within the ATP binding motif of the genomic *Clp1* locus. We show that extracts prepared from *Clp1K/K*embryonic fibroblasts display reduced TSEN cleavage activity, revealing an unexpected role for CLP1 during tRNA exon generation. Affinity purified kinase-dead CLP1 containing TSEN complexes were deficient in pre-tRNA cleavage, most likely due to reduced levels of TSEN2, TSEN34 and TSEN54 subunits. Thus CLP1 is an integral component of the TSEN complex and ATP binding and/or hydrolysis is crucial for complex assembly. Interestingly, loss of CLP1 activity causes increased cell death upon H_2O_2 challenge and leads to accumulation of an entirely novel set of small tRNA fragments, comprising 5' leader and 5' exon sequences derived from aberrant processing of mainly tyrosine pre-tRNA. Overexpression of such tRNA fragments results in enhanced p53 activation in response to H_2O_2 challenge. We thus hypothesize that inactivation of CLP1's kinase activity sensitizes cells to oxidative stress-induced p53 activation and p53-dependent cell death.

149 GENOME-WIDE IDENTIFICATION OF RECURSIVE SPLICING IN DROSOPHILA

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In *Drosophila*, the vast majority of introns are very short. As a result, the exons that are to be spliced together are in close proximity and can find one another easily. There are, however, many large introns (>50 kb) in *Drosophila* where it may be more difficult to juxtapose the exons that are to be spliced together. The 74 kb intron in the *Ultrabithorax* gene has been shown to be removed by a process called recursive splicing in which an upstream exon is spliced to a location within the intron, and the sequence of this splice junction re-creates a 5' splice site that can be subsequently respliced to another 3' splice site further downstream. The sites at which recursive splicing occurs are referred to as ratchet points. Though recursive splicing was discovered in 1998, only three additional genes (*kuzbanian, outspread,* and *frizzled*) have been experimentally shown to utilize recursive splicing to remove long introns. We have identified over 30 genes that undergo recursive splicing (and 70 ratchet points) by analyzing total RNA-Seq data generated from developmental time points, dissected tissues, and cultured cells. The recursive splicing events were identified by the presence of three specific features: 1) a novel splice junction that mapped to an annotated 5' splice site and an unannotated intronic 3' splice site, 2) the presence of the sequence AG/GT at the unannotated 3' splice site, and 3) a 5' to 3' gradient of RNA-Seq read intensity indicative of co-transcriptional splicing. We have validated many of these recursive splicing events by directed RT-PCR and sequencing. The average size of the intronic segments removed by recursive splicing is ~10 kb, and as many as 5 ratchet points have been identified in a single intron. Recursive splicing is detectable in all samples in which the host gene is abundantly expressed, indicating that recursive splicing is a constitutive process – we observe no evidence that recursive splicing occurs in some tissues or cell types, but not others. The ratchet points have a strong consensus sequence and are highly conserved among *Drosophila* species. We are currently performing RNA-Seq experiments in distantly related insects to investigate the functional conservation of recursive splicing. Together these results indicate that recursive splicing is commonly used in *Drosophila* and provides insight into the general mechanisms by which long introns are removed.

150 Isolated pseudo-RRMs of SR proteins can regulate splicing using a non-canonical mode of RNA recognition

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SR proteins, one of the major families of alternative-splicing regulators in Eukarya, have two types of RNA-recognition motifs: a canonical RRM and a pseudo-RRM. Although pseudo-RRMs are crucial for activity of SR proteins, their mode of action was unknown. By solving the structure of the human SRSF1 pseudo-RRM bound to RNA, we discovered a very unusual and sequence-specific RNA binding mode that is centered on one alpha-helix and does not involve the beta-sheet surface of the RRM. Remarkably, this mode of binding is conserved in all pseudo-RRMs tested. Furthermore, the isolated pseudo-RRM is sufficient to regulate splicing of about half of the SRSF1 target genes tested, and the bound alpha-helix is a pivotal element for this function. Our results strongly suggest that SR proteins with a pseudo-RRM frequently regulate splicing by competing with, rather than recruiting, spliceosome components, using solely this unusual RRM.

151 RBM5 OCRE domain modulates alternative splicing regulation by recognition of proline-rich motifs in spliceosomal SmN/B/B'

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RBM5 is a putative tumor suppressor gene frequently inactivated in cancers of the lung and other tissues and is down-regulated upon oncogenic Ras activation. Its main protein product is a 92 kDa, multi-domain protein that has been detected in pre-spliceosomal complexes and modulates cell proliferation and Fas mediated apoptosis. RBM5 was identified as a component of complexes involved in 3' splice site recognition, but contrary to classical mechanisms of splicing regulation, RBM5 does not affect early events of splice site recognition that lead to exon definition. Instead, RBM5 inhibits the transition between pre-spliceosomal complexes assembled around exons to mature spliceosome assembly on the flanking introns. Protein-RNA and protein-protein interactions are important for RBM5 regulation of alternative splicing of different target genes. Here, we examine the interaction of individual RBM5 domains with protein components of the spliceosome that are important to regulation of Fas exon 6 splicing.

The OCRE (OCtamer REpeat of aromatic residues) domain is important for RBM5 function in vivo and interacts with components of the U4/5/6 tri-snRNP. Using NMR spectroscopy, we show that the RBM5 OCRE domain adopts a novel β-sheet fold with a hydrophobic surface rich in tyrosine residues that are key determinants both for the interaction with protein partners and for splicing regulation in vivo. The structure of a complex of RBM5 OCRE with a proline-rich motif (PRM) from the core spliceosomal SmN proteins and mutational analysis reveal that tyrosine residues on the hydrophobic surface of OCRE recognize a poly-proline helix, while flanking arginines in the PRM define the orientation. Interestingly, OCRE binds to individual proline-rich motifs with mM affinity, but with μM affinity for the C-terminal tail of SmN, which contains multiple PRM motifs, suggesting avidity effects. Our data reveal how protein-protein interactions of the OCRE domain docks RBM5 to spliceosomal core protein for alternative splicing regulation.

152 GSK3-Induced Regulation of the Protein- and RNA-Binding Activity of PSF Modulates Signal-Induced Alternative Splicing.

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To maintain viability, virtually all cells in the human body must regulate their function in response to environmental stimuli. Such functional regulation requires control of gene expression in a signal dependent manner, including signal-regulated control of alternative splicing. However the molecular mechanisms by which signaling pathways impinge on splicing decisions remain largely unexplored.

We have recently identified hundreds of exons that display differential inclusion in response to antigen-stimulation of T cells. The best characterized of these T cell signal-regulated splicing events is the induced skipping of three variable exons of the CD45 gene. We have previously shown that signal-responsive repression of the CD45 exons is mediated in large part through the activity of the multifunctional RNA-processing protein PSF. PSF binds the exonic silencer elements of the CD45 pre-mRNA following T cell activation and favors variable exon exclusion. In resting cells, however, PSF is prevented from binding the ESS1 through a protein-protein interaction with TRAP150, which is dependent on phosphorylation of PSF at position T687 by the kinase GSK3.

Here we show that the two RNA recognition motifs (RRMs) of PSF are necessary and sufficient for binding to TRAP150. This interaction is direct and can be recapitulated with purified bacterial proteins. We further show that the RRMs of PSF interact in a mutually exclusive manner with TRAP150 and RNA, providing a mechanism for how TRAP150 blocks the function of PSF in the regulation of CD45 splicing. Surprisingly, the RRMs of PSF do not encompass the regulatory T687 residue, suggesting that phosphorylation of PSF-T687 regulates interaction with TRAP150 indirectly. Interestingly, limited proteolysis of PSF reveals a phosphorylation-dependent difference in digestion pattern, suggesting that phosphorylation of T687 induces a conformational change in PSF which controls accessibility to TRAP150. Together these data provide a unique example of signal-dependent control of an RNA binding protein through allosteric regulation of protein and RNA binding. Finally, we show that inhibition of GSK3, which occurs in response to T cell stimulation, is sufficient to account for approximately 40% of the alternative splicing events we observe during an antigen response in T cells. Thus we conclude that regulation of PSF by GSK3 plays a widespread role in shaping gene expression in T cells during an immune response.

153 Rhythmic U2AF26 alternative splicing regulates the circadian clock in mice

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The circadian clock is a cell-autonomous regulator driving daily rhythms in metabolism, behavior and physiology. At the molecular level, clock components of interlocking transcriptional feedback loops have been thoroughly investigated, whereas the impact of post-transcriptional regulation, especially alternative splicing, remains largely unexplored.

Here we describe a splice variant of mouse U2AF26 lacking exons 6 and 7 (U2AF26DE67) whose expression is strongly regulated with the circadian clock. Skipping of exons 6 and 7 induces a frameshift, thereby generating 144 novel C-terminal amino acids with interesting properties: the new C-terminus displays homology to the *D. melanogaster* core clock component Timeless and in addition substantially reduces the half-life of U2AF26DE67 protein, enabling diurnal expression of this specific isoform. Consistent with a function in regulating the circadian clock, we show that U2AF26DE67 interacts with and destabilizes Per1 protein in a cell culture model. To confirm this activity in vivo, we have generated U2AF26 deficient mice and analyzed the expression of core clock components at different circadian times. In line with our cell culture data, U2AF26 knockout mice display nearly acyclic expression of Per1 protein in the liver as well as a reduced and shifted peak of Per1 mRNA both in liver and cerebellum. mRNA expression of several other clock components such as Dbp, Npas2, and RevErba also showed a shifted expression profile, suggesting a broad circadian phenotype in U2AF26 deficient animals. We are now investigating whether these defects in the molecular clock of U2AF26 deficient mice lead to disturbed circadian locomotor activity.

In summary, we introduce U2AF26 as a novel component of the mammalian clock. We provide evidence for a circadian alternative splicing switch that directly regulates the expression of a central clock component in mammals. In addition, U2AF26 exon 6 and 7 exclusion is an interesting case for a splicing induced frameshift, resulting in translation into the 3'UTR to generate a protein with a new C-terminus with novel functions.

154 RNA Binding Protein Sfpq is required for the expression of neuron-specific long pre-mRNAs essential for brain development.

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Recent methodological advances using microarray, deep-sequencing and biochemical analysis combining bioinformatics provide growing evidence for the essential roles of mRNA processing on the neural development. The mRNA processing is mediated by the RNA-binding proteins which regulate the expression of many genes co-transcriptionally or post-transcriptionally through direct interaction with pre-mRNAs. Here we found that RNA-binding protein, Sfpq (splicing factor, proline/glutaminerich) plays an essential function in mammalian brain. In embryonic mouse brains, *Sfpq* is specifically expressed in nascent cortical plate neurons after they differentiate and migrate from neuronal progenitor cells, suggesting its crucial functions for the development of cerebrocortical neurons. To address the *in vivo* function of Sfpq, we disrupted the *Sfpq* gene in mice brain. Neuronal tissue specific knockout of *Sfpq* caused apoptosis in neurons and massive loss of brain tissues in the developing brains including neocortex, indicating that Sfpq is essential for differentiation or maturation of neurons. Next, we produced a specific antibody against Sfpq, and conducted the iCLIP analysis to identify the target of Sfpq in the mouse embryonic neocortex. Distribution of the iCLIP tags showed saw-tooth patterns on entire pre-mRNAs of more than 7400 genes with low sequence specificity. The density of the tags was highest in 5' end of introns, and gradually declined to 3' end. In these large populations of Sfpq-binding genes, only specific gene subsets which express long pre-mRNAs were significantly down-regulated in the *Sfpq*-disrupted mice brain. According to the Gene Ontology, these genes have essential functions for the brain development, such as cell-adhesion, cell motion, axon guidance, ion channel activity or receptor related molecules, and synaptic vesicle transport and related molecules. Our comprehensive transcriptome analysis showed that 17.4% of specifically expressed pre-mRNAs exceed 262k in differentiated neurons . These data indicate that the RNA binding protein Sfpq is required for the expression of long pre-mRNAs which play essential roles for the survival of neurons, especially in the cortical plate at the developmental stages of brain.

155 The SWI/SNF subunit Brahma modulates the choice of alternative terminal exons by recruiting the BRCA1/BARD1 ubiquitin ligase

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The choice of polyadenylation sites located in alternative terminal exons is a widespread phenomenon that increases transcript and protein diversity. The choice of alternative poly(A) sites generates different 3'UTRs that can affect translation, stability and localization of the mRNA. When coupled to the inclusion of an alternative last exon (ALE) alternative polyadenylation leads to the generation of mRNA variants that not only differ in their 3'UTR but may also encode proteins with different C-terminal regions. Whereas the molecular details of mRNA polyadenylation are rather well known, little is known about the choice between alternative polyadenylation sites.

Brahma (BRM), one of the two alternative ATPase subunits of the human SWI/SNF chromatin-remodeling complex, was previously shown to control alternative splicing by slowing down RNA polymerase II. Here we show that the effect of BRM on the selection of ALEs does not involve the modulation of the elongation rate of RNAPII but rather implicates a different molecular mechanism. We report that BRM favors the selection of the distal ALE by inhibiting 3' end processing at the proximal poly(A) site. BRM accumulates on the proximal ALE promoting an interaction between the 50 kDa subunit of the 3' end processing factor CstF and the BARD1/BRCA1 E3 ubiquitin ligase. Ubiquitination of CstF50 leads to dissociation of the CstF complex and consequently to the inhibition of 3' end processing. We propose a model in which commitment of the proximal ALE to splicing and 3' end processing would require a reduction of the RNAPII elongation rate. In contrast, selection of the distal ALE would necessarily require both a highly elongating polymerase and inhibition of 3' end processing at the proximal poly(A) site to allow transcription to continue past the proximal ALE. This would result in the recruitment of splicing factors on the downstream 3' splice site, resulting in the 3' end processing complex at the distal poly(A) site and the splicing out of the proximal ALE.

NOTES

POSTER ABSTRACTS

156 A Natural antisense transcripts in Neurospora crassa

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Antisense (AS) transcripts have been shown to play an important role in regulating the expression of genes in many organisms. Regulation of transcription, transcript splicing, stability and translation by AS transcripts has been reported. Here we report the prevalence of *cis*-AS transcripts in the model organism, *Neurospora crassa.*

RNA sequencing was performed using ABi SOLiD technology on RNA extracted from cultures grown in constant darkness at 25°C or exposed to a light or temperature pulse. The RNASeq reads were mapped to the genome and assembled into transfrags. We detected 6771 (~70%) transfrags arising from previously annotated genes that are expressed above a threshold level. Those transfrags that were not located close to a previously annotated transcript were considered to be novel transcripts. This led to the detection of over 1000 novel transcripts, only 60 of which were predicted to have significant coding potential. Further analysis led to the identification of more than 400 annotated sense/AS pairs and more than 450 novel transcripts that are expressed antisense to annotated genes. 57 and 34 novel transcripts were found to be differentially expressed in response to light and temperature respectively, approximately half of these were novel AS transcripts. The expression of several sense and AS transcripts was confirmed by RT-PCR. Hence, this study reveals the prevalence of novel *N. crassa* noncoding and AS transcripts and their expression in different environments.

157 B Overexpression of small noncoding RNAs and its effects on biofilm-related phenotypes in Escherichia coli

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It is undoubted that noncoding RNA has been deeply involved in various metabolic pathways in organisms ranging from bacteria to mammals many different organisms. Although nearly 100 small noncoding RNAs (sRNAs) have been experimentally verified in *E. coli*, knowledge about their roles has been continuously expanded through identification of functions of newly identified sRNAs as well as new roles of previously known sRNAs. Recently, roles of sRNAs in bacterial group behavior or pathogenesis have received a great deal of attention. Biofilm, a surface-bound and self-aggregate structure of bacteria embedded within extracellular polymeric substances, is one of their important group behaviors for survival in varying environmental challenges. In this study, a plasmid library expressing 99 experimentally verified *E. coli* sRNAs was constructed on IPTG-inducible RNA expression vector pHMB1/2. Using this sRNA-expressing library, changes of biofilm related phenotypes upon overexpression of each sRNA were examined. Especially flagella-based motility and cell surface appendages were analyzed in details.

158 C Defense against viral attack: single-molecule view on a bacterial adaptive immune system

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Escherichia coli maintain different strategies to protect the cell against invading foreign DNA. In a recently discovered adaptive immune system, fragments of foreign DNA are integrated into specific loci on the bacterial genome, known as clustered regularly interspaced short palindromic repeats (CRISPR). Short CRISPR-derived RNAs (crRNAs) are incorporated into the CRISPR-associated complex for antiviral defence (Cascade) and guide the complex's search for the DNA of returning invaders, which is targeted for destruction upon binding. Recent studies have shown that Cascade must recognize both a short "seed" sequence and an immediately adjacent PAM (protospacer adjacent motif) sequence in order for successful targeting of the foreign DNA. The mechanism and structural dynamics of this target recognition and binding process, however, are not well understood. Here we report a single-molecule FRET (F?rster resonance energy transfer) based assay to monitor in real time the target recognition and binding process of Cascade. We find that there is directionality to the base-pairing process between the crRNA and the target DNA. Upon binding, pairing occurs first over the seed region and then, after a time lag, proceeds through a region further downstream on the target sequence. This suggests a mechanism for target recognition wherein the seed/PAM region is identified first, followed by a distinct kinetic intermediate, perhaps a structural transition, before subsequent target pairing can occur. Our single-molecule study promises to reveal the mechanism of target DNA identification by the CRISPR immune system.

159 A TDP-43 regulates cancer-associated microRNAs

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MicroRNAs (miRNAs) play important roles in a wide range of biological processes. Aberrant regulation of miRNA genes contributes to human diseases, including cancers. The TAR DNA binding protein 43 (TDP-43), a DNA/RNA binding protein associated with neurodegeneration, is involved in miRNA biogenesis. Here, we systematically examined miRNAs whose expression levels are regulated by TDP-43 using RNA-Seq coupled with siRNA-mediated knockdown approach. Candidate TDP-43 targeted miRNAs were validated by quantitative RT-PCR. Some of TDP-43 regulated miRNAs appear to interact with TDP-43. Alterations in isomiR patterns and miRNA arm selections following TDP-43 down-regulation indicated TDP-43 is involved in miRNA editing. We examined correlation of selected TDP-43 regulated miRNAs and their candidate target genes and their expression patterns in human cancers. Our data show that TDP-43 promotes migration of lung cancer cell by regulating expression of TDP-43 target miRNAs. We have begun to analyze the correlation of expression of TDP-43 regulated miRNAs with disease outcome. Our experiments have also begun to reveal previous unknown miRNA-target gene pairs that may play important roles in cancer and other diseases.

160 B An RNA Degradation Machine Sculpted by Ro Autoantigen and Noncoding RNA

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Although the functions of many RNA-protein complexes (RNPs) are well understood, the roles of others are still being elucidated. One class whose function remains under investigation is the Ro class of RNPs. The major protein component, the ring-shaped Ro 60 kDa autoantigen (Ro60), is present in many animal cells and also in ~5% of sequenced bacterial genomes. In all characterized species, Ro60 binds ~100 nt noncoding RNAs (ncRNAs) of unknown function called Y RNAs. Because Ro60 also binds misfolded rRNAs and snRNAs in some animal cell nuclei, it is proposed to function in ncRNA surveillance. In the only bacterium in which Ro60 has been characterized, *Deinococcus radiodurans*, the ortholog Rsr functions with 3' to 5' exoribonucleases during some types of environmental stress. Specifically, Rsr and the exoribonucleases RNase II and RNase PH are required for efficient 23S rRNA maturation during heat stress. In addition, Rsr and the exoribonuclease polynucleotide phosphorylase (PNPase) are involved in rRNA degradation during stationary phase.

To understand how a Ro60 protein can influence the function of an exoribonuclease, we purified the Rsr/PNPase complex from *D. radiodurans* and examined its composition, molecular architecture and activity. We discovered that Y RNA tethers Rsr to PNPase to form an RNA degradation machine. Single particle electron microscopy (EM), followed by docking Ro and PNPase atomic structures into the three-dimensional reconstruction, revealed a double ring architecture, suggesting that Rsr channels RNA into the PNPase cavity for degradation. Biochemical experiments revealed that Rsr and Y RNA specialize PNPase for degrading structured RNA. Specifically, the Rsr/Y RNA/PNPase RNP is more effective than PNPase alone in degrading stemloop-containing RNAs, but is less active than PNPase on single-stranded RNA. These studies identify a role for Y RNA and also show that ncRNA, by tethering a protein cofactor, can alter the substrate specificity of an enzyme.

To determine if the role we identified for Rsr and Y RNA in *D. radiodurans* could be conserved in other bacteria, we examined *Salmonella* Typhimurium. We found that *S.* Typhimurium Rsr associates with two novel ncRNAs encoded 3' to *rsr*. One ncRNA, which we call YrlA, appears to be highly conserved, as potential orthologs are encoded adjacent to the Rsr ortholog in more than 100 bacterial genomes. Notably, by performing immunoaffinity experiments in *S.* Typhimurium, followed by glycerol gradient sedimentation, we found that Rsr and YrlA also associate with PNPase in this bacterium. Thus, assisting degradation of structured RNAs is likely to be a conserved role of bacterial Ro RNPs.

161 C The role of RNA degradation in moderating RNAi

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Endogenous antisense non-coding RNAs could theoretically hybridise to their cognate sense RNA, forming double-stranded RNAs that are substrates for the Dicer endonuclease. Therefore, antisense transcripts have the potential to trigger RNA interference responses (RNAi) that would lead to the degradation of both sense and antisense RNA from a locus, providing a potent negative regulatory capability. Although antisense RNAs have been characterised at many loci in many organisms, they are generally unstable which would limit entry of sense-antisense pairs into the RNAi system. Antisense RNAs are susceptible to various degradation pathways acting in both the nucleus and cytoplasm, raising the potential for wide ranging gene expression regulation through control of antisense degradation.

S. cerevisiae does not possess an endogenous RNAi system, but such a system can be reconstituted by introducing Argonaute and Dicer from a related yeast species to form an RNAi+ strain [1]. We have tested the efficiency of RNAi in these RNAi+ cells for a number of sense-antisense systems in which the antisense RNA is unstable. Stabilisation of the MAL32 antisense RNA by nuclear RNA degradation mutants increases the level of siRNAs produced from this locus, showing that stabilisation of antisense RNA in the nucleus can alter double stranded RNA formation as expected. However, altering cytoplasmic RNA levels by deletion of XRN1has more subtle effects; although levels of GAL4 sense and antisense are increased in this mutant, there is no increase in siRNA production. In contrast, stabilisation of the PDR3 antisense in the same cells leads to complete degradation of sense and antisense RNA by RNAi as predicted.

Our data show that RNA degradation can alter the susceptibility of mRNA to degradation by RNAi. This suggests a previously unrecognised role for the RNA degradation machinery in regulating entry of RNA from coding loci into the RNAi system. We are currently using high-throughput sequencing to elucidate genome-wide the effects of RNA degradation mutants on RNAi.

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162 A The role of non coding RNA at sites of DNA damage in the control of genome integrity

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The DNA damage response (DDR) is a signaling pathway that arrests the proliferation of cells undergoing genotoxic events to preserve genome integrity. DICER and DROSHA are crucial ribonucleases involved in RNA interference (RNAi). Components of RNAi are thought to have evolved to preserve genome stability from the attacks of viruses and mobile genetic elements. RNAi and DDR pathways had not been demonstrated to directly interact.

We have recently shown (Francia et al. Nature 2012) in cultured human and mouse cells and in zebrafish larvae that DICER and DROSHA, but not downstream elements of the RNAi pathway, are necessary to activate the DDR upon exogenous DNA damage and oncogene-induced genotoxic stress, as studied by DDR foci formation and by DNA-damage checkpoint assays. DDR foci are sensitive to RNase A treatment, and DICER- and DROSHA-dependent RNA products are required to restore DDR foci in RNase-A-treated cells. Through RNA deep sequencing and the study of DDR activation at a single inducible DNA double-strand break (DSB), we identified site-specific DICER- and DROSHA-dependent small RNAs, named DDRNAs, with the sequence of the DNA surrounding the DSB that are required for DDR foci formation. DDRNAs, either chemically synthesized or in vitro generated by DICER cleavage, are sufficient to restore the DDR in RNase-A-treated cells, even in the absence of other cellular RNAs. Therefore, DDRNAs are a novel class of ncRNAs that play a direct role in the control of DDR activation at sites of DNA damage.

Our progresses in understanding DDRNAs biogenesis and functions will be presented.

163 B The microRNA pathway mediates expression of yolk lipoproteins in the Caenorhabditis elegans intestine

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In the nematode *Caenorhabditis elegans*, yolk lipoproteins (VIT proteins) are synthesized in the hermaphrodite intestine, secreted, and internalized by the oocytes where their associated lipids provide nourishment throughout embryogenesis [1]. Here, we show that the microRNA (miRNA) pathway is required for proper production of the VIT lipoproteins, and consequently, for proper embryonic development. An expression analysis of the *vit* genes revealed that the miRNA pathway functions upstream of *vit* transcription. Moreover, two miRNAs, *let-7* and *lin-4*, are specifically required for proper endogenous *vit* gene expression, as well as activation of a GFP-based *vit-2* transcriptional reporter. Using the *vit-2* reporter, we have identified two gene inactivations that suppress the effects of a *let-7* mutation and partially restore *vit-2* gene expression. These two genes, *lin-14* and *lin-41*, are known targets of the *let-7* and/or *lin-4* miRNAs. We are currently investigating whether *let-7* and *lin-4* function within the intestine to control *vit* gene expression, or alternatively, whether they act in surrounding tissues to regulate intestinal development.

1. Kimble J, Sharrock WJ (1983) Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans*. Dev Biol 96: 189–196.

164 C The exosome subunit Rrp6 regulates the expression of retrotransposons and non-coding heterochromatic sequences in Drosophila melanogaster

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The exosome is a multiprotein complex with ribonuclease activity that is highly conserved from archaea to eukaryotes. The exosome has many functions and acts on many different transcripts, but the mechanisms by which the exosome is recruited to its different substrates are not fully understood. We have applied immnoprecipitation and mass spectrometry to identify proteins associated with individual exosome subunits in *Drosophila melanogaster* S2 cells. Among the many proteins associated with the catalytic subunit Rrp6, we have found two functionally related factors involved in heterochromatin formation: the histone deacetylase Rpd3 and the histone methyltransferase specific for H3-Lys9, SuVar3-9. We have also shown that the Heterochromatin Protein 1, HP1a, is associated with Rrp6. Moreover, immunofluorescent staining of salivary gland polytene chromosomes revealed that Rrp6 is associated with heterochromatic regions of the genome. Knock-down of Rrp6 by RNA interference in *Drosophila* S2 cells resulted in increased levels of both transposon transcripts and non-coding RNAs originated from heterochromatic regions of the genome, whereas overexpression of Rrp6 had the opposite effect. Moreover, reduced levels of Rrp6 rendered the heterochromatin less compact, as shown by experiments of micrococcal nuclease digestion. Ongoing research aimed at understanding the function of Rrp6 in heterochromatin regulation will be presented.

165 A Identification and Analysis of New Genes Targeted for sRNA Regulation in Bacteria

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Many bacteria use trans-encoded sRNAs to respond to environmental conditions. This pathway typically uses the RNA-binding protein Hfq to facilitate the identification of target mRNAs and to promote pairing between the sRNA and its mRNA partners. This interaction subsequently leads to altered translation or targeted RNA degradation. Because *trans*-sRNAs act by imperfect base pairing they often regulate multiple mRNAs, forming a network of regulatory actions. Annotation of sRNAs in bacteria has been extensive but it has been harder to identify and validate the direct targets of sRNA regulation.

Guided by knowledge of the motifs found on mRNAs and their common location within mRNA transcripts. We have identified a collection of new regulatory circuits. While the methodology for discovering these regulated genes still reports some false positives – validation shows that roughly 1/3 of the predicted targets do in fact undergo direct regulation when studied with reporter gene constructs.

The discovery of new mRNA targets demonstrates an increased complexity of the sRNA regulatory network in bacteria. More importantly, the ability to predict new mRNA targets provides significant benefits for genomes beyond *E. coli* where less is known about the biology of regulatory RNAs and provides a means to rapidly elucidate these pathways.

166 B Novel RNA Structures Controlling RIbosomal Protein Biosynthesis in E. coli and Beyond

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The control of ribosomal protein biosynthesis is tightly coordinated in eubacteria. In *E. coli* there are over 10 different RNA structures that act to autogenous regulate over half of the ribosomal protein genes. Each of these cis-regulatory RNAs interacts with a specific ribosomal protein to inhibit transcription or translation of an entire operon encoding multiple ribosomal proteins. Despite the important role these RNAs play in regulating the biosynthesis of an essential process, our recent work shows that many of them appear to be narrowly distributed to a few groups of closely related bacteria. This, in combination with the discovery of several putative RNA structures associated with ribosomal protein genes in different phyla of bacteria, suggests that there are numerous RNA structures responsible for regulation of ribosomal protein biosynthesis that remain to be discovered.

Using comparative genomics we identified several RNA structures associated with ribosomal protein operons, including one that precedes ribosomal protein genes *rpsF* and *rpsR,* encoding ribosomal proteins S6 and S18 respectively. This RNA structure is widely distributed to many bacterial phyla (it is found in both *E. coli* and *B. subtilis*), and overlaps a potential Shine-Dalgarno sequence in many organisms. Using *in vitro* assays we confirmed that this RNA interacts specifically with S18, and with an S6:S18 dimer suggesting that the RNA is a novel autogenous regulatory element responsible for coordinating the levels of ribosomal proteins in bacteria. This work shows that comparative genomic methodologies applied so successfully to identify riboswitch candidates may also be applied to the discovery of RNA regulators for ribosomal protein biosynthesis that may have been overlooked using previous approaches.

167 C The RNA subunit of RNase MRP: extra nucleotides at the 3' end

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RNase MRP is a eukaryotic ribonucleoprotein complex with specific endoribonucleolytic activity targeting a variety of vital cellular RNAs. A recent publication proposed a mechanism for regulation of the RNA subunit of MRP whereby RMRP RNA is a substrate for RNA dependent RNA polymerase activity when complexed with the protein subunit of human telomerase (hTERT)1. Our follow-up experiments demonstrate that, contrary to the previous report, the hTERT active site is not required for extension of RMRP. Instead, 3' extension activity is sensitive to alpha-amanitin and can be observed *in vitro* with purified RNA polymerase II. Further, traditional and next generation sequencing of RMRP 3' ends from total RNA of human cells reveals a distribution of terminal extensions from the annotated 3'-most nucleotide, including non-genomically encoded sequences. Sequencing of 3' ends of other common cellular RNAs yields somewhat more homogeneous profiles, notably lacking termini with complex non-genomically encoded sequences. Our current work aims to critically examine whether the *in vitro* additions recapitulate these produced *in vivo*, and ultimately characterize the significance of those extensions on RMRP function.

1. Maida, Y. *et al.* An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. *Nature* 461, 230-235, doi:10.1038/nature08283 (2009).

168 A Alu RNAs as possible modulators of microRNA function

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Alu elements are the most abundant repetitive elements in the human genome and belong to the class of short interspersed elements (SINEs) which are non-autonomous retrotransposons. Approximatively one million copies of Alu elements pervaded the genome throughout evolution making up more than 10% of it. They derive from the 7SL RNA gene, which encodes the RNA moiety of the signal recognition particle (SRP).

Depending on the site of integration during retrotransposition, Alu elements can be embedded in coding genes and are therefore co-transcribed by RNA polymerase II (Pol II). Additionally, Alu elements can be transcribed by RNA polymerase III (Pol III), since they have partially retained internal promoter regions.

Under normal conditions free Alu RNAs accumulate at low levels. Their abundance increases dramatically under various stress conditions such as heat shock, viral infection, and exposure to toxic agents. Little is known about the molecular function of free Alu RNAs. Some studies pointed out that they inhibit transcription by direct interaction with Pol II. Other groups reported naked Alu RNAs to have a stimulatory role for protein translation, while Alu RNAs bound to protein components of the SRP inhibit initiation of translation.

Recent data suggest that Alu RNA might have direct toxic effects in the pathogenesis of age-related macular degeneration, a disease leading to progressive loss of central vision. Here, decreased protein levels of the RNAseIII enzyme Dicer (Dcr), which is required for microRNA (miRNA) biogenesis, have been associated with accumulation of Alu RNAs while miRNA expression remained unaffected. The authors claimed for a direct role of Dcr in the degradation of Alu RNAs. Our study aims to determine if Alu RNAs are in fact degraded or processed to small RNAs by Dcr and if a crosstalk between Alu RNA expression and the miRNA regulatory pathway exists.

169 B RNA annealing activity of Hfq is sensitively modulated by various physical parameters

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Bacteria regulate their gene expression level in response to various environmental stresses. One of their regulation methods is small non-coding RNAs (sRNA) which acts at the post-transcriptional level by base-paring with their target mRNA. For example, *E. coli* DsrA sRNA is expressed under cold shock and up-regulates RpoS (Sigma-S) transcription factor by base-paring with *rpoS* mRNA. It is well know that this sRNA regulation process requires the RNA chaperone Hfq for efficient RNA duplex formation. However, how the RNA annealing activity of Hfq is modulated under varying physical parameters due to various environmental stresses is ill-understood. Here we investigate how the annealing activity of Hfq is modulated by various physical parameters by using single-molecule FRET assays. We use dye-labeled synthetic RNA fragments obtained from *E. coli* DsrA sRNA and from its target mRNA, *rpoS*. Our results show that low temperature increases the annealing efficiency by accelerating Hfq dissociation from DsrA:*rpoS*:Hfq ternary complex. Relative ratio between RNA and protein is also important as an excess Hfq lowers annealing efficiency by inhibiting DsrA:*rpoS*:Hfq ternary complex formation. The concentration of various salts greatly influences the annealing efficiency by increasing the turnover number of Hfq. Finally, the molecular crowding, which Hfq may encounter in vivo, enhances the annealing efficiency by increasing effective concentration of RNA and Hfq. Collectively, our results suggest that the activity of Hfq can be sensitively modulated by various physical parameters found *in vivo*.

170 C Identification of stage specific microRNAs during the developmental stages in Triops cancriformis (tadpole shrimp)

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microRNAs (miRNAs) are approximately 22 nucleotide non-coding RNAs that regulate gene expression at the post-transcriptional level, and play important roles in the cellular processes such as development and cell differentiation. Although many miRNAs are identified and analyzed in various model species during their development, it is unclear whether the miRNA functions are conserved among species or not. Here we focused on non-traditional model species *Triops cancriformis*, which is known as a "living fossil". *T. cancriformis* changes their morphology dramatically during the early larval developmetal stages. It was hypothesized that miRNA expression also dramatically changes in accordance with the morphological changes.

We first constructed small RNA libraries from six different stages of *T. cancriformis* development (egg, 1st to 4th instar larvae and adult). Deep sequencing analysis of these libraries resulted approximately 47 million reads. In parallel, we also performed deep sequencing analysis on genomic DNA that provided approximately 133 million reads. Bioinformatics analysis have shown 73 conserved miRNA sequences in *T. cancriformis*by using known miRNAs in other species registered in miRBase. It was confirmed that these 73 candidates were encoded on genomic DNA sequences and able to form a secondary structure of precursor miRNAs. Among these miRNA candidates, the expressions of eight *T. cancriformis* miRNAs were detected by northern blotting analysis among six developmental stages. We compared miRNA expression patterns of *T. cancriformis* with those of *D. melanogaster* reported from previous studies. As a result, *T. cancriformis* tcf-let-7 showed conserved expression profiles, whereas tcf-miR-87 showed different patterns. While tcf-miR-87 is strongly expressed in adult stage, *D. melanogaster* dme-miR-87 is detected in egg, 1st instar, 2nd instar, pupa, and adult stages. The inconsistency in the expression profile of conserved miRNAs suggest that these miRNAs may play different roles during development in each species, although they possess very similar sequences. The relationships between dynamic morphological changes and the miRNA expression pattern during the development will be discussed.

171 A A tandem-stem RNA motif mediates X-chromosome dosage compensation in Drosophila

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Long non-coding RNAs (lncRNAs) are emerging as important regulators of chromatin state and transcription in eukaryotic cells. They contribute to the regulation of single genes or whole chromosomes and can influence the 3-D structure of large genomic regions. Due to their length, which typically is in the range of kilobases, it is difficult to determine functional domains in these lncRNAs and therefore understand their exact contributions to transcriptional regulation. A prime example of lncRNAs regulating chromosome-wide gene expression is X-chromosomal dosage compensation. Sexually dimorphic organisms often correct for the unequal distribution of sex chromosomes in males and females in order to have a balanced gene expression between the sex chromosomes and the autosomes. This process, called X-chromosomal dosage compensation, requires the action of lncRNAs all the way from Humans to Drosophila. Interestingly though, the underlying mechanisms lie at the two opposing ends of the transcriptional spectrum: In humans Xist lncRNA (~17kb) plays a central role in repressing one of the two X-chromosomes in females; whereas in Drosophila, two redundant lncRNAs, roX1 (~3.7kb) and roX2 (~0.6kb) are necessary for the transcriptional up-regulation of the single X-chromosome in male flies. Dosage compensation in Drosophila is mediated by the Male-Specific Lethal (MSL) complex, which together with roX lncRNAs coats the male X chromosome and acetylates histone H4 lysine 16. Genetic evidence suggests that roX RNAs are particularly important for targeting the MSL complex to the X-chromosome, however, biochemical data detailing roX RNA – MSL complex interactions and how targeting of the MSL complex specifically to the X-chromosome can be aided by roX RNAs has been lacking.

Here, by using UV cross-linking followed by deep sequencing we show that two enzymes in the MSL complex, MLE RNA helicase and MSL2 ubiquitin ligase, bind evolutionarily conserved domains containing tandem stem-loops in roX1 and roX2 RNAs *in vivo*. These domains constitute the minimal RNA unit present in multiple copies in diverse arrangements for nucleation of the MSL complex. MLE binding to these domains is bimodal with distinct ATP-independent and ATP-dependent behavior. Importantly, we show that cooperative interaction of the different roX RNA domains is essential because combinatorial but not single mutations result in loss of dosage compensation and consequently male-specific lethality. Our study reveals that repetitive structural motifs in lncRNAs provide plasticity during multi-protein complex assembly to ensure efficient spreading *in cis* or *trans* along chromosomes.

172 B Long ncRNA NEAT1-dependent SFPQ relocation between nuclear body paraspeckle and gene promoter region mediates the transcription of IL8 gene in immune response

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Whole transcriptome analyses have revealed that novel classes of non-protein-coding transcripts, designated long noncoding RNAs (lncRNAs), were transcribed from mammalian genome. As the ratio of non-coding to protein-coding genomic regions increased as a function of developmental complexity, it has been assumed that the roles of lncRNAs transcribed from non-coding genomic regions are important to understand the genome function in higher organisms. NEAT1, a nuclear lncRNA, is essential for the formation of paraspeckle, one of nuclear bodies. NEAT1 consists two isoforms, 3.7-kb NEAT1v1 and 23-kb NEAT1v2, and the ratio between NEAT1v1 and NEAT1v2 are regulated by alternative 3'-end processing. NEAT1v2 but not NEAT1v1 is potent for the formation of paraspeckle, where NEAT1v2 interacts with splicing factor, proline/glutamine-rich (SFPQ) protein. However, the function of paraspeckles and NEAT1v2 are largely unknown.

We found that the levels of NEAT1v2 and IL8 mRNA were raised by poly I:C transfection. Poly I:C transfection induced excessive formation of paraspeckles without altered expression level of SFPQ. NEAT1 knock down decreased polyI:C-induced IL8 mRNA level. Conversely, solo overexpression of NEAT1v2 increased IL8 mRNA level and excessive formation of paraspeckles. These results suggest that NEAT1v2 regulates the expression of IL8 mRNA under polyI:C stimulation. Next, we investigated the mechanism how NEAT1v2 regulates IL8 mRNA expression. SFPQ knock down increased the *IL8* promoter activity as well as IL8 mRNA level, demonstrating that SFPQ repressed the transcription of IL8 mRNA. To test whether SFPQ directly binds the promoter region of *IL8* or not, we performed chromatin immunoprecipitation. Quantitative PCR analysis of the purified immunoprecipitated DNA binding with endogenous SFPQ showed that SFPQ bound the *IL8* promoter region in naive cells. In addition, either poly I:C stimulation or solo overexpression of NEAT1v2 decreased binding of SFPQ on the *IL8* promoter. Finally, we tested whether virus infection induced up-regulation of NEAT1v2. Influenza virus or herpes simplex virus 1(HSV-1) but not measles virus infection induced NEAT1v2. We confirmed that HSV-1 infection induced excessive formation of paraspeckles. These findings suggested that viral infection increases NEAT1v2 transcription and induces excessive formation of paraspeckles where SFPQ is relocated from promoter region of *IL8*, consequently, up-regulates IL8. We propose that paraspeckles function as a "molecular absorber" of paraspeckle proteins to regulate gene expression in response to stimuli.

173 C A Natural Antisense Transcript is Involved in the Destabilization of Cyclooxygenase 2 mRNA by Acetaminophen

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Acetaminophen is a popular antipyretic and analgesic drug. Acetaminophen has a low incidence of adverse effects, when compared with non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclooxygenase 2 (COX-2), the key enzyme in the biosynthesis of the inflammatory mediator prostaglandin. Because the mechanisms of action of acetaminophen are not fully understood, we sought to clarify the mechanism how acetaminophen affected the COX-2 expression. Similar to aspirin, a typical NSAID, acetaminophen reduced the levels of COX-2 protein and its mRNA in mouse macrophages treated with lipopolysaccharide (LPS). Acetaminophen suppressed the promoter activity of the *Cox-2* gene in the LPS-treated macrophages. We found that a natural antisense transcript (asRNA) was transcribed from the *Cox-2* gene in response to LPS, similar to the gene for inducible nitric oxide synthase, which produces another inflammatory mediator nitric oxide. The overexpression of COX-2 asRNA increased the expression of COX-2 mRNA. When we examined whether COX-2 asRNA modified the stability of COX-2 mRNA by reporter gene assays, acetaminophen canceled the effect of COX-2 asRNA and destabilized COX-2 mRNA. Our data suggest a possibility that COX-2 asRNA is a novel target of acetaminophen and a potent therapeutic target.

174 A Circular RNAs are Abundant, Conserved and Linked to ALU Repeats

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Formerly thought to be errors of splicing, endogenous circular RNAs have recently been shown by our group and other to be abundant and conserved. While circular RNAs have been identified by bioinformatic analysis, we sought to directly identify circular RNAs through an unbiased biochemical approach. Toward that end, we performed high-throughput sequencing (RNA-seq) on libraries derived from human and murine cells prepared from ribosome-depleted total RNA with or without digestion with RNA exonuclease. We identified >25,000 circular RNA species in human fibroblasts. Circular RNAs were usually composed entirely of exonic sequence. These exonic, circular RNAs (ecricRNAs) were identified by the presence of non-colinear exons (a "backsplice") and enrichment by exonuclease treatment. These species were validated as circular through biochemical approaches, and were considerably more stable than associated linear mRNAs. Expression of ecircRNAs ranged from very rare to highly abundant, with circular molecules exceeding the number of linear mRNA molecules by >10-fold for some transcripts. We did not identify ecircRNAs associated with ribosomes. Application of this method to murine testis RNA identified 69 ecircRNAs in precisely orthologous locations to human circular RNAs. We identified paralogous kinases, *HIPK2* and *HIPK3,* that produce abundant ecircRNA from their second, AUG-containing exon in both humans and mice. We noted degradation of circular RNAs by siRNAs, suggesting these species regulate gene expression by acting as competing endogenous RNAs. Analysis of backsplice sites utilized by ecircRNAs revealed shared features of circularized exons, including long bordering introns often containing complementary ALU repeats. EcircRNAs identified by this method were expressed in human cancer, with differential expression among tumor types. These data show that ecircRNAs are abundant, stable, and conserved products of RNA splicing that appear to regulate gene expression.

175 B Recognition of brain cytoplasmic 200 RNA by a human anti-RNA antibody

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It is known that diverse functional RNAs participate in a wide range of cellular processes. RNA structure plays an important role in their functions by themselves and as complex forms with proteins or ligands. Therefore, monitoring RNA conformation in the cell is essential for understanding their functional mechanisms, but any appropriate method is not established yet. Although hybridization is a general method used for analyzing specific RNA molecules through their base complementarity, it would be difficult to apply for monitoring RNA conformation because the hybridization requires partially denatured conditions, which could disturb their structural integrity. In the current study, we developed an efficient strategy for screening human monoclonal antibodies binding to RNA from a naïve antigen binding fragment (Fab) combinatorial phage library, using brain cytoplasmic 200 (BC200) RNA as a bait. BC200 RNA is a neuron-specific non-coding RNA that operates as a translational modulator, implicated in the inhibition of local synaptodendritic protein synthesis, in human cells. The neuron-specific BC200 RNA has been reported to be also expressed at high levels in invasive carcinomas than benign tumors of the breast. We biopanned a large human Fab combinatorial phage library ($5x10^{11}$ recombinants), and isolated two antibodies that recognize BC200 RNA, and one of them was selected for further affinity maturation by modifying residues in LCDR3. The best binding antibody, MabBC200-A3, binds specifically to the two regions of BC200 RNA (residues 76 to 85 and 96 to 104) with dissociation constant of about 7 nM. In the secondary structure model, the two regions were separated by approximately one-half helix-turn so that they could be placed in the same surface for interaction with the antibody. Various breast cancer cell lines were examined for their BC200 RNA expression using conventional hybridization, and BC200 RNAs expressed in those cells were analyzed with MabBC200-A3. MabBC200-A3 discriminates BC200 RNA from homologous 7SL RNA in purified total cellular RNA. The amount of BC200 RNA recognized by MabBC200-A3 in the purified total RNA pool was proportional to the cellular level of BC200 RNA, but the amount of antibody-recognizable BC200 RNA in the cell was not, suggesting that BC200 RNA can exist in distinct states in the cell. Our data show that anti-RNA antibody can provide a novel tool for RNA-detecting and analyzing that hybridization cannot provide.

176 C Evf2 (Dlx6AS) long non-coding RNA regulation of interneuron gene expression and behavior

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The role of anti-sense (AS) gene regulation in controlling neural circuits and complex behavior are poorly understood. We previously showed that *Evf2 (Dlx6AS)* long non-coding RNA (lncRNA) represses Dlx6 in embryonic brain, affecting circuitry in adult hippocampus. However, behavioral effects of *Evf2* loss in mice are not known. A battery of behavioral tests reveals that mice lacking *Evf2* have reduced immobility in the forced swim test (FST), showing altered response to acute stress. Evf2 mutant mice behave normally in learning, motor, anxiety, and social interaction tests. Interneuron genetic fate-mapping analysis indicates significant loss of interneurons in Evf2 mutant adult brain. However, calcium binding protein expression is dramatically increased in remaining Evf2 mutant interneurons. Thus, elevated calcium binding proteins in Evf2 mutants are likely to be responsible for normal responses in many of the behavioral assays, as well as reduced immobility in FST. Truncation of *Evf2* that retains anti-sense Dlx6 transcription does not affect Dlx6 expression or FST immobility times. Together, these data indicate that loss of *Evf2(Dlx6AS*) anti-sense gene regulation can affect interneuron number and subtype, with behavioral consequences in adult mice.

177 A The double stranded RNA transcriptome of E. coli reveals novel antisense RNAs

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Antisense RNAs are transcribed from the DNA strand opposite another gene and have perfect complementarity to the corresponding sense mRNA. Advances in high-throughput transcriptome analyses have revealed hundreds of antisense RNAs for many bacteria, although few have been characterized, and the number of functional antisense RNA remains unknown. We developed a method to identify potentially functional antisense RNAs in *Escherichia coli*. Most mechanisms of regulation via antisense RNAs require a RNA/RNA interaction with its target RNA. We hypothesized that a functional antisense RNA would be found in a double stranded RNA (dsRNA), duplexed with its cognate mRNA in a single cell. Therefore, we developed a method of enriching dsRNAs from total RNA by immunoprecipitating with a monoclonal antibody specific for dsRNA. Total RNA and dsRNA fractions from RNase III wild-type and mutant strains were converted to cDNA libraries, deep sequenced and many novel, potentially functional antisense RNAs were identified. The sense strand of many newly identified dsRNAs are known, functional sRNAs including: DsrA, Spot42, MicA, MicM, CyaR, ArcZ, MgrR and RydC. In addition, we identified many asRNAs encoded opposite of 5' ends of mRNAs and gene junctions of operons. Northern blot analyses have confirmed the presence of over 20 of the novel antisense RNAs and functional and mechanistic characterization is underway.

178 B Roles for roX RNA and the RNA helicase MLE in the assembly of the dosage compensation complex in Drosophila

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Dosage compensation in *Drosophila melanogaster* involves the transcriptional activation of genes on the single male X chromosome to match their expression levels to those in females, where both X chromosomes are transcribed.

The regulatory dosage compensation complex (MSL-DCC) consists of five so-called male-specific lethal (MSL) proteins and two long, non-coding RNA, *roX1* and *roX2*, whose function is enigmatic. RNA helicase maleless (MLE) is among the MSL subunits. It is crucial for dosage compensation, but its role remains unclear. Due to its unwinding activity and its multiple RNA-binding domains, MLE is well suited to play a key role in*roX* functionality and may be involved in regulating the association of *roX* with the MSL components. Up to now, biochemical analyses had found that MLE associates only weakly with the other MSL proteins in nuclear extracts and *in vitro* binding studies failed to reveal any specific interaction of MLE with *roX* RNA.

We report on the results of our recent efforts to characterize the interactions of MLE with roX RNA, combining by RNA pull-down assays, RNA 2D structure analysis and footprint experiments. We found that MLE is able to recognize specific aspects of *roX* RNA and to change its 2D structure to form that is competent to initiate MSL assembly. Our data strongly suggest that the ATP-dependent remodeling of *roX* RNA by MLE may be rate limiting for MSL-DCC assembly.

179 C Molecular Mechanism Involved in Antisense-Mediated Transcriptional PHO84 Gene Silencing

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The development of new technologies such as tiling arrays and RNA-seq has revealed that eukaryotic genomes are extensively transcribed. In *Saccharomyces cerevisiae* a class of non-coding transcripts produced by RNA pol II, called "cryptic unstable transcripts" (CUTs), is stabilized upon deletion of the nuclear exosome subunit Rrp6(1). Whether this broad and unstable transcriptome has a regulatory role in gene expression is still unclear.

It has been previously shown that the *PHO84* gene is transcriptionally regulated by an antisense RNA that accumulates upon deletion of Rrp6, causing the recruitment of the histone deacetylase complex Hda1/2/3 which leads to the transcriptional repression of the *PHO84* gene(2).

A genetic screen aimed at further characterizing this mechanism has identified new factors that could be involved in antisense RNA-mediated silencing of the *PHO84* gene. Among those is the histone chaperone complex Hir, originally described as a key factor in the regulation of the histone gene expression during the cell cycle. However, more recent data support the idea of a possible global requirement of this complex in chromatin remodeling (3). Our aim is to better characterize the role of this complex in the regulation of the *PHO84* gene in the absence of Rrp6. We hypothesize that antisense RNA production may facilitate the recruitment of the Hir complex to the *PHO84* gene promoter and contribute to its silencing.

1. Neil et al., *Nature*, (2009):1038-1042
2. J.Camblong, et al., *Cell*. (2007):706-717
3. Fillingham J,et al., *Mol. Cell*. (2009): 340-51

180 A MiR-19 and miR-155 role in oncogene-induced senescence bypass

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Cancer establishment and progression is a multi-step process that requires various concerted modifications. Fortunately, cells have evolved many different mechanisms to counteract tumorigenesis. Oncogene-induced senescence (OIS) is one of these mechanisms and it has been shown to be triggered early on in tumorigenesis. Its establishment is mainly carried through the activation of two important tumor suppressor pathways: the p53/p21 pathway and the p16/RB pathway. Thus in cancer progression, key players in these pathways must be altered in order to bypass this barrier.

Leukemia is often characterized by an over-expression of STAT5A. The latter is known to induce sets of genes that are antagonistic leading to either cell proliferation or senescence. Accumulating reports also provide evidence that microRNAs (miRNAs) are upregulated in various leukemias suggesting an oncogenic role for these. We propose that normal STAT5 signaling includes the regulation of the tumor suppressor SOCS1 that prevents uncontrolled cell proliferation and triggers senescence in part by regulating p53. We think that during leukemia, constitutively active STAT5A induces the oncomirs (oncogenic miRNAs) miR-19 and miR-155 resulting in down-regulation of SOCS1 by the miRNAs and subsequent bypass of the oncogene-induced senescence.

In order to study the role of the oncomirs in leukemia we used microRNA sponges that inhibit miRNA activity by titrating their levels. Our results show that sponges against miR-19 or miR-155 stabilized SOCS1 and enhanced p53 activation as shown by measuring p53 phosphorylation at serine 15 and its transcriptional target p21. Although the development of therapeutics to induce miRNA down-regulation is currently underway, or work suggests that inhibition if miR-19 and miR-155 can reactivate endogenous tumor suppressors in leukemia.

181 B Fission yeast Cactin silences chromosome ends and retrotransposons and links heterochromatin establishment to telomere length regulation

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Eukaryotic telomeres are transcribed by RNA polymerase II into diverse long non-coding RNA (ncRNA) molecules forming the telomeric transcriptome. Among these ncRNA species, telomeric repeat-containing RNA (TERRA) is conserved in eukaryotes and remains associated to telomeres post-transcriptionally, suggesting that TERRA is an evolutionarily conserved integral component of telomeric heterochromatin. Still, the functions and the mechanisms of regulation of the telomeric transcriptome remain enigmatic. We have screened a complete *Schizosaccharomyces pombe* gene-deletion collection and identified several mutants with increased TERRA cellular levels. One of these strains is deleted for the gene encoding 'Fission yeast Cactin-like protein 1' (Fyc1), the *S. pombe* member of the eukaryotic Cactin protein family, which comprises poorly characterized polypeptides possibly involved in cell cycle progression and cell growth. Consistently, *fyc1*Δ yeasts grow slower compared to wild type counterparts and are sensitive to a cold environment. Fyc1 binds telomeric and subtelomeric sequences at low levels *in vivo*, and *fyc1*Δ mutants accumulate subtelomeric and telomeric RNA and fail to silence subtelomeric reporter genes. Thus, Fyc1 is necessary to silence chromosome ends and to establish telomere position effect. In absence of Fyc1, telomere elongation by telomerase is exaggerated and the telomeric G-overhang is longer, suggesting that telomere transcription may stimulate telomerase-mediated telomere elongation. Intriguingly, cells deleted for *fyc1+* also accumulate RNA deriving from all Tf2 retrotransposons and numerous solo LTR elements scattered throughout the genome, implying that Fyc1 promotes concomitant silencing of telomeres and elements of retroviral origin. Mechanistically, Fyc1 sustains establishment of heterochromatin at telomeres and retrotransposon-containing loci by restricting the levels of acetylated histone H3 at lysine 9 (H3K9) and, conversely, promoting accumulation of trimethylated H3K9. Our findings reveal that Fyc1 is a novel regulator of telomeric heterochromatin establishment and telomere length maintenance, and link silencing of telomeres and retrotransposons. We are investigating whether and to what extent the different phenotypes observed in *fyc1*Δ cells are causally linked.

182 C Revealing the elusive molecular biology of the vault RNA

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Recently several novel and previously reported non-protein-coding RNAs (ncRNAs) have been identified to be upregulated upon Epstein-Barr virus (EBV) infection in human B-lymphocytes. A group of these significantly upregulated ncRNAs are called vault RNAs (vtRNAs).[a,b] Only about 5% of the total cellular vtRNAs are connected to the vault particle, the largest known ribonucleoprotein particle (RNP) in eukaryotic cells. However the function of this ncRNA family and moreover of the vault particle remains still rather unclear. Our previous findings suggest a link between EBV infection and vtRNA expression. Consequently we are interested which part of the viral genome is responsible for the upregulation and moreover which function the vtRNAs might possess during virus propagation.

To address this question we have separately overexpressed specific EBV-encoded, latently expressed proteins in BL2-cells to determine the influence on the vault RNA levels. Thereby we identified one EBV-encoded protein, called Latent Membrane Protein 1 (LMP1), which significantly contributes to the vtRNA upregulation. We used LMP1 mutants to characterize the region of the protein and the responsible pathway for triggering the elevated vtRNA expression. Our results suggest that the NFkB-pathway might be involved in this process. To investigate a possible functional connection between the vtRNA and EBV infection, we have overexpressed vtRNA1-1 in BL41, a cell line usually not expressing this vault RNA. We show that overexpression of vtRNA1-1 leads to a better viral establishment and markedly protects cells from undergoing apoptosis. Knock-down of the major vault protein, the main component of the vault particle, had no effect on EBV infection and apoptosis resistance. Thus these results support the view that the observed phenotype is caused by the vtRNA rather than the vault particle.

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[b] Nandy C., Mrázek, J., Stoiber H., Grässer F.A., Hüttenhofer A., Polacek N. (2009) Epstein-barr virus- induced expression of a novel human vault RNA. J Mol Biol. 388 (4)

183 A Structure and function of Zucchini endoribonuclease in piRNA biogenesis

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PIWI-interacting RNAs (piRNAs) silence transposons to maintain the integrity of the genome in animal germlines. piRNAs are divided into primary and secondary piRNAs depending on the biogenesis machinery. Primary piRNAs are processed from long non-coding RNA precursors transcribed from piRNA clusters in the genome through the primary processing pathway. Although the existence of a ribonuclease participating in this pathway has been anticipated, its molecular identity remains elusive. Here, we show that Zucchini (Zuc), a mitochondrial phospholipase D (PLD) superfamily member, is an endoribonuclease essential for primary piRNA biogenesis. We solved the crystal structure of *Drosophila melanogaster* Zuc (DmZuc) at 1.75-Å resolution. The structure revealed that DmZuc has a positively charged, narrow catalytic groove at the dimer interface, which could accommodate a single-stranded, but not a double-stranded, RNA. DmZuc and the mouse homolog MmZuc (also known as Pld6 and MitoPLD) exhibited endoribonuclease activity for single-stranded RNAs *in vitro*. The RNA cleavage products bear a 5′-monophosphate group, a hallmark of mature piRNAs. Mutational analyses showed that conserved active-site residues of DmZuc are critical for ribonuclease activity *in vitro*, and for piRNA maturation and transposon silencing *in vivo.*We propose a model for piRNA biogenesis in animal germlines, in which Zuc endoribonuclease plays a key role in primary piRNA maturation.

184 B Malignant Transformation Changes Packaging and Targeting of Extracellular MicroRNAs

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Cells regulate each other remotely in many ways. An emerging mechanism is the exchange of microRNA (miRNA) packaged into membrane-coated vesicles. We reported that several cellular processes associated with malignant transformation change the export of extracellular miRNAs (ex-miRs) by affecting whether a particular miRNA species is released selectively or retained by the cell. We here describe the basis of selective ex-miR release and transfer to target cells.

We show that ex-miRs are packaged mutually exclusively in different carriers released from breast cancer cells. miR-451, which is selectively released from transformed cells associates with exosomes, miR-1246 with nucleosomes, and neutrally released miR-16 associates with unconventional "L-" exosomes. In contrast, normal cells release these ex-miRs in a single type of vesicle. Stresses upon the donor cells, including DNA damage, affect the export of miR-16. We further demonstrate that uptake of ex-miRs and its consequences are cell-type and carrier-type specific. For example, T-cells accumulate ex-miR-16 and ex-miR-451, while megakaryocytes internalize miR-16 but not miR-451. The received miR-16 represses BCL2 in T-cells, triggering apoptosis, but causes cell cycle arrest in megakaryocytes. Finally, monocytes do not acquire miR-16 or miR-451 from the extracellular environment, and the cells differentiate.

Malignant transformation induces *de novo* extracellular vesicles, into which some ex-miRs are assorted mutually exclusively. This separation is key in determining the cell-type specific delivery of extravesicular cargo, and can explain how ex-miRs can simultaneously activate cancer-promoting cells and block anti-cancer cells. This relay is dynamic, as changes in the ex-miR population due to DNA damage in the cancer cell of origin is relayed to other cells and affects their function. We propose a model wherein ex-miR-cell signaling occurs similar to virus-cell interactions, including the need for ligand-receptor interactions, and subsequent cellular trafficking.

185 C Identification and functional characterization of the long non-coding RNA in myogenesis

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Eukaryotic genomes express a diverse range of non-coding RNAs as well as protein-coding mRNAs. Among them, some of long non-coding RNAs are known to have functions to regulate diverse biological processes, such as epigenetic control of chromatin, X-chromosome inactivation, and nuclear body formation. However, comparing to the large portion of long non-coding RNAs in entire genome, their functions remain largely uncharacterized. In particular, the potential contribution of long non-coding RNAs to the cellular differentiation is rarely elucidated. Myogenesis, the muscle differentiation, is a multistep process during which pluripotent mesodermal cells give rise to myoblasts that eventually differentiate into myotubes. During the course of myogenic differentiation, many muscle genes are up-regulated including MyoD, Myogenin, and MCK. Here, we took the advantage of genome-wide transcriptome analysis and found that the expression of not only muscle genes but many long non-coding RNAs is changed during myogenesis. There were many non-coding RNAs which are up-or down-regulated during muscle differentiation. Among the non-coding RNAs, we show the long non-coding RNA transcript, UT-10, which is increased during myogenesis has a potential role in muscle differentiation by regulation of muscle genes

186 A Degradation of ribosomal RNA and ribosomal proteins constitute separate pathways of ribophagy

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Nutritional deprivation leads to severe changes in cell homeostasis, ultimately promoting destruction of cellular components and organelles to prevent death. Recently, a deubiquitination-dependent selective degradation of ribosomal proteins in yeast vacuoles, which is activated by nitrogen starvation and termed ribophagy, has been reported. We describe a separate ribophagy mechanism, wherein robust fragmentation of mature ribosomal RNA in autophagic yeast cells is independent of protein degradation. This ribosome recycling mechanism is highly specific, requires a subset of autophagic regulators, Atg proteins, is dependent on the TOR (target of rapamycin) regulatory pathway, but proceeds efficiently in the absence of the Ubp3-Bre5 complex required for the ribophagic degradation of ribosomal proteins. This indicates that the concurrent response on the protein part of ribosomal subunits is not a prerequisite. RNA cleavages take place on intact ribosomes in the cytoplasm and are carried out predominantly, but not solely, by the vacuolar ribonuclease Rny1, which is released from the vacuole to the cytosol upon activation of autophagy. Endonucleolytic cleavages generate specific and stable RNA fragments, which are further digested by exonucleases, including Nuc1, 5'-3' exonuclease Xrn1 and the 3'-5' exosome complex. In contrast, components of both 18S and 25S "non-functional rRNA decay" (NRD), that eliminates malfunctionning ribosomes, are not required for rRNA destruction during autophagy. The existence of the autonomous rRNA ribophagic pathway substantiates the importance of ribosome breakdown to survive the shortage of nutrients.

187 B Discovery of Hfq-binding nanoRNAs in Escherichia coli

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RNA-based regulation enables exquisite control over the extent and timing of gene expression, thereby enabling bacteria to rapidly respond to their environment. The bacterial host factor Hfq acts as a post-transcriptional regulator of such changes in gene expression. This cellular activity likely stems from the ability of Hfq to function as a generic RNA chaperone. Previous work has found that Hfq preferentially interacts with A/U-rich RNAs. In this study, we discovered an Hfq that interacts with short U/C-rich nanoRNAs, when recombinantly expressed in Escherichia coli. These Hfq-binding nanoRNAs interact with high affinities (nanomolar-scale) and feature specific RNA end-chemistries (5'-monophosphate, 3'-hydroxyl). These unanticipated Hfq···nanoRNA associations may represent a novel mechanism by which Hfq and Hfq•sRNA complexes modulate RNA-based regulatory circuits in vivo.

188 C Mutations in the 5'UTR of SERPINA1 transcripts are involved in the disease associated mechanisms

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Single Nucleotide Polymorphisms (SNPs) are found throughout the human genome. SNPs implicated in disease-associated mechanisms that map to coding regions of the genome generally alter the function of the protein. However, disease-associated SNPs that map to non-coding regions can affect the translation regulation and/or stability of the mRNA. Novel high-throughput mappings of RNA binding protein sites found that most UnTranslated Regions (UTR) of mRNA are often targeted by proteins involved in the regulation of translation, stability of mRNA, and mRNA localization. Not only that many RNA binding proteins recognize specific primary sequences of the mRNA, but is also now well established that the secondary structure plays a critical role in the accessibility of a binding site. Thus, SNPs that significantly alter the structure of UTRs could affect post-transcriptional regulation.

Alpha 1-antitrypsin deficiency (A1AD) is an autosomal recessive genetic disorder caused by the defective production of alpha 1-antitrypsin (A1AT), a protein encoded by *SERPINA1*. Severe deficiency of A1AT causes panacinar emphysema or Chronic Obstructive Pulmonary Disease (COPD) as well as various liver diseases. It has been shown that certain mutations (Glu342Lys) in the coding regions of *SERPINA1* led to severe cases of COPD. A recent genome wide-association study identified a SNP in the 5' UTR of SERPINA1 associated with increased risk of developing COPD. SHAPE structure mapping analysis reveals that this SNP alters RNA structure acting forming a RiboSNitch – a SNP that induces a large conformational change of RNA resulting in an altered function of RNA. Thus, SNP induced RNA structure change likely plays an important role in COPD predisposition.

189 A RNA Structure and Ligand Interactions Probed by Strategically Positioned 15N-Labels

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To explore folding and ligand recognition of metabolite-responsive RNAs is of major importance to comprehend gene regulation by mRNA riboswitches. On the secondary structure level, folding events often lead to changes in the H-bond mediated Watson-Crick base pairing pattern. The individual base pairs can be monitored by the NMR spectroscopic HNN-COSY experiment which detects and quantifies magnetically active ^{15}N nuclei of the H-bond donor and acceptor.

Here, we present the synthesis of all four ribonucleoside phosphoramidites carrying single ^{15}N-labels at the pyrimidine-3 or purine-1 positions. Their site-specific introduction into RNA has been achieved by solid-phase synthesis using a combination of 2'-*O*-TOM and 2'-*O*-TBDMS RNA chemistry.

To furthermore demonstrate the power of the labels for NMR spectroscopic applications, we investigate pseudoknot folding and ligand interaction of transcriptionally [1] as well as translationally acting $preQ_1$ class-I aptamers in a Mg^{2+} and temperature dependent manner. The study provides detailed insights into the ligand recognition mode of $preQ_1$ riboswitches.

1. T. Santner, U. Rieder, C. Kreutz, R. Micura; Pseudoknot preorganisation of the $preQ_1$ class I riboswitch; J. Am. Chem. Soc. 2012, 11928-11931.

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190 B Integrated genome-wide in silico and capture array approach discovers a large spectrum of novel structured RNAs associated to regulatory elements

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Computational predictions and high-throughput sequencing techniques, such as RNA-seq, have recently given rise to the discovery of many non-coding RNAS (ncRNAs). In silico methods have in particular focused on predicting RNA structure, which is a functional characteristic of many ncRNAs. However, these screens have primarily searched in regions of high evolutionary sequence conservation and lack of large scale experimental follow up analysis.

To overcome these limitations, we introduce a genome-wide (on all regions covered by MAF blocks) in silico screen for ncRNAs based on structural RNA alignments (of corresponding sequence from vertebrates) in direct combination with the design of a capture array for expression analysis. The in silico screen resulted in ~600,000 highly structured candidate regions in the human genome of an estimated false discovery rate of 26%. For the top ~60,000 regions the FDR is less than 10%. We also predict a genome-wide coverage by conserved RNA structures of around 14%. The identified regions are primarily intergenic and are enriched for untranslated regions (UTRs) in mRNAs. We observe good overlap to known ncRNAs and recently identified long ncRNAs. The study reveals that structured RNAs are most adequately predicted from structural alignments and, interestingly, comprise RNA structures of down to 20% sequence identity.

Functional indications of the conserved RNA structures are given by their location adjacent to many regulatory features such as transcription factor binding sites and the evidence for their negative selection. To provide further confidence on the in silico predictions we performed the first large-scale experimental analysis of structured ncRNA candidates by probe design of ~80,000 structured candidate regions. The designed capture array reveals the expression of ~8,000 of these regions. The chosen strategy of RNA capture array and high-throughput sequencing found a number of low expressed ncRNAs and RNA structures in extended UTRs which have not been found by ordinary RNA-seq experiments. Further experiments (qPCR, RACE and structure probing) in human and mouse support our hypothesis of transcripts weakly conserved in sequence but with a highly conserved RNA structure.

191 C Identification and characterization of rice non-coding RNAs involved in nitrogen-starvation stress response

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MicroRNAs and long non-coding RNAs are key regulators that control the expression level of genes by transcriptional and post-transcriptional regulation in animals and plants. Both of the non-coding RNAs are also involved in regulating plants' responses to stress conditions. Recent studies showed that non-coding RNA pool changes in some environmental conditions, implying that non-coding RNAs play key roles in responding to stress condition in plants. In this study, we aim to identify and characterize microRNAs and long non-coding RNAs induced by nitrogen-starvation stress in *Oryza sativa*. To do this, we performed RNA-Seq and small RNA-Seq using nitrogen-starvation stress-treated rice samples, and analyzed the expression profiling of the non-coding RNAs and their target genes. With this study, we investigate the relationship between the expression of nitrogen-starvation stress-specific non-coding RNAs and their effects on genes in *Oryza sativa*.

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192 A Divergent transcription of long noncoding RNA/mRNA gene pairs in embryonic stem cells

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Many long non-coding RNA (lncRNA) species have been identified in mammalian cells, but the genomic origin and regulation of these molecules in individual cell types is poorly understood. We have generated catalogs of lncRNA species expressed in human and murine embryonic stem cells (ESCs) and mapped their genomic origin. A surprisingly large fraction of these transcripts (>85%) originate from divergent transcription at promoters or enhancers of active protein-coding genes. The divergently transcribed lncRNA/mRNA gene pairs exhibit coordinated changes in transcription when ESCs are differentiated into endoderm. Down-regulation of a divergently transcribed lncRNA by RNAi reduces the efficiency of differentiation of hESCs into the endoderm. Our results reveal that transcription of most lncRNA genes is coordinated with transcription of protein-coding genes.

193 B Uncovering novel microRNAs involved in homeostatic plasticity

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Synaptic scaling is a homeostatic mechanism responsible for the adjustment of the overall synaptic strength in a neuron's synapses to a set point, in order to stabilize neuronal firing. Dendritic protein synthesis is crucial for synaptic scaling but the mechanisms that regulate the de-repression of mRNAs are still unclear. MicroRNAs, well known posttranscriptional regulators, are modulated upon changes in neuronal activity, which indicates that miRNAs may have a role in the regulation of homeostatic plasticity. Our main goal is to unveil novel miRNA players during synaptic scaling and to further investigate their role in this form of plasticity.

We performed a gene expression microarray analysis of rat hippocampal neurons under chronic blockade of activity and submitted the results to biological interpretation using the GoMiner tool. Several gene categories, relevant for synaptic events, were selected and miRNA target sites were predicted for those genes using the following algorithms: miRanda, TargetScanS and MirTarget2. Focusing on a restricted group of altered genes with a crucial role in synaptic scaling and/or in synaptic function, we identified a group of 18 predicted miRNAs, and performed a screening panel for their expression levels in primary cultures of rat hippocampal neurons subjected to synaptic scaling up conditions. This analysis revealed that several miRNAs present altered expression in scaling up conditions and therefore suggests an important role for these miRNAs in synaptic scaling mechanisms.

194 C Transcriptional regulation and non-coding RNA: The Steroid Receptor RNA activator

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The steroid receptor RNA activator (SRA RNA) is a unique non-coding RNA which has been shown to stimulate hormone-mediated transcriptional responses. The SRA RNA is a member of the diverse group of factors known as the nuclear receptor co-regulators. The RNA has a complex secondary structure with multiple stem-loop structures which are the landing platform for its partners. Understanding the specific association between the SRA RNA and its regulatory partners will provide critical tools to modulate hormone-mediated transcriptional responses. Increased understanding of these signalling pathways has great potentials for breast and pancreatic hormone-responsive cancer treatment for example. We are studying the specific association of the SRA RNA with two transcriptional regulators: the SRA stem-loop interacting RNA binding protein (SLIRP) and the *SMRT*/*HDAC*-associated repressor *protein* (SHARP). Both proteins are classified as transcriptional repressors. SLIRP is a small RRM-containing protein shown to specifically associate with SRA in the nucleus and to be a partner of the mitochondrial protein LRPPRC. SLIRP is binding to the SRA RNA and is thought to outcompete other transcriptional activators. The SHARP protein belongs to the split end protein family, initially characterized in Drosophila where it was shown to affect homeotic patterning. SHARP recruits the SMRT protein and histone deacetylases to the promoter regions, which in turn blocks the transcriptional machinery by promoting chromatin compaction.

We are characterizing *in vitro* the binding properties of these factors with their RNA binding sequences in the SRA RNA. Furthermore, we have obtained an atomic model of the N-terminal region of SHARP using X-ray crystallography. This region contains three RRM domains (RRM2, RRM3 and RRM4) which are conserved within the entire SHARP protein family. Two of these RRMs (RRM3-4) are forming a stable entity using a previously uncharacterized region. The third RRM (RRM2) is tethered to the others via a helical linker. Small angle X-ray scattering experiments indicates that the linker could be used by the protein single RRM to reach two preferential orientations located on both side of the stable block formed by the RRM3-4. Structural comparison, mutagenesis and *in vivo* experiments are validating the importance of the RRM organization and binding ability for the co-repressor activity mediated by SLIRP and SHARP.

195 A RNA polymerase-binding elements attenuating transcription in E. coli

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Transcription is a highly controlled step of gene expression. A number of diverse RNA-based transcription regulatory mechanisms have been discovered to date. However, in contrast to numerous well-studied protein factors, there are only few known regulatory RNAs tuning transcription by a direct interaction with the RNA polymerase machinery. Addressing the question if such type of transcriptional riboregulation could be a frequent phenomenon in bacteria, we combined *E. coli* RNAP - genomic SELEX with next-generation sequencing. The obtained data reveal a large group of polymerase binding RNA elements (PBEs) located within diverse coding sequences that bind RNAP with high affinity. Using different *in vitro* and *in vivo* techniques we demonstrate that a subgroup of these PBEs attenuate transcription of the nascent RNA by promoting premature termination events under relevant biological conditions. Our findings let us propose a novel and potentially widespread mechanism of transcriptional regulation by the transcribing RNA itself. Detailed single-molecule mechanistic elucidation of the process is underway.

196 B A link between long intervening noncoding RNAs and microRNA regulation

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Thousands of long intervening noncoding RNAs (lincRNAs) have been identified in mammals. To better understand the evolution and functions of these enigmatic RNAs, we identified more than 550 distinct lincRNAs in zebrafish. Although these shared many characteristics with mammalian lincRNAs, only 29 had detectable sequence similarity with putative mammalian orthologs, typically restricted to a single short region of high conservation. Other lincRNAs had conserved genomic locations without detectable sequence conservation. Morpholinos targeting conserved regions of two zebrafish lincRNAs caused developmental defects. Morpholinos targeting splice sites caused the same defects and were rescued by adding either the mature lincRNA or its human or mouse ortholog. In one of the lincRNAs, called *cyrano*, the conserved region extensively pairs to miR-7, and this pairing has been essentially unchanged since the dawn of vertebrates. Our experiments confirm that the conserved site is a bona fide microRNA target site that is bound by argonaute proteins and confers regulation by miR-7. This regulation is part of a larger network of miRNA-lincRNA regulations, which is revealed by multiple types of experimental evidence. We also demonstrate how direct comparison of lincRNA sequences across distant species can uncover miRNA complementary sites and other conserved elements that are missed in whole-genome alignments.

197 C Evolutionary relationships between PB1 mRNA of Influenza A virus and host microRNAs among vertebrates

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Influenza A virus (IAV) undergoes rapid evolution to evade the host defense system, whereas hosts have developed several antiviral responses. Recently, it has been reported that three human miRNAs (miR-323, miR-491 and miR-654) are able to bind to almost the same site in the viral polymerase basic protein 1 (PB1) mRNA and inhibit H1N1 IAV replication (Song *et al*., *J. Virol*. 2010). Thus, these miRNAs have been suggested as potential novel antiviral factors against IAV. Meanwhile, some viruses are known to subvert host antiviral function of miRNAs, indicating that miRNAs play critical roles in complicated host-pathogen interaction networks.

To better understand the intricate host-IAV interactions via miRNAs, here, we investigated the evolutionary relationships between host miRNAs and PB1 mRNA. Firstly, to characterize the dynamic nature of human IAV evolution, we classified a variety of human IAV (7,368 strains) from 1918 to 2012 based on the amino acid sequence similarities. Consequently, large-scale spectral clustering analysis visualized a comprehensive picture of IAV evolution in whole segments, showing not only process of reassortment but also gradual mutations simultaneously. Secondly, conservation analysis of miRNA-target sites in PB1 mRNA sequences based on Shannon's information theory suggested that the miRNA-target sites were significantly conserved in human IAVs from 1918 to 2012. In addition, both swine and avian IAVs (1,412 and 8,257 segments, respectively) also possessed the miRNA-target sequences in PB1 mRNA. As for host miRNAs, both miR-323 and miR-491 were conserved in human and swine genome. Furthermore, we predicted that several other host miRNAs hybridized with the conserved miRNA-target sequences in human, swine and avian.

On the basis of these results, we suggested that regulatory relationships between host and IAV via miRNAs had been evolutionary conserved among vertebrates, and, therefore, we proposed the following hypotheses: (1) host miRNAs targeted immutable regions of IAV, or (2) IAV regulated appropriately its own replication in order to maintain the persistent infection by utilizing the host defense system of miRNAs. In this conference, evolutionary advantage of host miRNA regulations will be discussed from the standpoints of both hosts and viruses.

198 A Rat mir-155 generated from the lncRNA Bic is 'hidden' in the alternate genomic assembly and reveals the existence of novel mammalian miRNAs and clusters

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MicroRNAs (miRNAs) are a class of small noncoding RNAs acting as post-transcriptional gene expression regulators in many physiological and pathological conditions. During the last few years, many novel mammalian miRNAs have been predicted experimentally with bioinformatics approaches and validated by next-generation sequencing. Although these strategies have prompted the discovery of several miRNAs, the total number of these genes still seems larger. Here, by exploiting the species conservation of human, mouse, and rat hairpin miRNAs, we discovered a novel rat microRNA, mir-155. We found that mature miR-155 is overexpressed in rat spleen myeloid cells treated with LPS, similarly to humans and mice. Rat mir-155 is annotated only on the alternate genome, suggesting the presence of other "hidden" miRNAs on this assembly. Therefore, we comprehensively extended the homology search also to mice and humans, finally validating 34 novel mammalian miRNAs (two in humans, five in mice, and up to 27 in rats). Surprisingly, 15 of these novel miRNAs (one for mice and 14 for rats) were found only on the alternate and not on the reference genomic assembly. To date, our findings indicate that the choice of genomic assembly, when mapping small RNA reads, is an important option that should be carefully considered, at least for these animal models. Finally, the discovery of these novel mammalian miRNA genes may contribute to a better understanding of already acquired experimental data, thereby paving the way to still unexplored investigations and to unraveling the function of miRNAs in disease models.

199 B HEN1-directed labeling of small non-coding RNAs

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microRNAs and siRNAs are 21-25 nucleotides long non-coding RNAs described in viruses, unicellular eukaryotes and a broad range of multicellular organisms, ranging from plants and insects to mammals. microRNAs control a variety of biological pathways including development, apoptosis, metabolism or immunological response by inhibiting protein translation and, in some cases, leading to degradation of mRNA transcripts. In view of numerous functions the connection of microRNAs with a wide range of human diseases comes as no surprise. Differences in microRNA expression patterns are significant in cancer, diabetes, heart malfunctions, neurodegenerative diseases *etc*. For this reason detection techniques for high-throughput microRNA profiling need to be developed. The novel small RNA labeling technology recruits the HEN1 methyltransferase to attach the extended side chains with functional group towards 3'-end of microRNA or siRNA. The method provides two strategies: quick and effortless one-step labeling through the direct attachment of the relevant reporter group which is embedded in the transferred radical. Alternatively, the two-step approach extends a choice of coupling strategies for manifold label conjugation to functional group.

200 C Noncoding RNA-mediated chromosomal fusions

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Chromosomal fusion, a common occurrence in normal and cancer cells, can lead to the formation of aberrant gene products and chimeric transcripts. The mechanisms driving these fusions are poorly understood, but recurrent fusions are widespread. Here we describe chromosomal fusion events that are driven by either long or short aberrant RNAs during somatic differentiation in the ciliate *Oxytricha trifallax*. Exposure of the germline to either long noncoding "template" RNAs that specify rearrangements (Nowacki et al. 2008 *Nature*) or to 27 nucleotide piRNAs that protect DNA sequences against deletion (Fang et al. 2012 *Cell*) can lead to fusion of chromosomes in the offspring, or even the formation of circular chromosomes, in one case. Furthermore, we demonstrate that these RNA-mediated inter- and intra-chromosomal fusions are heritable over multiple sexual generations, illustrating the ability of noncoding RNAs to program and reprogram genome architecture, including chromosome fusion and circularization, and expanding the known repertoire of RNA-mediated, transgenerational, epigenetic inheritance.

201 A Regulation of transcription by Pol II-binding RNA aptamers

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Transcription by RNA polymerase II (Pol II) is the first step in eukaryotic gene expression and one of the most important ones to regulate. As abundance of non-coding RNAs with diverse functions have been identified, it has become clear that RNAs regulate every step in gene expression including transcription. To investigate the regulatory potential of the human transcriptome, we performed Genomic SELEX with Pol II as a bait and identified many high-affinity RNA aptamers (Polymerase-binding elements or PBEs). PBEs can be pulled down with Pol II from HeLa cells and they abolish transcription when fused to a reporter gene in an *in vivo*expression system. Interestingly, many PBEs are located in repetitive regions of the human genome, with most prominent ACRO1 satellites, previously uncharacterized repeat elements that cluster in the pericentromeric region of chromosome 4 and can be found on chromosomes 1, 2, 19 and 21 as well. Another class of PBE-containing repeats are the L1 retrotransposons, which had been shown to inhibit their own expression by an unknown mechanism. Our results suggest a novel way of *in-cis* transcription regulation by RNAs, wherein nascent transcripts bind Pol II to interfere with elongation.

202 B A short guide to human long non-coding RNA gene nomenclature

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The HUGO Gene Nomenclature Committee (HGNC) is the only organisation authorised to assign standardised nomenclature to human genes. Of the 34,000 approved gene symbols in our database (www.genenames.org) the majority represent protein-coding (pc) genes; however we also name pseudogenes, phenotypic loci, some genomic features, and to date have named over 5,000 human non-protein coding RNA (ncRNA) genes and ncRNA pseudogenes. We have already established unique names for most of the small ncRNAs by working with experts for each class: for example we have named the ~1,500 human microRNA genes in collaboration with miRBase. Small ncRNAs can be defined into their respective classes by their shared homology and common function. In contrast long non-coding RNA (lncRNA) genes represent a disparate set of loci related only by their size, over 200 bases in length, share little conserved sequence homology, and have variable functions. As with pc genes wherever possible lncRNAs are named based on the known function of their product, e.g. '*XIST*' 'X (inactive)-specific transcript' is involved in transcriptionally silencing one of the pair of X chromosomes in females. We have currently named ~100 lncRNA genes that encode a transcript with published evidence of function. There are, however, potentially thousands of lncRNAs, and for the vast majority their function remains unresolved. Such lncRNA genes are named based on their genomic context. If there is a proximal pc gene then the lncRNA genes are given a gene symbol beginning with the pc symbol and assigned a suffix according to whether they are: antisense (AS) e.g. *BACE1-AS*; intronic (IT) e.g. *SPRY4-IT1*; or overlapping (OT) e.g. *SOX2-OT*. Whereas long intergenic lncRNAs (lincRNAs) that lie between pc gene loci are named with a common root symbol (*LINC: "long intergenic non-coding RNA"*) and an iterated, numerical suffix. We present a short guide to the nomenclature of lncRNA genes and provide examples of some of the genes named to date. For further information on ncRNA nomenclature please see the HGNC RNA webpage: www.genenames.org/rna or email us at hgnc@genenames.org

203 C RNA functional profiling by gene deletion in S. cerevisiae

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New sequencing technologies and high-resolution microarray analysis have revealed that an large portion of the genome is transcribed, generating a significant number of RNAs with non-coding capacity. The focus of current debate is how many of these non-coding RNAs are functional, and what is their function. The yeast *Saccharomyces cerevisiae* is an important model organism for investigating gene functions. Protein-coding gene deletion strains have provided a valuable research resource for studying essential cellular processes and understanding the mechanisms of drug action. However, the pervasively transcribed RNA-coding genes are absent from deletion strain collections, making it difficult to study the contribution of RNA-coding genes in biological processes. Therefore, we have constructed molecular barcoded RNA gene deletion strains in *S. cerevisiae*, including annotated snRNAs, snoRNAs, tRNAs and recently identified stable unannotated transcripts (SUTs) and cryptic unstable transcripts (CUTs). In addition, the verified RNA hemizygotes have been sporulated and dissected to generate haploid mutant collections and homozygous deletion strains. The majority of RNAs studied are non essential under nutrient rich (YPD) conditions. Furthermore, these RNA deletion strains will be used for RNA fitness profiling to explore, by next generation sequencing of barcodes, the role of individual RNAs to growth of yeast under a variety of conditions and treatments. Nevertheless, selected RNA genes will be investigated for their potential roles in regulating expression of genes nearby. Finally, all RNA deletion strains will be deposited with international repositories for yeast strains to allow distribution of this new resource to provide a comprehensive gene deletion collection for genome-wide analysis.

204 A Scaffold function of long noncoding RNA HOTAIR in protein ubiquitination

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Although mammalian long noncoding (lnc)RNAs are best known for modulating transcription, their post-transcriptional influence on mRNA splicing, stability, and translation is emerging. Here we report a post-translational function for the lncRNA *HOTAIR* as an inducer of ubiquitin-mediated proteolysis. *HOTAIR* associated with E3 ubiquitin ligases bearing RNA-binding domains, Dzip3 and Mex3b, as well as with their respective ubiquitination substrates, Ataxin-1 and Snurportin-1. In this manner, *HOTAIR* facilitated the ubiquitination of Ataxin-1 by Dzip3 and Snurportin-1 by Mex3b in cells and *in vitro*, and accelerated their degradation. *HOTAIR* levels were highly upregulated in senescent cells, causing rapid decay of targets Ataxin-1 and Snurportin-1, and preventing premature senescence. These results uncover a role for a lncRNA, *HOTAIR*, as a platform for protein ubiquitination.

205 B Towards improved shRNA inhibitors with a Dicer-independent processing route

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Towards improved shRNA inhibitors with a Dicer-independent processing routeBen BerkhoutLaboratory of Experimental Virology, Academic Medical Center, University of Amsterdam, The Netherlands (b.berkhout@amc.uva.nl) Short hairpin RNAs (shRNAs) are widely used to induce RNA interference (RNAi). The shRNA is processed by the Dicer endonuclease into an siRNA duplex. One strand of the duplex instructs the RNA-induced silencing complex (RISC) with the catalytic AGO2 protein to mediate cleavage of the complementary mRNA target. Although shRNAs can potently and specifically suppress target genes, RNAi may also cause serious side-effects, which is an siRNA-sequence and dose-dependent phenomenon. Thus, there is a need to select potent shRNAs in order to reduce the required shRNA concentration. To date, only few shRNA designs have been tested. We tested a variety of shRNAs that differed in stem length and terminal loop size and revealed strikingly different RNAi activities and shRNA processing patterns. Interestingly, we identified a specific shRNA design that uses an alternative Dicer-independent processing pathway, which also resulted in potent knockdown of the target gene. Detailed shRNA analyses indicated that a short stem length is critical for avoiding Dicer processing and activation of the alternative processing route, in which the shRNA is incorporated into RISC and processed by the AGO2-mediated slicer activity. Such alternatively processed shRNAs (AgoshRNAs) yield only a single RNA strand that effectively induces RNAi, whereas conventional shRNA processing results in an siRNA duplex of which both strands can trigger RNAi. These results have important implications for the future design of more specific RNAi therapeutics.

206 C AGO1 requires interaction with GW182 to repress translation of miRNA targets

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Animal miRNAs silence the expression of mRNA targets through translational repression, deadenylation and subsequent mRNA degradation. Silencing requires association of miRNAs with an Argonaute protein (AGO) and a GW182 family protein. In turn, GW182 proteins interact with PABP and the PAN2-PAN3 and CCR4-NOT deadenylase complexes. These interactions are required for the translational repression, deadenylation and decay of miRNA targets (Huntzinger at al., 2013). Recent studies have indicated that miRNAs can also repress translation in a GW182-independent but AGO-dependent manner (Fukaya and Tomari, 2013). AGO1 and GW182-mediated repression was reported to occur by distinct mechanisms in *Drosophila melanogaster* (Dm) cells (Fukaya and Tomari, 2013). However, the contribution of these two alternative mechanisms to silencing has remained unclear. To address this question, we characterized the interaction of *Dm* AGO1 and GW182. Based on the recent structure of human AGO2 (Schirle and MacRae, 2012), we designed mutations that disrupt *Dm* AGO1 interaction with GW182. Functional assays in *Dm* cells indicate that *Dm* AGO1 requires interaction with GW182 to mediate translational repression and degradation of mRNA targets and does not possess independent repressive activity. We are combining cellular, biochemical and structural approaches to investigate how the GW182 proteins repress translation of miRNA targets.

207 A Argonaute-associated factors required for translational repression in plant RNA silencing

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In *Arabidopsis thaliana*, microRNAs (miRNAs) are loaded in ARGONAUTE 1 (AGO1) as part of RNA induced silencing complexes (RISCs) to regulate target messenger RNAs (mRNAs) via base-pairing. AGO1 possesses an intrinsic endonuclease activity responsible for the 'slicing' of mRNA targets, an activity that is abrogated when bulges or mismatches face nucleotides 10-11 of the miRNAs. Such mismatches promote in turn alternative forms of target repression such as the translational inhibition and/or decay of the mRNAs. Intriguingly, the fact that in *Arabidopsis* most of the miRNAs regulates their target mRNAs via perfect or near-perfect complementarity has contributed to the widespread belief that plant miRNAs, unlike their animal counterparts, exert their effect mostly through target mRNA slicing. Yet, a previous work notably carried out in our lab suggests that these miRNAs can concurrently slice and transnationally inhibit a given pool of mRNAs. This raises the fundamental question of how slicing is avoided during translational inhibition. One possibility is that translational repressor proteins actually associate to AGO1 in order to change further the fate of the target mRNAs. Following this hypothesis, we have biochemically isolated three AGO1-associated factors. Here, we show that these proteins genetically interact with AGO1; they do not affect the miRNAs biogenesis, nor the stability of the main silencing factors, but clearly change the mode of action of AGO1. Indeed, upon deletion of these proteins, the slicing activity of AGO1 is greatly improved, favouring the cleavage of the target mRNAs. Surprisingly, this property also gives to these mutant plants a stronger resistance upon infection by the *Tobacco rattle virus* (TRV). Together, our results revealed the existence of three AGO1 partners that seem important to modulate the slicing activity of AGO1. As multiple pools of AGO1 co-exist in *Arabidopsis*, we propose that these factors act as guides and drive AGO1 toward a specific protein complex in which the translational inhibition is favoured over slicing.

208 B Endogenous RNA interference is driven by copy number

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A plethora of non-protein coding RNAs (ncRNA) are produced throughout eukaryotic genomes, many of which are transcribed antisense to protein-coding loci. A direct interaction between antisense ncRNA and protein-coding mRNA would form double-stranded RNA that could instigate an RNA interference (RNAi) response, resulting in mRNA down-regulation. However, this occurs very rarely and the variables controlling such an RNAi response remain undefined. Here we use a minimal reconstituted RNAi system in budding yeast to show that gene copy number is a key factor controlling the RNAi response to transcripts from endogenous loci, and that increasing copy number is sufficient to cause the degradation of both sense and antisense RNA by RNAi. This is observed for loci expressing ncRNA or mRNA, and occurs even with rare or unstable antisense transcripts. Importantly, increased RNA abundance does not account for this effect as multi-copy loci produce more small interfering RNAs (siRNA) than single-copy loci with equivalent RNA expression. The difference can instead be explained by the ability of multi-copy loci to simultaneously transcribe sense and antisense RNA. Cells are able to identify high-copy DNA, which is an essential step in the suppression of newly evolved transposable elements; our experiments clearly demonstrate that identification of the products of high-copy DNA is an emergent property of a minimal RNAi system. Efficient surveillance of high-copy sequences by RNAi would however require genome-wide transcription, suggesting a function for the pervasive transcription of eukaryotic genomes. We propose that pervasive transcription is part of a defence mechanism capable of directing a sequence-independent RNAi response against transposable elements amplifying within the genome.

209 C Identification of anhydrobiosis-related genes using RNA interference with 27-bp RNA duplexes

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Anhydrobiosis is a highly stable state of biological organization that is achieved by certain species when exposed to extreme water stress. During anhydrobiosis, specimens can be submitted to diverse abiotic stresses such as UV light, radiation and pressure, and still remain viable. RNA interference (RNAi) has been used to identify anhydrobiosis-related genes in order to allow the development of future technologies enabling more efficient methods for conserving organs, vaccines, enzymes and molecules of interest.

RNAi can be triggered in the anhydrobiotic nematode *Panagrolaimus superbus* through many ways, as i) micro-injection, ii) feeding or iii) soaking. We decided to do a screening of 25 genes that have been described as up-regulated during desiccation in other anhydrobiotic species, using soaking with 27 bp siRNAs (dicer substrates), approach that is much simpler since dicer substrates can be commercially purchased and was shown by our group as an efficiently technique of triggers RNAi.

Worms were grown at 20°C in the dark, collected from culture plates and transferred to tubes containing siRNAs against a target gene in the final concentrations of 1 mM. They were kept in the dark for 24 hours at 21°C. Dicer substrates were designed in the program Strand Analysis. After soaking, to determine whether the knockdown leads to lethal effects, survival percentages were determined by staining with Erythrosin B. To determine the effect of RNAi knockdown on desiccation resistance, the worms were immobilized on 0.45 µm Supor filter membranes by vacuum filtration with a Sartorius funnel, then placed into 1.5 mL test tubes. Worms were then subjected to the following conditions: 98% relative humidity (RH) for 24 h (in a saturated solution of copper sulphate); 10% RH for 24 h (in dry silica gel) and pre-hydration in 100% RH for 24 h (in distilled water vapour). Rehydration was achieved by adding 1.5 mL of M9 buffer to the tubes. Determination of viability after desiccation was done by staining with Erythrosin B.

On this screening, we were able to identify that 12% of the selected genes showed significant reduction in viability after silencing and desiccation. This study is important to guide our next steps on the comprehension of the anhydrobiotic process. Our findings may accelerate the process of identifying anhydrobiosis-related genes.

210 A Human Dicer caught in the act via single-molecule fluorescence spectroscopy

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The microRNAs are endogenous small non-coding RNAs generated through a series of enzymatic processing steps. After an RNA transcript is processed by the Drosha complex, the precursor miRNA (pre-miRNA) is exported to the cytoplasm where it is cleaved by the Dicer complex. The latter contains Dicer and several non-catalytic accessory proteins such as TRBP and PACT. Despite the discovery of the key actors in the miRNA biogenesis, the molecular basis of miRNA maturation remains unclear. In order to unveil the molecular mechanisms of pre-miRNA processing by the Dicer complex, we have developed a single-molecule technique by combining fluorescence spectroscopy with a protein complex pull-down assay. Using this new technique, we have shown step by step the action mechanism of human Dicer and its cofactors – from RNA binding and cleavage to product release. Our single-molecule data highlights novel coordination between Dicer and its cofactors.

211 B Single-molecule view on the action of Drosophila Dicer-2

Anna C. Haagsma, Mohamed Fareh, Chirlmin Joo

#N/A

RNA interference is a novel gene regulation process which involves small interfering RNAs (~20-30 nucleotides). The small interfering RNAs contribute to a multitude of cellular processes including development, metabolism and stress responses. The RNase III family enzyme Dicer is a specialized class of endonucleases that cleave double-stranded RNAs into small interfering RNAs [1, 2]. Despite the importance of the enzyme in RNA interference, the mechanism of Dicer is not yet fully understood. *Drosophila* has two types of Dicer with distinct biological functions. Among these two, Dicer-2 plays a critical role in transposon control and defense against viral infection. Dicer-2 processes a long double-stranded RNA into many short interfering RNAs. For efficient processing of a long double-stranded RNA, Dicer-2 may utilize a translocation mechanism through its helicase domain [3, 4]. By pulling down Dicer-2 out of insect cell extracts and immobilizing on a single-molecule imaging surface [5], we attempt to visualize the putative translocation process of the Dicer-2 protein for the first time.

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212 C Analysis on viral suppressors of RNA silencing in plant cell-free RISC assembly system

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Small RNAs including small interfering RNAs (siRNAs) and microRNA (miRNAs) play large roles in both plant and animal gene expression. The effector complex of posttranscriptional gene silencing (PTGS), RNA-induced silencing complex (RISC), contains a single-stranded small RNA bound to an ARGONAUTE family protein (AGO). RISC represses gene expression, guided by the small RNA to complementary sequences on target RNAs. RISC assembly is a key step of RNA silencing. On plant RISC assembly, small RNA duplexes are loaded onto AGOs in a manner dependent on molecular chaperone machinery, followed by removal of one strand of the duplex. PTGS is an important mechanism for antiviral defense in plants. To counteract this defense, viruses express viral suppressors of RNA silencing (VSRs). Despite accumulating evidence of VSRs, it is not clear how VSRs act on host PTGS. The VSR functions have not been hitherto addressed in a plant cell-free system that recapitulates RISC assembly. This study analyzes the roles of several VSRs, including*Tomato bushy stunt virus* P19, *Turnip crinkle virus* P38, *Peanut clump virus* P15, *Cucumber mosaic virus* 2b, and *Potato virus Y* HC-Pro, in a cell-free RISC assembly system that has been recently developed by using extracts of evacuolated tobacco BY-2 protoplasts (BYL). All VSRs synthesized by *in vitro* translation inhibited RISC assembly, but did not affect target RNA cleavage by preassembled RISC. The inhibitory effect of VSR on RISC assembly was different among tested synthetic small RNA duplexes. The present study can reveal novel characteristics of VSRs, and further help dissect molecular mechanisms underlying PTGS.

213 A Real-time observation of target binding and dissociation of Argonaute-guide complex

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Argonaute (Ago) is the key component of the RISC (RNA-induced silencing complex) which is an effector of small RNA(20–30nt)-based gene regulation. In the regulatory process, target recognition by Ago-guide is important for controlling gene expression vigorously. Target dissociation is also crucial for the recycling of Ago in the regulation. Here, by using single-molecule fluorescence resonance transfer (FRET) assays and *Thermus thermophilus* Ago (*Tt*Ago), we monitor the target binding/dissociation kinetics of Ago-guide and reveal that different regions of guide-target base pairing have different roles in their binding and dissociation.

214 B Investigating the potential role of an archaeal Argonaute in the subtype III-B CRISPR system of M. piezophila

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Members of the Argonaute protein family are found in all higher eukaryotes and have important functions in translational regulation of gene products, as well as defense against viruses or transposable elements. In processes known as RNA interference (RNAi) and microRNA (miRNA) mediated silencing, Argonaute proteins associate with small non-coding RNAs and use them as guides to bind to complementary RNAs.(*1*) Instead of using RNAi as a defense mechanism against non-self genetic elements, archaeal and many bacterial genomes contain Clustered Regularly Interspaced Short Palindrome Repeats (CRISPR) and variable arrays of the CRISPR-associated (cas) genes, which together form the CRISPR-associated system (CASS).(*2*) In a manner analogous to RNAi, this adaptive immune system uses small RNAs to specifically target and degrade foreign genetic elements derived from phages and/or plasmids.

Despite the absence of homologous RNAi pathways, Argonaute proteins have been identified in prokaryotes. The crystal structures of several bacterial and archaeal Argonautes revealed the same domain architecture as their eukaryotic homologs. Furthermore, catalyltically active prokaryotic Argonautes have been shown to bind small nucleic acid guides for endonucleolytic cleavage of complementary targets *in vitro*. However, the biological function of prokaryotic Argonaute proteins remains unknown.(*3*)

Recent phylogenetic analyses by *Makarova* et al. revealed the genomic colocalization of members of the Argonaute family and CASS in archaea.(*4*) Potentially active Argonaute proteins are encoded in the CRISPR subtype III-A and III-B loci in the archaeal species *Methanopyrus kandleri* and *Marinitoga piezophila*, respectively. In both genomes, the Argonaute protein is in the same operon as Cas1 and Cas2, which are likely to be responsible for spacer acquisition. Here, I present my recent efforts towards characterizing the CRISPR subtype III-B operon of *M. piezophila* and elucidating the role of Argonaute in this system.

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215 C NMR structural study of the two N-terminal dsRBDs of TRBP in complex with siRNA.

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RNA interference mediated by small interfering (si)- or micro (mi)- RNA is a cellular mechanism of gene expression regulation at the post-transcriptional level. The molecular effector is the RISC (RNA InducedSilencing Complex), a ribonucleoparticle comprising the protein Argonaute (Ago) and a single strand RNA called the siRNA 'guide strand'. Messenger-RNA recognition by the RISC, occurring through the sequence specific annealing with the guide strand, leads to translation inhibition and/or mRNA degradation.

Si-RNA guide strand precursors are RNA duplexes of ca. 20 base pairs. However, only one strand is selected and loaded into Ago during each RISC formation event. For a particular class of siRNA called asymmetric siRNA, the guide strand selection process is strongly biased, with one strand of the siRNA duplex being preferentially loaded into Ago. Although this property has been shown to correlate with the thermodynamics stability of the siRNA extremities, the molecular mechanism of the selection process is not understood yet. The key protein factors involved in the guide strand selection process are Ago, TRBP, and Dicer, associated into the RLC (RISC Loading Complex), but the relative contribution of these is yet unknown.

In order to understand better the contribution of TRBP in RISC loading, we carried out a structural study of its two N-terminal dsRBDs (dsRNA Binding Domains) (dsRBD12) in complex with various siRNAs by NMR spectroscopy. Three-dimensional structures of the each dsRBD were solved in their RNA bound form, revealing the presence of an additional α-helix at the N-terminus of the first dsRBD. Intermolecular contacts observed between dsRBD12 and a highly asymmetric siRNA indicate the presence of two major dsRBD12 binding sites. Furthermore, RDC (Residual Dipolar Coupling) data measured in two different media suggest that the two dsRBDs of TRBP do not bind siRNA independently, but rather adopt a well-defined orientation relative to one another. Interested by the impact of siRNA methylation in silencing activity, we tested whether siRNA methylation would also have an impact on TRBP-siRNA binding. Our NMR data show that position specific methylation of RNA actually affects TRBP binding on siRNA. Overall, by helping to characterize siRNA recognition by TRBP, our results contribute to the understanding of the role of TRBP in RNA silencing.

216 A crRNA-guided R-loop formation and the architecture of the Type I-C Cascade

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CRISPR-Cas system serves as an RNA-based immune system against invasive genetic elements. In several Type I CRISPR-Cas systems, the subtype-specific Cas proteins assemble around the CRISPR RNA (crRNA) to form Cascade, which recognizes the target ds-DNA in a sequence specific fashion. Here we reveal the discrete biochemical steps leading to the target DNA recognition by the *B. halodurans* Type I-C Cascade, report its low resolution EM structure, and the crystal structure analysis of the scaffolding protein Csd2. We provide evidence that I-C Cascade actively promotes the base-pairing between crRNA spacer and the target ds-DNA, forming the R-loop structure. The architecture of *B. halodurans* Type I-C Cascade resembles *E. coli* Type I-E Cascade, with minor architectural differences. The Csd2 crystal structure containss a ferredoxin fold and a positively charged groove, which upon oligomerization forms an extended nonspecific binding surface for crRNA or crRNA/DNA duplex. Mutagenesis guided by the crystal structure and EM docking further provides insights about the nucleic acid binding and oligomerization mechanisms in Csd2. Overall, our results define the molecular function of Type I-C Cascade.

217 B P body-associated RNA silencing complex PRSC of Cryptococcus effects transposon suppression

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We recently described a novel RNA silencing system in the yeast *Cryptococcus neoformans* (Dumesic, Natarajan et al., *Cell*, 152, 957-968, 2013), in which stalled spliceosomes serve as a signal for RNAi-mediated genome defense. In addition to the nuclear SCANR complex required for siRNA accumulation, we described PRSC, a P body-associated RNA silencing complex. PRSC is dispensable for siRNA accumulation, suggesting it is an effector complex. PRSC contains two subunits: the Argonaute, Ago1 and a glycine-tryptophan(GW) motif protein, Gwo1. Gwo1 and mammalian GW182 family members are best reciprocal protein search hits suggesting they are orthologs. Together with their similar association with Argonaute proteins and their localization to P-bodies, the effector complexes from the yeast and mammalian systems appear to be striking similar. Consistent with this view, PRSC physically associates with mRNAs targeted by small RNAs in a siRNA-dependent manner. To probe the biological function of RNA silencing, we developed a quantitative *in vivo* transposon mobilization assay using a marked *Harbinger* DNA tranposon. We found PRSC and SCANR are both required for suppression of transposon mobilization *in vivo*: specifically, we observed a 1000-fold increase in the frequency excision events in null mutants in either complex, supporting the view that PRSC is a genome defense effector complex. Despite the magnitude of its effect on transposon mobilization, cells lacking PRSC display only modest increases in target RNA levels. This raised the possibility that that PRSC, like mammalian GW182-Argonaute complexes, controls the translation of targets mRNAs. To probe the mechanistic role of PRSC, we successfully implemented ribosome profiling in *Cryptococcus* to examine the impact of PRSC on transcriptome-wide ribosome occupancy. Insights into the role of PRSC obtained from these experiments will be described.

218 C The rough Endoplasmatic Reticulum is the central nucleation site of siRNA-mediated RNA silencing

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Despite of progress in mechanistic understanding of the RNA interference (RNAi) pathways, the subcellular sites of RNA silencing still remain under debate. We will present a quantitative tracing of the fate of exogenous siRNA within the cell upon lipid delivery. Our data demonstrate that entry of siRNA in the RNAi pathway with current delivery vehicles is highly inefficient, whereas trafficking of the siRNA to the site of RISC (RNA induced silencing complexes) loading emerges as one major rate limiting step. We will further present data which reveal that loading of lipid-transfected siRNAs and endogenous microRNAs into RISC, encounter of the target mRNA, and Ago2-mediated mRNA slicing in mammalian cells are nucleated at the cytosolic membrane of the rough endoplasmic reticulum (rER). We identify TRBP and PACT as key factors anchoring RISC to ER membranes in an RNA-independent manner. Finally we will discuss implications of our work on the development of next generation, subcellular targeted delivery strategies for RNAi therapeutics.

219 A Functional characterization of DCL1 and DCL2 proteins from Medicago truncatula

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In eukaryotic organisms several types of 21-24 nucleotides long RNA molecules (called small regulatory RNA; srRNA) are involved in the sequence-specific regulation of numerous biological processes, including developmental and stress response pathways or pathogen defense. This widespread mechanism of RNA-mediated regulation is facilitated by a range of proteins which participate in processing of srRNA precursors, and enable functioning of the mature products. Among them ribonuclease III Dicer was identified as the key enzyme involved in the last step of srRNA biogenesis. Mammals and many other animals use a single Dicer enzyme to generate a complex spectrum of srRNAs. In contrast, there are several Dicer-like proteins (DCL) typically found in plants. In *Arabidopsis thaliana* four DCL proteins were identified (AtDCL1-4). The consecutive studies demonstrated that each AtDCL protein is predominantly specialized in the production of different class of small RNAs. AtDCL1 mainly produces 21 nt long microRNAs, while the products of AtDCL2, AtDCL3, and AtDCL4 are various small interfering RNAs (siRNAs) of 22, 24, and 21 nucleotides in length, respectively. However, some functional overlaps for Arabidopsis DCL proteins were also showed. Considerably less information concerning Dicer-like proteins from other plants is available.

In order to increase our knowledge on DCL proteins in legume plants we attempted to determine how many members of a DCL family are encoded in the genome of *Medicago truncatula*. We obtained the full-length cDNAs coding for MtDCL1 and MtDCL2 proteins and determined the profile of their genes expression in different plant tissues and at different developmental stages. Next, we produced the peptides representing the "catalytic cores" of both proteins (MtDCL1-CC and MtDCL2-CC) and characterized *in vitro* their enzymatic activity and substrate specificity. Finally, we compared the enzymatic activity of MtDCL1-CC and MtDCL2-CC with the activity of two commercially available enzymes: human Dicer and parasitic protozoan *Giardia intestinalis* Dicer. The former is composed of a full set of functional domains typically found in Dicer-type proteins, while the latter can be classified as a minimal Dicer, similar to MtDCL1- and MtDCL2-CC.

220 B Distinct activities of the Dictyostelium discoideum RNA-dependent RNA polymerases in post-transcriptional gene regulation

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Cellular RNA dependent RNA polymerases (RdRPs) are involved in various processes of gene regulation and different mechanisms for their action have been proposed. To study the function of the three RdRPs in the amoeba *Dictyostelium discoideum*, we have deleted the encoding genes *rrpA*, *rrpB* and *rrpC* in all possible combinations. We show that the two RdRPs RrpA and RrpC of the amoeba exert specific, non-overlapping roles in the regulation of retrotransposons and microRNAs.

Strains lacking RrpC strongly accumulate transcripts of the centromeric retrotransposon DIRS-1 and show a dramatic loss of DIRS-1 small RNAs, which are asymmetrically distributed in the wild type, as shown by deep sequencing. We report the discovery of an hitherto unknown long antisense DIRS-1 transcript that is driven by the promoter activity of the right inverted long terminal repeat. Fluorescence *in situ* hybridization shows both this long antisense transcripts and the sense RNA in nuclear spots. We propose that in wild type cells, both DIRS-1 transcripts are generated, but post-transcriptionally degraded by the action of RrpC, possibly with the help a Dicer-related nucleases in the amoeba. By this, RrpC apparently serves to prevent retrotransposition, as we observe a strong accumulation of DIRS-1 copies in the genome of the rrpC gene deletion strain.

In contrast to this, the silencing of another retrotransposon, Skipper, appears to be mediated by RrpA and the Dicer-related nuclease DrnB. Deletion strains of the respective genes display strongly increased Skipper transcript levels, but here, surprisingly, also small RNA levels are elevated. Such increased RNA levels in strains lacking RdRPs were also observed for other types of small RNAs, including microRNAs.

To study the activity of the RdRPs molecularly, we have investigated in the rrp gene deletion strains the appearance of small RNAs derived by antisense or hairpin RNA constructs against the transgene *lacZ*. Both types of constructs led to a similar reduction of the enzymatic activity of β-Galactosidase. However, only in *rrpC* knock out strains, low levels of β-gal small interfering RNAs (siRNAs) could be detected in antisense RNA expressing strains. In contrast to this, and at considerably higher levels, all hairpin RNA expressing strains featured β-gal siRNAs. Spreading of the silencing signal to mRNA sequences 5' of the original hairpin trigger was observed in all strains, except when the *rrpC* gene or that for the Dicer-related nuclease DrnB was deleted, indicating that transitivity of an RNA silencing signal exists in *D. discoideum* and that it requires the two enzymes RrpC and DrnB.

In summary, our data indicates that the RdRPs RrpA and RrpC have specific endogenous targets in *D. discoideum* and that gene silencing triggered by antisense RNA and hairpin RNA works through different mechanisms despite an overall similar efficiency.

221 C Computational Analyses and Experiments Indicate Role of Guide RNA Structure in MicroRNA::Target Interaction

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MicroRNAs (miRNAs) are small noncoding RNAs which regulate expression of numerous metazoan genes posttranscriptionally. Binding of mature miRNAs guides RNA-induced silencing complexes to complementary messenger RNA targets. We investigated the role of guide structures in miRNA::target interactions using computational RNA secondary structure analyses and experimental gene knockdown assays. The computational investigation revealed characteristic common and species-specific features. Virtually all structures contained at least one unpaired site suitable for nucleation suggesting that the kinetics of RNA-RNA interactions contribute to RISC binding. Structures of mature human miRNAs were found to have a preference for U/A-rich unpaired 5'ends and U/G-rich paired 3'ends. Unpaired sites matching with validated targets were rich in base-pairing-competent bases G and U and poor in A and C as compared to nonmatching sites indicating the involvement of such sites in miRNA::target interactions. One group of miRNAs preferred a single nucleation site, located either 5' terminal, 3' terminal or central, whilst others appeared to be bi- or multivalent. Conversely, local mRNA targets allowed only one kind of nucleation indicating that mRNAs select their miRNA partners as much as the other way around. We experimentally confirmed this hypothesis by comparing wild-type miRNAs with mutants in which target base-pairing was unchanged but which comprised reprogrammed guide structures and nucleation sites. Removal of nucleation sites resulted in reduced silencing, whereas switch from one to another allowed interactions, did not affect silencing. Nucleation site changes yielding site extensions enhanced silencing. We suggest a two-phase control mechanism of miRNA::target interactions: First, a kinetic nucleation-based control dictated by the mRNA target site repelling false-positive seed matches and second a thermodynamically controlled seed match verification process dominated by the sequence of the mature miRNA.

222 A The CCR4-NOT complex releases PABP from silenced miRNA targets in the absence of deadenylation

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GW182 family proteins interact with Argonaute proteins and are required for the translational repression, deadenylation and decay of miRNA targets. To elicit these effects, GW182 proteins interact with PABP and the CCR4-NOT deadenylase complex. Although the mechanism of miRNA target deadenylation is relatively well understood, how GW182 proteins repress translation is not known. Here, we demonstrate that GW182 proteins decrease the association of PABP with silenced targets in the absence of deadenylation. We further show that the dissociation of PABP contributes approximately 2-fold to repression, indicating that additional repressive mechanisms are used by miRISCs to achieve maximal target silencing. Remarkably, PABP dissociation requires the interaction of GW182 proteins with the CCR4-NOT complex but not with PABP. Accordingly, NOT1, POP2 and CCR4 cause dissociation of PABP from bound mRNAs in the absence of deadenylation. Our findings indicate that the recruitment of the CCR4-NOT complex to miRNA targets by GW182 proteins releases PABP from the mRNA poly(A) tail, thereby facilitating translational repression and deadenylation. To gain further insight into the mechanism of PABP release by the CCR4-NOT complex, we are currently investigating which subunit(s) of the CCR4-NOT complex contacts directly PABP and is responsible for PABP release. Given the central role of the CCR4-NOT complex in post-transcriptional mRNA regulation, we anticipate that this novel activity of the complex will contribute to the translational repression of a large variety of different mRNAs.

223 B Characterization of small RNAs that contribute to ribosomal RNA gene silencing through the siRNA machinery

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Cellular growth and division is limited by the number of ribosomes actively translating mRNA in the cell. The ribosomal RNA gene is highly transcribed making up to 80% of total RNA transcribed in the cells with the deregulation thereof leading to abnormal cellular growth and cancer. In normal human haploid cells there are approximately 200 copies of the ribosomal RNA gene of which half are maintained in a silent and heterochromatic state. Maintaining the ratio of active to silent ribosomal DNA loci seems to be critical in maintaining normal cellular homeostasis. The major Polymerase that transcribes the ribosomal DNA locus is RNA Pol I. Data suggest that Pol II is also localized to the ribosomal DNA locus and that the low level of transcription by Pol II aids in maintaining the silent loci in a heterochromatic state. These Pol II transcripts of the ribosomal DNA locus provide Argonaute 2 with small RNAs which allow it to be in turn targeted to the ribosomal DNA. Argonaute 2 then recruits the necessary silencing machinery to maintain the silent loci in their heterochromatic state thus maintaining a normal cellular growth rate. The major goal of this work is to characterize the small RNAs transcribed by Pol II and how they feed into the ribosomal DNA silencing pathway.

224 C Structural Characterization of the RNA, Cyp33, MLL, Histone H3 Interaction Network

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The Cyclophilin 33 (Cyp33) is a member of the *cis-trans* peptidyl-prolyl isomerases family of cyclophilins. Members of this family possess various additional domains. Cyp33 includes an N-terminal RNA Recognition Motif (RRM) domain next to the conserved cyclophilin domain with PPIase activity.

It was shown that the RRM domain of Cyp33 mutually exclusive binds to RNA and the third Plant Homeobox domain (PHD3) of the Mixed Lineage Leukemia factor (MLL) [1]. This competitive binding makes Cyp33 unique among the RNA recognition motif. Further it was demonstrated that the binding of Cyp33 to the Mixed Lineage Leukemia factor down-regulates the expression of some targets of MLL, which is a transcription activator for a series of genes from the HOX family. RNA binding sequences of Cyp33-RRM can be found in an non-coding RNA (ncRNA) located in the 3'-UTR of HOXC8, which itself is a target of MLL. Addition of this ncRNA rescues the expression of HOXC8 in cells with a silent HOXC locus leading to the theory that Cyp33 bound to MLL is sequestered by the ncRNA and thereby allow MLL to switch back to it activation state. In its transcriptional active state MLL is bound to a specific epigenetic mark on Histone H3 (H3K4me3) mediated by the PHD3 domain, the same domain which also interacts with the RRM of Cyp33. MLL is an oncoprotein and it fusion variants are associated with infant leukemia. All of the fusion variants of MLL lack the cassette of the homeobox and all subsequent domains. It was shown that reinsertion of the third PHD domain (PHD3) rescues the aberrant transcription caused by MLL fusion proteins [2]. This emphasizes the importance of this interaction network between RNA, Cyp33, MLL and H3K4me3.

In this project we studied the various structural aspects of all four component of the beforehand described interaction network using Nuclear Magnetic Resonance spectroscopy and Isothermal Titration Calorimetry. Using different labeling schemes we expressed the RRM domain of human Cyp33 and in addition to the PHD3 domain of human MLL. Further we used different RNAs (AAUAAA and UAAUGUCG) and the Histone H3 peptide with ammonium tri-methylation at lysine four (H3K4me3).

The structure of Cyp33-RRM in free state revealed a third alpha helix in the conserved C-terminal extension of the RRM which folds back on the RRM beta-sheet. Different binding registers precluded an accurate structure determination of the RRM bound to RNA. After all we obtained a model for such a complex which shows that the third helix has to dissociate from the beta-sheet in order to allow complexation with RNA. Structure determination of the Cyp33-RRM : MLL-PHD3 complex in combination with ITC experiments involving H3K4me3 revealed an allosteric effect in ternary complex formation of Cyp3-RRM, MLL-PHD3 and the epigenetically marked tail of Histone H3.

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225 A The Igf2as transcript is exported into the cytoplasm and is associated with polysomes

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Murine insulin-like growth factor 2 antisense (*Igf2as*) transcripts originate from the opposite strand of the same *Igf2* locus as the *Igf2* sense mRNA. *Igf2as* transcripts are located in a cluster of imprinted genes together with *Igf2*, Insulin 2 (*Ins2*) and *H19* genes on chromosome 7. Loss of imprinting of *IGF2* locus in humans is associated with Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS) as well as with Wilm's tumor and colorectal cancer. We developed a RNA-FISH protocol to detect and determine the location of *Igf2as* and *Igf2* transcripts. The results from the RNA-FISH were confirmed with quantitative real-time PCR and clearly indicate that *Igf2as* transcripts are predominantly located in the cytoplasm of C2C12 cells. A polysome association study was used to determine if the *Igf2as* had coding potential. We showed that *Igf2as*sedimented with polysomes in a sucrose gradient. The cellular localization of *Igf2as* transcripts together with polysome fractionation analysis provides compelling evidence that *Igf2as* is protein coding. We performed expression analysis of *Igf2as* transcripts in muscle, brain and liver tissues at different stages of development. The results showed high *Igf2as* expression in tissues of fetuses and newborns. Contrary, in adult tissues the*Igf2as* expression was low. These expression patterns indicate that *Igf2as* plays a role in the early developmental stages. To study the function of the new putative protein we are using two approaches. First, we are overexpressing *Igf2as* in transfected C2C12 cells to investigate its effects and to characterize putative proteins. Second, we perform an RNA-Seq experiment with RNA from primary muscle cells from DMR1-U2 knockout mice compared to wild type cells. Differentially expressed transcripts will be used to conduct a pathway analysis indicating potential networks in which *Igf2as* transcripts may be involved. Results from these experiments will be presented and discussed.

226 B Human Argonaute Suppresses Cryptic RNA Polymerase II Transcription and Chromatin Structure of the Silent rRNA Genes.

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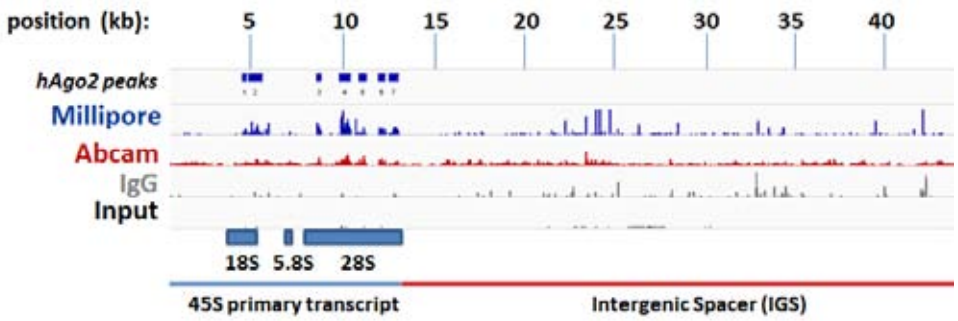
Eukaryotic cells package much of their genome into a heterochromatic structure in order to facilitate the proper regulation of gene expression and nuclear organization. In S. pombe, the initiation of heterochromatin structure is mediated by the highly conserved RNAi machinery but it remains unclear if RNAi performs a similar function in humans. To examine this question, we performed a genome-wide analysis of the chromatin binding sites of hAgo2 in the human erythroleukemia cell line, K562. Our results suggest that hAgo2 is found almost exclusively within repetitive DNA sequences, with a strong preference for the coding region of the silent ribosomal DNA loci (Figure 1, below). This localization is Dicer-dependent, and correlates with Dicer-processed, hAgo2- bound, small RNAs. Knockdown of hAgo2 causes a ~10% increase in the synthesis rate of the mature 28S and 18S rRNAs. However, a knockdown of hAgo2 causes a ~3-fold increase in synthesis of cryptic RNA species originating from the rRNA gene body. This increase corresponds with a 3-fold increase in Pol-II localization. Ago2 is needed to maintain the proper histone modification pattern, as a loss of hAgo2 causes a loss of H3K9me2 and an increase in H4ac throughout the locus. This change in histone modification pattern is likely a consequence of the loss of Suv39H1 recruitment in hAgo2 KD cells. Consistent with a role in regulating only the silent rRNA genes, hAgo2 does not localize within the nucleolus and has no effect on the recruitment of the RNA Polymerase I transcription factor, UBF1. This interaction is cell cycle specific; hAgo2 is lost from the rRNA genes during M-phase. We present our model of cooperation between hAgo2 and Pol-II in maintaining the proper chromatin structure among the silent rRNA genes.



227 C Histone replacement reveals distinct essential functions for H3K36 methylation

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Histone post-translational modifications (PTMs) are known to function importantly in regulating gene expression, primarily at the level of DNA promoters and cis-regulatory elements. However, recent evidence suggests that PTMs may also play important co-transcriptional roles related to RNA processing. Methylation of lysine 36 of the histone H3 subunit (H3K36) in particular has been implicated in co-transcriptional suppression of cryptic transcription initiation and, more recently, in regulation of alternative splicing. Though the complexity of co-transcriptional RNA-level regulation necessitates a robust, controlled *in vivo* model to study the effect of depleting histone PTMs upon splicing, to date such a model has not been developed. Crucially, existing studies that link H3K36 methylation with RNA processing rely upon perturbation of PTM-catalyzing "writer" enzymes, many of which are known to have non-histone substrates that can confound interpretation of phenotypes. Here we present a novel set of tools in *Drosophila melanogaster* that enable full genetic replacement of endogenous histones with transgenic copies bearing PTM-inactivating mutations. We have generated endogenous histone deletion lines complemented by transgenic histones containing mutant H3K36 alleles to facilitate *in vivo* analysis of the RNA processing consequences of eliminating H3K36 methylation. We report that two H3K36 alleles, alanine (H3K36A) and arginine (H3K36R), rescue full histone deletion phenotypes to vastly different degrees, suggesting a separation of essential functions for H3K36. Interestingly, H3K36R animals exhibit locomotion defects two days prior to lethality, suggesting potential defects in neuronal processes in which alternative splicing is known to play a prominent role. We intend to use RNA-seq to comprehensively evaluate expression and splicing phenotypes manifested in H3K36 alleles.

228 A Transcriptome-wide Analysis of the m6A Landscape Reveals Pervasive Adenosine Methylation in 3′ UTRs and near Stop Codons

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Methylation of the N^6 position of adenosine (m^6A) is a post-transcriptional RNA modification whose prevalence and physiological relevance are poorly understood. The recent discovery that *FTO*, an obesity risk gene, encodes an m^6A demethylase implicates m^6A as an important regulator of physiological processes. Here we show that m^6A is a highly prevalent base modification which exhibits tissue-specific regulation and is markedly increased throughout brain development. Using a novel method for transcriptome-wide m^6A localization termed MeRIP-Seq, we identify mRNAs of 7,676 genes which contain m^6A, indicating that m^6A is a common modification of mRNA. We find that m^6A sites are enriched near stop codons and in 3' UTRs, and we uncover an association between the presence of m^6A residues and microRNA binding sites within 3' UTRs. These findings are the first global identification of the transcripts that are substrates for adenosine methylation and reveal novel insights into the epigenetic regulation of the mammalian transcriptome.

229 B Single cell analysis reveals aspects of antisense RNA regulation and mode of action in PHO84 transcription repression

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A large number of non-coding RNAs (ncRNAs) are transcribed in yeast; many of them are rapidly degraded by the nuclear exosome. The function of most of these ncRNAs, however, is still largely unknown, although a subset has been implicated in modulating transcription regulation. Loss of nuclear exosome component Rrp6 results in the accumulation of long *PHO84* antisense RNAs and repression of sense transcription in a mechanism that involves *PHO84* promoter deacetylation by the Hda1/2/3 histone deacetlylase complex. Moreover, the Set1 H3K4 methyl transferase stimulates antisense RNA production, correlating with *PHO84*repression, the precise mechanisms that orchestrates *PHO84* transcriptional silencing however is still not fully understood. Here, we use single molecule resolution fluorescent in situ hybridization (smFISH) to further investigate ncRNA mediated transcription regulation of *PHO84* at the single cell level. We show that *PHO84 AS* transcription acts as a bimodal switch in *PHO84* regulation, where low frequency *PHO84* AS transcription efficiently represses sense transcription within individual cells. Surprisingly, *PHO84* AS RNAs do not accumulate at the *PHO84* gene but are exported to the cytoplasm where they are degraded by the 5'-3' RNA degradation machinery. Furthermore, we show that loss of Rrp6 promotes antisense production by reducing early transcription termination by the Nrd1/Nab3/Sen1 complex, rather than by stabilizing the *PHO84* AS RNA, and that loss of Set1 enhances early termination by promoting Nrd1 recruitment. These observations suggest that antisense-mediated repression is regulated, at least in part, at the level of antisense early termination, and that *PHO84* silencing results from low frequency yet constant antisense transcription through the promoter rather than its static accumulation at the repressed gene.

230 C Regulation of piRNA production

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Setting germ cells apart from other somatic cells in the body is crucial for all sexually reproducing organisms. This process of germ cell specification occurs early in embryogenesis and requires the conserved process of transcriptional silencing. In *Drosophila* germ cells global transcriptional silencing is mediated by the gene *polar granule component* (*pgc*) that codes for a small 7 kDa protein. *pgc* mutant germ cells show precocious active chromatin marks that result in the transcription of somatic genes that lead to their death. Thus transcriptional silencing mediated by *pgc* feeds into the epigenetic pathway and plays a critical role in specifying a germ cell fate by suppressing a somatic one. We have found that in *Drosophila* repressive transcriptional mechanisms also play an important role at a different stage of germ cell development, namely germ line stem cell (GSC) differentiation. Repressive marks consistent with heterochromatin, namely H3K9me3, H4K20me3 and HP1, are enriched in the nuclei of the differentiating GSC daughter. Interestingly, H3K9me3 enriched chromosomal sites correlate with the location of piRNA production. The piRNA pathway is a small RNA-based mechanism that is a major component of the defense against transposable elements (TE) in the germ line. In mutants defective in heterochromatin formation, differentiation of GSC is blocked, piRNA levels are reduced, and TE activity is increased. While we thus know that heterochromatin protects the germ line by activating the piRNA pathway and repressing transposable elements, we do not understand what controls heterochromatin formation in the GSC daughter. Surprisingly, we have observed that the transcriptional silencer *pgc* that has a known role in germ cell specification is also expressed during GSC differentiation. We hypothesize that *pgc* causes transient transcriptional silencing that allows heterochromatin formation on the piRNA producing clusters and thus permits proper GSC differentiation. We have shown that the expression of the transcriptional silencer *pgc* in the GSC daughter is controlled by translational control factors Nanos and Pumilio.

231 A Mmi1, an RNA-binding protein, mediates heterochromatin gene silencing by recruiting the nuclear exosome to long non-coding RNAs in fission yeast

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RNA mediates epigenetic modifications and drives transcriptional gene silencing in a broad range of eukaryotes. In the fission yeast *Schizosaccharomyces pombe*, heterochromatic gene silencing requires a close interplay between RNAi-dependent and -independent processes. However, while the RNAi-dependent process is relatively well understood, the RNAi-independent one remains largely unknown. Our studies show that the RNA-binding protein Mmi1 and its associated RNA surveillance machinery, which are known to play a central role in fission yeast sexual differentiation control, are also involved in the deposition of the heterochromatin H3K9 methylation mark and the recruitment of RNAi machinery at specific meiotic genes. In addition and quite unexpectedly, they reveal that Mmi1 also acts at non-coding regions to mediate heterochromatic gene silencing in parallel of RNAi. We will present our latest data defining this new RNA-based RNAi-independent epigenetic silencing mechanism.

232 B Introns of plant pri-miRNAs are required for proper biogenesis and function of miRNAs

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Plant *MIR* genes are independent transcription units that encode long primary miRNA precursors which usually contain introns. For two miRNA genes, *MIR163* and *MIR161*, we show that introns are crucial for the accumulation of proper levels of mature miRNA. Removal of the intron in both cases led to a drop-off in the level of mature miRNAs. We demonstrate that the stimulating effects of the intron mostly reside in the 5'ss rather than on a genuine splicing event. Our findings are biologically significant since the presence of functional splice sites in the *MIR163* gene appears mandatory for pathogen-triggered accumulation of miR163 and proper regulation of at least one of its targets.

233 C Common regulation of micro-RNAs by oncogenic transcription factors in B-cell lymphomas

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micro-RNAs (miRNA) are regulators of many processes including the development and maintenance of cancer. Many important targets of oncomiRs and tumor suppressive miRNAs have been identified; however, the regulation of these miRNAs in cancer is not well understood. Here we demonstrate the common regulation of miRNAs by the oncogenes v-Rel, c-Myc and the AP-1 transcription factor family. We performed miRNA-seq analysis, comparing v-Rel and c-Myc transformed chicken B-cell lines to a normal bursal (B-cell) control and have identified many miRNAs that are differentially regulated. More miRNAs were upregulated than downregulated in this analysis. Using EST databases and ChIP-seq data generated by the ENCODE project, we predicted the promoters of 600 human miRNAs and found that they are conserved across vertebrates. Further, Rel, Myc, Jun and Fos bind promoters of a common set of human miRNAs. Interestingly, we found that most miRNAs upregulated by v-Rel and c-Myc had binding sites in human ChiP-seq data, indicating conserved regulation of miRNAs in chickens and humans. Furthermore, some of the downregulated miRNAs inhibit Rel transformation capability in an in vitro transformation assay.

234 A RNA-binding proteins vs. microRNAs: how IGF2BP1 modulates tumor-suppressive microRNA-action in tumor cells

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The RNA binding protein IGF2BP1 (insulin-like growth factor 2 mRNA binding protein) is highly expressed during embryonic development and is often de novo synthesized in cancer. This trait is correlated with tumor progression and lower patient survival rates. In the cytoplasm, the protein supports the expression of oncogenic factors like MYC, PTEN or ZEB1 and represses the mRNA translation of MAPK4 and ACTB. Through this post-transcriptional gene regulation IGF2BP1 promotes cell proliferation, migration and invasion of tumor-derived cells. Most interestingly, 'aggressive' tumor cells frequently express IGF2BP1 mRNA with shortened 3'untranslated region (UTR). These shorter isoforms usually result from alternative cleavage and poly-adenylation. Notably, IGF2BP1 comprises four APA-signals in an approximately 6.7kb long 3'UTR. The use of APA-signals eliminates large parts of the 3′UTR and thus shortens transcripts. This consequently causes the loss of inhibitory microRNA targeting 3'-UTR. In the case of IGF2BP1, inhibitory microRNAs include the let-7 family, suggesting regulation of oncofetal IGF2BP1 expression by tumor-suppressive microRNAs.

To identify additional regulatory microRNAs targeting the IGF2BP1 3'UTR in tumor-derived cells, we used the complete 3'UTR of IGF2BP1 as a bait in an *in vitro* microRNA trapping RNA affinity purification assay, termed miTRAP. Next-generation sequencing identified co-purified microRNAs and confirmed the association of let-7 family members, but also identified various candidate microRNAs regulating IGF2BP1 expression. Significantly, these data indicate the microRNA-family 181 as putative strong posttranscriptional regulators of IGF2BP1 expression. Several of the identified microRNAs were previously described as tumor-suppressive microRNAs, e.g. miR-181-family and miR-191, -196, -203, -205, -24-3p and miR-34a. We hypothesize that IGF2BP1 downregulation is partly responsible for the tumor-suppressive nature of these microRNAs. Additionally, the transcription of some of these microRNAs is reduced by the oncogenes MYC and ZEB1. By upregulating the expression of both these oncogenes, IGF2BP1 may inhibit the expression of the same tumor suppressive microRNAs which we found to target its mRNA.

In brief, we have identified powerful regulatory feed-back loops potentially modulating tumor progression. Current studies focus on quantifying the role of IGF2BP1 3'UTR length as a possible oncogenic 'break' in these loops and understanding the role of these candidate microRNAs in suppressing the oncogenic function of IGF2BP1.

235 B miRNA profiles characterise distinct states of cellular pluripotency

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Reprogramming of fibroblasts through induction of the Yamanaka factors (Myc, Sox2, Klf4 and Oct4) is a powerful approach to delineate the molecular characteristics of the pluripotent cellular state. We used an efficient secondary reprogramming system to monitor whole culture reprogramming over time, allowing us to characterise intermediate states as well as to identify several distinct pluripotent cell states. As part of this investigation we measured the miRNA profiles of these cell states by next-generation sequencing, which feature marked changes in the expression of many of the previously described core miRNA-mediators of pluripotency. We uncover the timing of their involvement in the process of pluripotent cell generation, which sheds light on their individual roles in the process, as well as on how miRNAs are regulated during reprogramming. We describe the miRNA processing variants that exist in these cell states, including 5' and 3' isomiRs, non-templated addition, editing and unusual strand bias. Many of the core reprogramming miRNAs have isomiRs, which can alter their targeting spectrum and lead to reinterpretation of their specific roles. Broader analysis of the dataset also suggests that other small RNA species may be involved in pluripotency as, like the miRNA population itself, the small RNA profile changes markedly after induction of the Yamanaka factors. This work is part of a larger collaborative study, which co-ordinately measured protein, miRNA, mRNA, DNA methylation and histone modification in this model of pluripotent cell generation and related data will be discussed in the context of miRNA regulation and function.

236 C Quantitative analysis reveals extensive target specificity of individual let-7 miRNA family members in vivo

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Recognition and silencing of miRNA targets is thought to rely chiefly on basepairing of the miRNA 'seed' (nucleotides 2...8) with target sequence. Since miRNAs can occur in 'families', defined by a shared seed sequence, members of a given family are considered to function interchangeably in silencing specific targets. Here, we have used the *let-7* miRNA family in *C. elegans* as a paradigm to test this hypothesis rigorously and under physiological conditions. To this end, we have developed a quantitative imaging assay that examines the effects of endogenous miRNAs on reporter transgenes, which we express in various tissues at defined, constitutive but low (physiological) levels through targeted single copy transgene integration. We find that *let-7* and its sisters act in a strikingly non-redundant manner. In particular, when examining a number of distinct targets, we observe that they differ not only in their spatial repression patterns but also in their response to the loss of individual *let-7* family members. This specificity is a function of target site sequence, which we demonstrate by reengineering a target of *let-7 proper* to be repressible by distinct family members. Importantly, family member specificity is extensive but not absolute and appears to further depend on the cellular levels of a miRNA. Thus, miRNA targeting may simultaneously be more restricted as well as dynamic (e.g. across tissues, developmental stages, or in response to environmental cues) than anticipated, impacting on both our understanding of miRNA regulatory networks and experimental procedures for reliable and meaningful miRNA target validation.

237 A Piwi-Interacting RNAs Protect DNA Against Loss During Oxytricha Genome Rearrangement

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Genome duality in ciliated protozoa offers a unique system to showcase their epigenome as a model of inheritance. In *Oxytricha,* the somatic genome is responsible for vegetative growth, while the germline contributes DNA to the next sexual generation. Somatic nuclear development eliminates all transposons and other "junk DNA", which constitute ~95% of the germline. We demonstrate that Piwi-interacting small RNAs (piRNAs) from the maternal nucleus can specify genomic regions for retention in this process. *Oxytricha* piRNAs map primarily to the somatic genome, representing the ~5% of the germline that is retained. Furthermore, injection of synthetic piRNAs corresponding to normally-deleted regions leads to their retention in subsequent generations. Our findings highlight small RNAs as powerful transgenerational carriers of epigenetic information for genome programming.

238 B Positive regulation of inflammation by miR-19

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microRNAs (miRNAs) are critical for normal cell function, and several miRNAs (e.g. miR-146a) have already been found to be involved in the repression of inflammation. To identify miRNAs that enhance inflammation, we performed *in silico* bioinformatic analyses of several known negative regulators of NFκB activity. Putative targeting by miRNAs from the oncogenic miR-17~92 cluster was enriched in such genes. Selective inhibition of each member of the miR-17~92 cluster demonstrated an important role for miR-19b in the positive regulation of NFκB signaling. We confirmed direct targeting of several key negative regulators of NFκB signaling by miR-19b, including A20 and its partner RNF11. Specific depletion of miR-17~92 in primary mouse macrophages of miR-17~92 conditional knock out mice resulted in dampened production of pro-inflammatory cytokines following innate immune activation. In addition, transfection of rheumatoid arthritis primary synovial fibroblasts with miR-19b mimics resulted in increased IL-8 production by these cells, establishing a pro-inflammatory effect of miR-19b in the context of disease. Our results thereby establish the coordinate regulation of a network of regulators of NFκB activity by miR-19, promoting inflammation.

239 C Characterization of microRNAs derived from the HIV-1 TAR RNA hairpin

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The transacting responsive (TAR) hairpin is present at the 5' and the 3' end of the HIV-1 RNA genome. This TAR hairpin is essential for HIV-1 replication because it binds the viral Tat transcriptional activator protein. Several groups have demonstrated the presence of small TAR-derived RNAs in HIV-1 infected cells. Supposedly, these small RNAs are microRNAs (miRNAs) produced by the RNAi pathway. We used the sensitive SOLiD ultra-deep sequencing method to characterize these small RNAs in HIV-1 expressing cells. This analysis revealed the presence of miRNAs corresponding to both the 5' and the 3' side of the TAR stem, with the 3' fragments being more abundant. The cleavage pattern of the miRNAs differed from the patterns expected for the Drosha/Dicer mediated RNAi pathway and suggests the involvement of another nuclease. Northern blot analysis of the intracellular RNA confirmed the presence of the TAR miRNAs and their TAR RNA precursor. Analysis of TAR-mutated HIV-1 variants revealed that these TAR RNAs and miRNAs are produced exclusively from the TAR element present at the 5' end of the viral transcripts. The role of Tat in the production of these small RNAs is currently investigated.

240 A miRNA degradation during C. elegans development

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miRNAs are small non-coding RNAs that regulate gene expression at the posttranscriptional level. These endogenous small RNAs have been shown to be involved in numerous physiological and pathological processes in many different organisms. Their biogenesis has been widely studied and the main factors involved are conserved among species. Mature miRNAs can act on their targets by either translational repression or inducing transcript degradation. miRNAs can be specifically degraded and typical half-lives of miRNAs vary from several hours to days. In *C. elegans*, miRNAs are important players in the heterochronic pathway that regulates larval development. A strict regulation of transcription as well as biogenesis has been shown to be responsible for this tight control of mature miRNA levels. To study the impact of regulated miRNA degradation on miRNA levels during *C. elegans* development we performed small RNA sequencing at hourly intervals spanning two larval transitions. We found that a number of miRNAs are rapidly and extensively degraded at specific timepoints with half-lives of down to approximately one hour. In a particularly striking example we observed a >40-fold down-regulation of a specific miRNA over six hours. The factors that are involved in this rapid degradation as well as the functional relevance of this tight regulation are currently being investigated.

241 B Temporal expression of tRNA fragments in development of *Triops cancriformis* (Tadpole shrimp)

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It is well known that microRNAs, which are 18-24 nucleotide (nt) regulatory RNAs, are deeply implicated in development, especially in morphogenesis. However, the relationship between other sizes of small RNAs (sRNAs) and development remains obscure. In this research, we chose *Triops cancriformis* (Tadpole shrimp) for target organisms since its morphology changes dramatically during the development, and focused on stage-specific sRNAs range in sizes from 25 to 45 nt.

In order to find the stage-specific sRNAs, we performed deep sequencing analysis of sRNA libraries constructed from each six developmental stage (egg, 1^{st}-4^{th} instar larvae, and adult) of *T. cancriformis*. After removing unreliable sequencing reads, novel sRNA candidates of 25-45 nt long were extracted. Based on the analysis of their gene expression (read counts) as well as comparative genomics between the candidate sRNAs and known non-coding RNAs in other species, many putative tRNA fragments were detected in any one of six developmental stages. To reveal the exact origin of these tRNA fragments, it is necessary to know the mature tRNA sequences. Therefore, we also conducted deep sequencing analysis of genomic DNA isolated from *T. cancriformis* and predicted a set of tRNA genes using tRNAscan-SE. As a result, at least five tRNA fragments were found to be derived from either $tRNA^{Gly}$(CCC), $tRNA^{Lys}$(CUU), $tRNA^{Glu}$(CUC), $tRNA^{Asp}$(GUC), or $tRNA^{Phe}$(GAA). Moreover, northern blot analysis showed that these tRNA fragments were actually expressed in *T. cancriformis.* Interestingly, in the case of the $tRNA^{Lys}$(CUU), several tRNA fragments in different length were produced depending on the developmental stages. These results suggest that tRNA fragments are not random degradation products, but may have some important role(s) in eukaryotic development. Recently, it has been demonstrated that tRNA fragments are generated by endonucleolytic cleavage of tRNAs under specific conditions such as age, cancer, oxidative stress and amino-acid-starvation in various cell types. The possible function(s) of tRNA fragments in the development of *T. cancriformis* are discussed in the conference.

242 C Highly potent and specific siRNAs isolated from E. coli with endogenous p19 expression

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Most synthetic siRNAs have been produced by chemical synthesis. Here we present a method to produce highly potent siRNAs in E.coli. This method relies on ectopic expression of p19, an siRNA-binding protein found in a plant RNA virus. When expressed in E. coli, p19 stabilizes an ~21-nt siRNA-like species produced by bacterial RNase III. These siRNAs are then isolated from bacterial total RNA by binding to p19-coupled magnetic beads. When mammalian cells are transfected by siRNAs generated in bacteria expressing p19 and a hairpin RNA encoding 200 or more nucleotides of a target gene, they selectively knock down expression of the specific target gene by ~90% without immunogenicity or off-target effects. The bacterial expression of p19 can also be used to isolated novel endogenous siRNAs made from anti-sense transcripts.

243 A Novel NFL mRNA targeting microRNAs in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease in which the low molecular weight neurofilament (NFL) mRNA level is suppressed in motor neurons. Based on previous findings, we hypothesized that microRNAs (miRNAs) will play a central role in NFL transcript stability, and may lead to the decrease in NFL mRNA. MiRNAs are small 20-25 nucleotide molecules and act as post transcriptional gene regulators by targeting 3' untranslated region (UTR) of mRNA resulting in mRNA decay or translational silencing. We profiled the expression of small RNA from spinal cord lysates from sporadic ALS and neurologically intact controls and determined which of these small RNA were alternatively expressed in ALS. Out of 304 differentially expressed small RNA determined to be miRNA, a total of 42 (30 known, 12 novel) were predicted to target NFL mRNA (determined by targetscan and manual screens). Of these, 12 were found to be down-regulated in sporadic ALS and the remaining were up-regulated compared to neurologically intact controls. Real time PCR analyses confirmed that the majority of these miRNAs were up-regulated in ALS except block294889, block1336549, block11865845, block4652280, block 2403141 and Sblock659 which were down-regulated. Functional analysis of these miRNAs by reporter gene assay confirmed a subset of these miRNAs play a potential role in NFL mRNA decrease in ALS. This is the first report describing these novel sequences as miRNAs and their altered expression in ALS spinal cord lysates.

244 B A Specialized Mechanism of Translation Regulation in Quiescence

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Quiescence (G0) represents an assortment of reversible, proliferation-arrested states, implicated in the persistence of clinically resistant cancer cells, dormant stem cells and other distinct, arrested cells in development and in the body. The G0 state involves a discrete gene expression program with selective mRNA expression while decreasing general translation. One subset of such transcripts includes select mRNAs recruited by a distinct microRNP (microRNA-protein complex) containing Argonaute 2 (AGO2) and a specific isoform of Fragile X-mental-retardation-related protein 1, isoform-a (FXR1a). The AGO-FXR1a microRNP lacks the repressor, GW182, and promotes translation of associated mRNAs in G0 mammalian cells and in G0-like immature *Xenopus laevis* oocytes. Our data reveal that microRNA-mediated upregulation is dependent on nuclear entry of the microRNA in immature oocytes; cytoplasmic injections result in repression. FXR1a overexpression rescues translation upregulation of cytoplasmically-injected RNAs and in low density, proliferating cells. Consistently,*in vivo* crosslinking-coupled nuclear-cytoplasmic fractionation and immunoprecipitation demonstrate significant interaction of AGO with FXR1a in the nucleus compared to the cytoplasm. MicroRNA targets for upregulation, Myt1 and TNFα mRNAs and reporters bearing their target sequences, are associated with the nuclear AGO-FXR1a microRNP. mRNAs that are repressed or lack target sites are not associated with this nuclear microRNP, indicating the importance of a compartmentalized AGO-FXR1a microRNP for selective mRNA recruitment for translation upregulation. The AGO-FXR1a microRNP interacts with a specialized translation factor in G0 and in immature oocytes, thereby connecting mRNAs recruited by this microRNP with the translation machinery for selective expression. Importantly, disruption of this mechanism in G0 abrogates microRNA-mediated translation upregulation and decreases G0 cell viability, indicating its relevance for maintenance of the quiescent state.

245 C X-ray crystal structure of Maelstrom

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Transposons occupy a large part of our genome and the mobilization of these elements induces genetic instability. PIWI-interacting RNAs (piRNAs) associate with PIWI proteins and silence transposable elements, thus maintaining the genomic integrity of the germ line. Maelstrom (Mael) plays a critical role in the piRNA pathway. Mael consists of a HMG-box domain and a MAEL domain. The HMG-box domain binds the *miR-7*promoter and represses *miR-7* expression, ensuring proper differentiation of *Drosophila* germline stem cell lineage. Bioinformatics analysis suggested that the MAEL domain has an RNase H-like fold but lacks catalytic residues highly conserved among RNase H proteins. The MAEL domain is also characterized by a conserved ECHC motif. A recent study using ovarian somatic cells (OSCs) showed that Mael participates in Piwi-mediated epigenetic regulation of transposons but is not required for piRNA biogenesis. However, the mechanism by which Mael is involved in the piRNA pathway remains unknown. Here, we report the crystal structure of the *Drosophila melanogaster* MAEL domain at 1.6 A resolution. The crystal structure revealed that the MAEL domain has an RNase H-like fold with a zinc ion coordinated by the ECHC motif. A structural comparison of the MAEL domain with RNase H proteins showed that the MAEL domain lacks any catalytic residue. Instead, the MAEL domain has a conserved molecular surface, which may serve as a platform for interacting with nucleic acids and/or proteins in the nucleus of OSCs. Functional analyses based on the crystal structure are undergoing.

246 A DmGTSF1 is essential for effective retrotransposon silencing by Piwi in Drosophila ovarian somatic cells

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Drosophila Piwi associates with PIWI-interacting RNAs (piRNAs) and silences transposons to maintain the integrity of the genome in the germline. Previous studies have shown that nuclear localization, but Slicer activity, of Piwi is necessary for Piwi-mediated transposon silencing in ovarian somatic cells (OSCs). However, the underlying mechanism remains unclear. We have screened 100 genes by RNA interference in OSCs and so far identified 12 genes required for transposon silencing in the cells. Of those, we currently focus on DmGTSF1, the *Drosophila* homolog of mouse gametocyte-specific factor 1 (GTSF1), because DmGTSF1 tightly associates with Piwi and is required for Piwi-mediated transposon silencing in OSCs. Mouse GTSF1 has been shown to be essential for transposon silencing in the testes; yet, the molecular function remains unclear. To reveal the role of DmGTSF1 in OSCs, we produced anti-DmGTSF1 monoclonal antibodies. Immunofluorescence on OSCs using the antibodies showed that DmGTSF1 localizes in the nucleus of OSCs. Disruption of DmGTSF1 affected neither the Piwi nuclear localization nor the expression level of piRNAs; however, Piwi-mediated transposon silencing became defective. We are currently analyzing how DmGTSF1 enhances Piwi-mediated nuclear transposon silencing in OSCs, which will be discussed at the meeting.

247 B Competition between spliceosome and microprocessor complex regulates processing of Splice site Overlapping (SO) 34b pri-miRNA

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Several coding or non-coding PolI precursor transcripts are processed into both spliced mRNAs and micro RNAs (miRNA) but the mechanism that coordinates the spliceosome and the Drosha-DGCR8 Microprocessor complex (MPC) activities are not completely understood. We have explored the functional relationships between these two machineries in a peculiar class of miRNAs, we named Splice site Overlapping (SO)-miRNA, whose pri miRNA hairpins overlap with splice sites. We found 17 pri-miRNA hairpins overlapping with splice sites, 11 SO pri-miRNAs contain a 3'ss, 6 a 5'ss and 8 are evolutionarily conserved among vertebrates. We focussed on the evolutionarily conserved SO miR-34b whose non-canonical 3'ss lacks a polypyrimidine tract. miR34, originally identified as a tumour suppressor miRNA, is involved in several physio-pathological conditions including spermatogenesis, neurodegeneration, central stress response and neural stem cell differentiation.

SO pri-miR-34b hairpin is located in the last exon of a non-coding transcript and we show that the embedded non-canonical acceptor site is correctly spliced *in vivo* in human tissues and in minigene systems. Through mutational analysis we identify two indispensable elements for the recognition of the non-canonical site: a strong branch point located in the hairpin, 18 bp upstream of the 3'ss AG dinucleotide, and a downstream purine-rich exonic splicing enhancer (ESE). Interestingly, in minigene systems, splicing inhibition due to ESE deletion or direct disruption of the AG 3'ss increases miR-34b levels. On the other hand, siRNA-mediated silencing of Drosha and/or DGCR8 improves splicing efficiency and abolishes miR-34b production. Thus, the processing of this 3' SO miRNA is regulated in an antagonistic manner by the MPC and the spliceosome. We propose that in SO miRNAs, competition between these two machineries on the nascent transcript represents a novel mechanism to regulate miRNA biosynthesis.

248 C Molecular mechanisms of the piRNA biogenesis machinery

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Epigenetic silencing of transposons by Piwi-interacting RNAs (piRNAs) constitutes an RNA-based genome defense mechanism. Piwi endonuclease action amplifies the piRNA pool by generating new piRNAs from target transcripts by a poorly understood mechanism. Here, we identified mouse Fkbp6 as a factor in this biogenesis pathway delivering piRNAs to the Piwi protein Miwi2. Mice lacking *Fkbp6* derepress LINE1 (L1) retrotransposon and display reduced DNA methylation due to deficient nuclear accumulation of Miwi2. Like other co-chaperones, Fkbp6 associates with the molecular chaperone Hsp90 via its tetratricopeptide repeat (TPR) domain. Inhibition of the ATP-dependent Hsp90 activity in an insect cell culture model results in the accumulation of short antisense RNAs in Piwi complexes. We identify these to be by-products of piRNA amplification that accumulate only in nuage-localized Piwi proteins. We propose that the chaperone machinery normally ejects these inhibitory RNAs, allowing turnover of Piwi complexes for their continued participation in piRNA amplification.

249 A Abstract Withdrawn

250 B Poly(A) and histone mRNA processing factor Symplekin is involved in endo-siRNA biogenesis

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CPSF73, CPSF100 and Symplekin form a protein complex required for 3' end processing of both histone mRNAs and canonical polyadenylated mRNAs. CPSF73 is the cleavage factor, CPSF100 forms a heterodimer with CPSF73 and Symplekin is a scaffolding protein that binds CPSF73, CPSF100 and several other proteins. This "core cleavage complex" interacts with various accessory factors to cleave mRNAs with different 3' ends. In an attempt to isolate other proteins involved in histone 3' end processing, we performed a large-scale immunoprecipitation of Symplekin from nuclear extracts of *Drosophila* tissue culture cells. A protein specific antibody was used to isolate endogenous Symplekin, samples were separated on an SDS-PAGE gel and mass spectrometry was performed to identify bound proteins. Several known Symplekin-interacting proteins, including CPSF160, CPSF100, CPSF73 and CstF77, were isolated in addition to many factors previously not reported to be involved in polyadenylation. I also found an unexpected binding partner: Dicer-2 (Dcr-2). Dcr-2 processes dsRNAs into both endo- and exo-siRNAs in *Drosophila melanogaster*. To confirm the Symplekin-Dcr-2 interaction, I did the reciprocal IP with an anti-Dcr-2 antibody. This experiment confirms the Dcr-2-Symplekin interaction. To determine if Symplekin is necessary for the production of endogenous siRNAs, I RNAi-depleted Dmel-2 tissue culture cells of Symplekin and performed a northern blot with a probe to esi-2.1, an endogenous siRNA. This experiment shows that when Symplekin is RNAi-depleted, the amount of esi-2.1 produced is drastically reduced. Preliminary data indicate that Symplekin may be required for small RNA biogenesis, in addition to its role in 3' end processing.

251 C Sensitized Backgrounds Reveal Critical Roles for microRNA Families

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MicroRNAs (miRNAs) are a class of small regulatory RNAs that have been implicated in the control of many cellular functions including cell specification, differentiation, proliferation, and metabolism. A smaller number of miRNAs are expressed in stem cells and at early stages of development, including some that are thought to maintain pluripotency. Interestingly, even though many *C. elegans* miRNAs are highly conserved, for many single deletion knockouts or even family knockouts, phenotypes are subtle or not evident. *C. elegans* have 23 miRNA families, 9 of which are conserved through humans. The Horvitz group showed that most miRNAs are not essential for development or viability on their own. This indicates a high degree of functional redundancy among miRNA family members, and between unrelated miRNA families in the worm. Recent findings from the Abbott group illustrated that sensitizing the worm may reveal several mutant phenotypes associated with loss of individual miRNAs or families.

Here, I examined the phenotypes associated with several critical miRNA effector complexes to determine whether combining reduced effector complex expression with miRNA family knockouts were able to alleviate or exacerbate the observed phenotypes. For instance, RNAi of Alg-1 or Alg-2 (Argonaute-like genes) produces characteristic developmental delays and disruptions that include problems with molting, slow growth, absent or missing alae, and protruding vulva. These abnormalities are occasionally severe enough to produce embryonic or larval lethality. Examining the effects of Alg-1 or Alg-2 RNAi on miRNA family mutants produces an interesting picture. Most notably, the loss of some miRNAs appears to alleviate the impact of RNAi on Alg-1 or Alg-2 to produce a less severe phenotype as compared to RNAi on wild-type animals. Moreover, some family mutants are uniquely sensitive to Alg-1 versus Alg-2 RNAi or vise versa. These results produce a larger picture of the interaction of unique miRNA families with different effector molecules that allow for a tightly regulated environment to control highly specific and reproducible events throughout development.

252 A A proteomic screen identifies novel regulators of micro-RNA biogenesis

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MicroRNAs (miRNAs) are a class of small noncoding RNAs that negatively regulates gene expression by destabilization or translational inhibition of mRNAs. miRNAs are encoded in the genome as part of longer primary transcripts and subsequently processed by two nucleolytic events catalyzed by the RNase III enzymes Drosha and Dicer. The expression of miRNAs is tightly regulated, and in many cases deregulation of miRNA abundance has been linked to diseases including cancer. Apart from transcriptional regulation of the primary miRNAs it has become evident that also the maturation steps can be specifically regulated by RNA binding proteins. However, only a small number of posttranscriptional miRNA regulators has been discovered to date. Therefore, we have set up a proteomics-based screen to identify proteins specifically binding to individual miRNA precursors. In a pulldown approach we have screened a set of 72 in vitro transcribed miRNA precorsor sequences against a panel of 11 cancer cell lines and have analyzed the bound proteins by mass spectrometry.

The obtained data revealed about 150 proteins that bind specfically to one or a small number of the tested RNA sequences and thus are candidates for a sequence specific recognition and regulation of individual miRNA precursors. Analysis of the interaction motives suggests that the terminal loop of the hairpin-shaped miRNA precursors serves as main interaction-site in our assay.

In a second screening stage we are currently validating a test set (30) of the candidates in knock down assays to investigate their role in the biogenesis of the bound miRNAs. Initial results indicate that a number of the identified candidates indeed regulates the processing of recognized miRNAs, already now expanding the range of regulatory factors in miRNA biogenesis markedly. Together with additional validation experiments, this project aims to for the first time generate a comprehensive view of posttranscriptional regulation of miRNA biogenesis.

253 B The role of the RNA chaperone protein Hfq in the translation regulation by small noncoding RNAs targeting ompD mRNA from Salmonella typhimurium.

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The RNA chaperone protein Hfq is a hexameric Sm-like protein, which acts as a major-regulator of bacterial gene expression. Hfq facilitates the binding of sRNAs to their target mRNAs and affects the stability of their complexes. It has been proposed that Hfq is recruited to the regulated mRNAs through $(ARN)_n$ motifs, and that the mRNA-sRNA annealing is most accelerated when Hfq binds mRNA immediately 3' of the sRNA binding site. Interestingly, the mRNA of *Salmonella* outer membrane protein OmpD is regulated by four Hfq-dependent sRNAs (InvR, MicC, RybB, SdsR), which bind partly overlapping sites located downstream of the AUG codon. The region of *ompD* mRNA containing the sRNA binding sites also includes several ARN repeat sequences, which could serve as potential Hfq binding sites.

The aim of this project is to elucidate the role of Hfq for the binding of different sRNAs to the *ompD* mRNA sequence. To characterize the structures of interacting molecules the *in vitro* structure probing of 5'end-labeled RNAs in the absence or presence of Hfq was performed. The comparison of cleavage patterns allowed us to determine sRNA secondary structures and their Hfq binding sites. As the mRNA binding sequences of SdsR and InvR sRNAs are located in base-paired regions it appears possible that the Hfq role in facilitating the sRNA-mRNA annealing could be related to unfolding these regions. Next, we are planning to analyze the role of potential Hfq binding sites on *ompD* mRNA for promoting the sRNA-mRNA annealing. The results of these experiments could allow us to better understand the role of the *Salmonella* Hfq protein in facilitating sRNA interactions with their target mRNAs.

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254 C Novel stress-induced smRNAs from Brachypodium distachyon

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Small non-coding RNAs (smRNAs), which include miRNAs, siRNAs, as well as other classes of endogenous smRNAs, have been firmly established as key regulators of the cellular and genome functions in diverse eukaryotic organisms, including all land plants. However, it is challenging to study common monocot plants in standard laboratory settings due to their large genomes, physical size, as well as demanding growth conditions. During the past decade, *Brachypodium distachyon* has emerged as a new model organism for studying functional genomics of grasses. The goal of this study was to investigate how smRNA transcriptome of temperate grasses responds to various biotic and abiotic stresses.

To address this question *Brachypodium* plants were challenged with different abiotic stresses such as heat, cold and salt, as well as with infection by the plant pathogen fungus *Magnaporthe grisea*, and their smRNA transcriptomes were examined by deep sequencing. To analyze the populations of smRNAs we deployed proximity-based algorithm, and also classified smRNAs on the basis of their size, the nature of their first nucleotide, and their genomic features. We found that challenging plants with stresses resulted in upregulation of the population of smRNAs ranging from 21 to 25nt in length, while the populations of smRNAs shorter than 20nt in length were downregulated.

In addition to previously reported plant miRNAs and siRNAs, we also identified new groups of stress-specific smRNAs. Among the group of upregulated smRNAs we discovered a class of novel 24nt smRNAs. These smRNAs exhibit several distinct patterns and originate in a strand specific manner from 3'-UTRs of a subset of mRNA genes. The analysis of this group of stress-induced smRNAs and their targets will be presented.

255 A The novel Tetrahymena gene COI12 is crucial for siRNA loading into the Argonaute protein Twi1p

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RNA interference pathways have important functions in the regulation of diverse cellular processes. The ciliated protozoan Tetrahymena utilizes a complex RNAi pathway during its sexual reproduction, called conjugation, as a defense mechanism against transposon like sequences. In the somatic genome these sequences are packed into heterochromatin which eventually leads to elimination of the DNA. The correct targeting of these sequences is thought to be achieved via base pairing of ~29nt siRNAs with nascent transcripts.

At the onset of conjugation the nuclear Dicer like enzyme Dcl1p produces the ~29nt siRNAs, so called scan RNAs (scnRNAs). In order to form a functional effector complex these scnRNAs have to be loaded into the Argonaute protein Twi1p. This process takes place in the cytoplasm and is therefore clearly separated from the production of scnRNAs. However, how the loading is regulated is not well understood.

Here we report the characterization of the conjugation-induced gene 12 (*COI12*) which is essential for this loading process. At early stages of conjugation Coi12p localizes to the cytoplasm where scnRNA loading occurs. In the absence of Coi12p immunopurified Twi1p is not in a complex with scnRNAs. As a consequence these small RNAs as well as the Argonaute protein get degraded. Coi12p has a TPR domain, which interacts with Hsp90, and an Fk-binding domain with a yet unclear function. We established in vitro and in vivo systems to manipulate and analyze the loading process at a mechanistic level in more detail and to investigate a possible function of Coi12p and Hsp90p in the loading process. This study could shed more light on the suggested role of the Hsp90 chaperone machinery in the loading process of small RNAs to Argonaute proteins.

256 B An Exchange-Induced Remnant Magnetization Technique for MicroRNA Detection

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We present a novel technique of exchange-induced remnant magnetization (EXIRM) for label-free microRNA (miRNA) detection. The characteristics of this technique are the following: ultrahigh sensitivity, broad dynamic range, single-base specificity, and no use of amplification or washing. MiRNAs are important regulators of genes and biomarkers for cancer, but their detection and sequencing remain challenging for existing techniques. The short length of miRNAs, which usually contains 18-25 nucleotides, makes precise sequencing difficult using conventional techniques. The expression levels of miRNAs are highly heterogeneous, which requires high sensitivity and broad dynamic range for the detecting methods.

The EXIRM technique is based on a novel concept that can be described in the following three stages. First, RNA duplexes are prepared on the sample well surface. One of the two strands is complimentary to the target miRNA and immobilized on the surface; the other strand has one mismatching base and is labeled with magnetic particles. Second, the sample containing various target miRNAs are incubated in the sample well with the initial RNA duplex. Exchange reaction takes place because the target miRNA has stronger binding with its complimentary RNA than the mismatching strand has. Third, the dissociated mismatching strand undergoes Brownian motion, causing randomization of the magnetic dipoles of the magnetic particles. Therefore, the quantity of the target miRNA is measured as a magnetic signal decrease. The detection of magnetic signals is achieved by an atomic magnetometer, the most sensitive device for magnetic sensing. The dynamic range of detection is at least five orders of magnitude and can be expanded by varying the size of the magnetic labels. The unique combination of zeptomole sensitivity, single-base specificity, and broad dynamic range makes EXIRM a well suited technique for miRNA identification.

257 C Nonstop decay in C. elegans: examination of a possible role for 22G RNAs

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In *C. eleg*ans, small noncoding RNAs known as 22G RNAs are synthesized by RNA-dependent RNA polymerases and target thousands of protein-coding mRNAs, repetitive sequences, and cryptic transcripts throughout the genome. In some cases, 22G RNA synthesis appears to be triggered as a secondary event downstream of targeting by *C. elegans* piRNAs (known as 21U RNAs) in a pathway that defends against non-self RNA species. (*1, 2*) However, the synthesis of the majority of 22G RNAs is unaffected by loss of 21U RNAs, and the triggers for biosynthesis of these 21U-independent 22Gs remain elusive. Using a comparative genomic approach in geographically distributed wild isolates of *C. elegans*, we have identified a locus (F43E2.6) at which loss of the stop codon is correlated with increased production of 22G RNAs. The F43 mRNA has a short 3'UTR with no further in frame stop codons, raising the intriguing possibility that 22G RNAs could be involved in a nonstop decay pathway in *C. elegans*. F43 is also targeted by an unusual Dicer product; however this Dicer product does not appear to act as a primary siRNA to trigger F43 22G RNAs. First, the Dicer product is expressed equally in strains with large differences (more than 10 fold) in 22G RNA levels. Second, this product is expressed only during embryogenesis, whereas F43 22G RNAs are expressed most strongly in adults. We have demonstrated that the 22G RNA expression phenotype at the F43 locus is genetically linked to the locus itself, and are currently assaying transgenic worms carrying F43 reporter constructs that bear or lack a stop codon to determine whether the loss of a stop codon is indeed causal for increased 22G RNA production. In addition, we are generating reporter constructs that will allow us to ask whether 22G RNAs play a functional role in the nonsense-mediated and no-go decay pathways.

258 A Spliceozymes: Ribozymes that act like Spliceosomes

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The spliceosome is a large, dynamic RNA-protein particle (RNP) that evolved from a common ancestor with self-splicing group II intron ribozymes. Self-splicing intron ribozymes contain the larger group II intron ribozymes and the smaller group I intron ribozymes. To model the evolution of the spliceosome in the lab we generated a variant of trans-splicing group I intron ribozymes that acts like a spliceosome: The ribozyme variant uses its 5'- and 3'-terminus to recognize two splice sites on a target RNA, catalyzes the removal of the internal sequence, and joins the flanking exons. These 'spliceozymes' appear general with regard to intron length and intron sequence because introns with lengths between 64 and 329 nucleotides were removed, with diverse intron sequences. To test whether these spliceozymes could function like spliceosomes inside a cell we inactivated the mRNA of chloramphenicol acetyl transferase (*CAT*) with an intron and targeted a spliceozyme to remove this intron. In *E.coli* cells, the spliceozyme processed this *CAT* pre-mRNA to functional *CAT* mRNA efficiently enough to mediate *E.coli* growth on medium containing chloramphenicol. We used this growth phenotype in *E.coli* cells to create a model system for the evolution from spliceozymes to spliceosomes: Mutations were introduced into the spliceozyme sequence by mutagenic PCR, and the most efficient spliceozymes were selected by plating the *E.coli* cells on medium containing chloramphenicol. Over multiple cycles of this evolution, the population of spliceozymes accumulated mutations and sampled the protein repertoire of the *E.coli* cells for interactions that benefit the expression of CAT enzyme. The preliminary results of this evolution will be presented at the conference. In summary, our results show that trans-splicing ribozymes can be used to model the first steps in the evolution of the spliceosome.

259 B Molecular Mechanism of preQ1 Riboswitch Action: a Molecular Dynamics Study

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Riboswitches often occur in the 5'-untranslated regions of bacterial mRNA where they regulate gene expression. The preQ1 riboswitch controls the biosynthesis of a hypermodified nucleoside queuosine in response to binding the queuosine metabolic intermediate. Structures of the ligand-bound and ligand-free states of preQ1 riboswitch from *Thermoanaerobacter tengcongensis* were determined recently by crystallography.1 We used multiple, microsecond-long molecular dynamics simulations (29 μs in total) to characterize the structural dynamics of preQ1 riboswitche in both states. We observed different stability of the stem bearing part of the Shine-Dalgarno sequence in the bound and free states, resulting in different accessibility of the this ribosome-binding site. These differences are related to different stacking interaction between nucleotides of the stem and the adjacent RNA loop, which itself adopts different conformations in the bound and free states.1,2 We suggest that the loop serves not only to bind preQ1 but also transmits information about ligand binding from the ligand-binding pocket to the stem, which has implications for mRNA accessibility to the ribosome.2 We explain functional results obscured by a high salt crystallization medium and help to refine regions of disordered electron density, which demonstrates the predictive power of our approach. 2

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260 C Characterization of Metal Ion Binding Sites in the P4 Helix of Bacillus subtilis RNase P

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The endoribonuclease P, RNase P, is responsible for catalyzing the 5'-end maturation of precursor tRNAs. Like many large ribozymes, divalent ions stabilize the folded structure and enhance catalytic function of RNase P. P4 helix, the most highly conserved region in PRNA, is essential for RNase P activity and has been suggested to contain catalytic and/or cocatalytic metal ion binding sites. The crystal structure of *T. maritima* RNase P (4.2 Å) proposed a metal ion directly coordinates the O4 of the bulged uracil (U51 in *B. subtilis*) in the P4 helix[1]. NMR spectroscopy of a P4 stem-loop mimic suggested an inner-sphere metal interaction with the O6 of the base corresponding to G379 in the P4 helix of *B. subtilis* RNase P[2].

To evaluate the structure and function of these putative metal ion sites, 4-thiouracil and 2-aminopurine are specifically substituted for U51 and G379, respectively. These substitutions decrease the pre-tRNA binding affinity at low concentration of metal ion but not at high concentration. Single turnover kinetics show that these substitutions do not affect the rate of the conformational change step in the RNase P - pre-tRNA complex. However, the 4-thiouracil substitution at U51 and 2-aminopurine substitution at G379 decrease the cleavage rate constant in Mg^{2+} by 20-fold and 10-fold, respectively, without altering the *K1/2* for the Mg^{2+}-dependent activation of cleavage. These data indicate that O4 of U51 and O6 of G379 are important both for stabilizing pre-tRNA affinity in a metal-dependent fashion and for enhancing catalytic activity. The magnitudes of these effects are smaller than expected for an inner-sphere metal ion interaction but are consistent with outer-sphere metal ion coordination.

In contrast, the rSpacer substitution of U51 does not affect the cleavage step but decreases the conformational change rate by 6-fold. Furthermore, the rSpacer substitution of G379 decreases the cleavage rate constant by 3-fold. The modest effect of deletion of the base of U51 and G379 might be due to additional water molecules filling the space allowing a catalytic metal ion to be optimally positioned. These results further suggest that O4 of U51 and O6 of G379 form hydrogen bonds with waters coordinated to a magnesium ion.

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261 A Ligand-induced stabilization of the aptamer terminal helix in the adenine riboswitch

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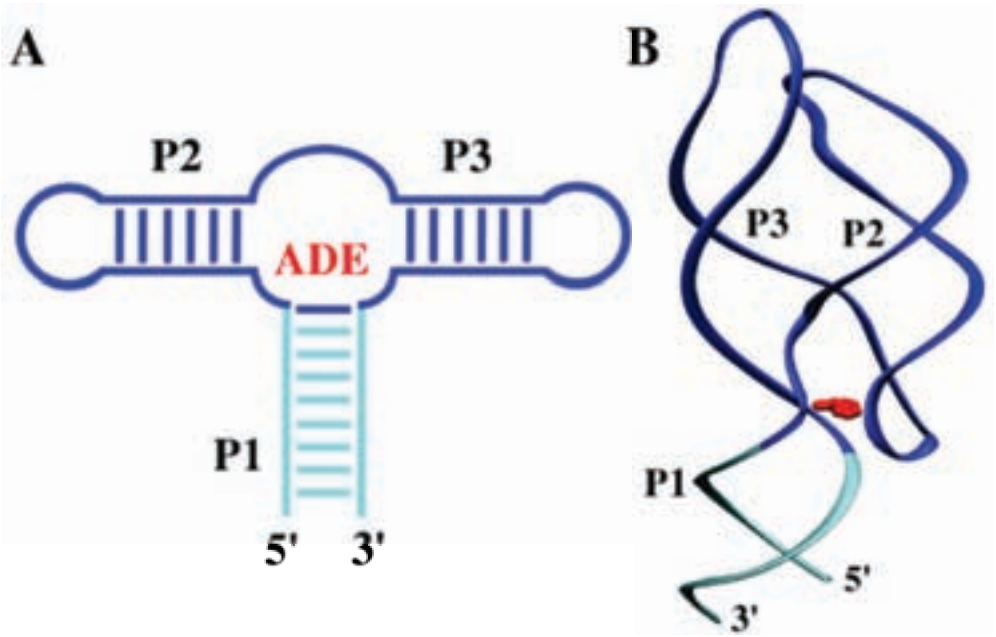
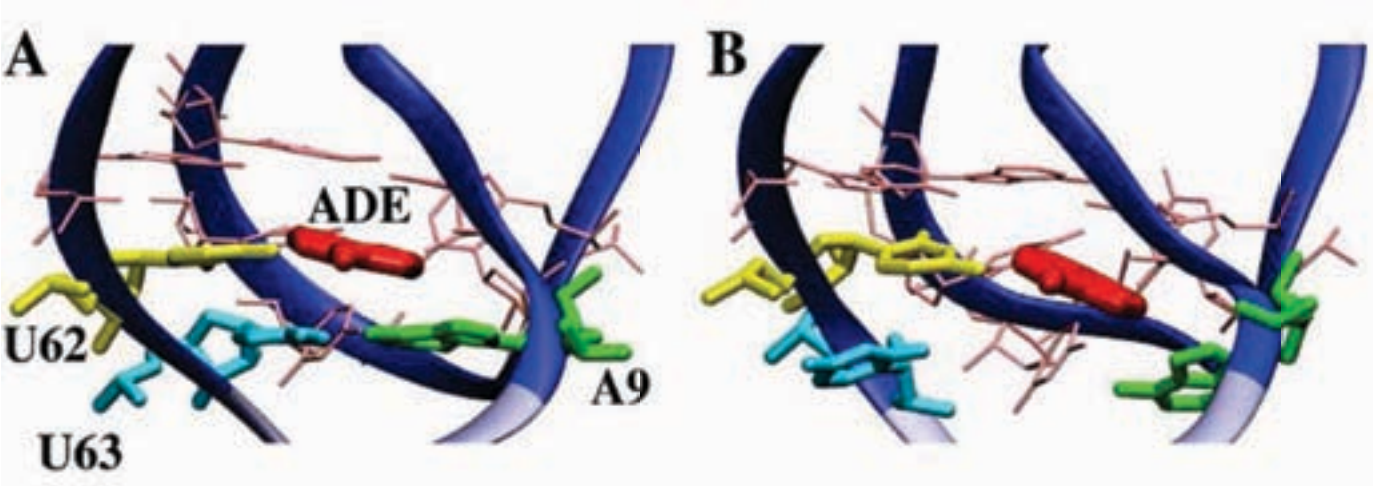
Riboswitches are structured mRNA elements that modulate gene expression. They undergo conformational changes triggered by highly-specific interactions with sensed metabolites. Among the structural rearrangements engaged by riboswitches, the forming and melting of the aptamer terminal helix, the so-called P1 stem, is essential for genetic control. The P1 stem, which competes with the repressor, is formed in the ON-state, whereas it is disrupted in the OFF-state. It has been proposed that P1 is stabilized by the ligand and that this could be a common feature in many riboswitch classes. In this context a quantitative estimation of the energetic contributions associated to ligand binding, in particular regarding the role of direct P1-ligand interactions, has not yet been provided and the structural mechanisms by which this conformational change is modulated upon ligand binding mostly remain to be elucidated. State-of-the-art-free-energy methods combined with atomistic simulations can bridge the gap providing an unparalleled perspective on the mechanism and dynamics of the biomolecular process of interest. Here we used steered molecular dynamics simulations to study the thermodynamics of the P1 stem formation in the presence and in the absence of the cognate ligand for the *add* adenine riboswitch. The P1 ligand-dependent stabilization was quantified in terms of free energy (-4.4 kJ/mol) and compared with thermodynamic data from previous dsRNA melting experiments and single-molecule experiments, resulting in nice agreement with them. Our work provides atomistic details and energetic estimates of the process of interest and, altogether with the related experimental works, it suggests a model for the aptamer folding in which direct P1-ligand interactions play a minor role when compared with those related to the ligand-induced aptamer preorganization. Because the structural/functional role of the aptamer terminal helix is a common feature in the "straight junctional" riboswitches, we foresee a wider validity of this model.





262 B Studying Conformational Changes in the Glycine Riboswitch using Electron Paramagnetic Resonance Spectroscopy

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Electron Paramagnetic Resonance (EPR) spectroscopy is used to study dynamic conformational changes in the RNA glycine riboswitch. The dynamic role of the leader-linker interaction within glycine riboswitch conserved sequences is probed through site directed spin labeling and continuous wave EPR. Inter-aptamer and aptamer-expression platform interactions are elucidated through double electron-electron resonance spectroscopy. Incorporation of spin labels is achieved through optimized ligation methodologies allowing synthetically modified RNA to be joined to larger RNA sequences. Expected folding and burial of riboswitch elements will lead to restricted motion of the spin label and, additionally, pulsed EPR experiments yield distance distribution profiles indicating conformational exchange between states in the absence and presence of glycine.

263 C In vitro evolution of a calcium ion-sensing ribozyme from the natural glmS riboswitch-ribozyme

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The *glmS* ribozyme is a natural catalytic RNA widely distributed in Gram-positive bacteria that undergoes self-cleavage under physiologic divalent metal ion concentrations upon binding to its cognate coenzyme glucosamine-6-phosphate (GlcN6P) (*1*). This allows bacteria to sense their intracellular GlcN6P concentration and control its biosynthesis at the mRNA level through negative feedback. To address the evolution of its coenzyme dependence, we previously evolved a mutant ribozyme (*glmS*^{AAA}) that is only 3 adenine mutations away from the wildtype ribozyme, and catalyzes the same RNA cleavage chemistry in the absence of GlcN6P. This mutant, in contrast to wildtype, has a strong preference for Ca^{2+}, and requires higher divalent metal ion concentrations (>100 mM) for maximal activity. To further characterize the metal ion specificity of*glmS*^{AAA} and to generate specific metal ion-sensing ribozymes that could function *in vivo*, we have now performed two additional parallel selections. These experiments aimed to evolve self-cleaving ribozymes from*glmS*^{AAA} that are functional in either 2 mM Mg^{2+} or 2 mM Ca^{2+}. From the Mg^{2+} selection, we isolated a fast ribozyme that is equally active in either 2 mM Mg^{2+} or 2 mM Ca^{2+}. The Ca^{2+} selection, on the other hand, produced a Ca^{2+} specific ribozyme that discriminates strongly against Mg^{2+} (more than 10,000-faster in 2 mM Ca^{2+} than in 2 mM Mg^{2+}), and has a higher apparent affinity to Ca^{2+} than *glmS*^{AAA} ($K_{1/2}$ of 19 mM and 93 mM, respectively). This ribozyme is non-specific towards the sequence upstream of its cleavage site, and is not further activated by GlcN6P or physiological concentrations of Mg^{2+}. Our work suggests possible evolutionary links between wildtype *glmS* ribozyme and different ligand independent self-cleaving ribozymes, providing clues as to the origin of the wildtype. Our new selection experiment has generated the first example, to our knowledge, of a self-cleaving ribozyme with such high specificity for Ca^{2+}. We envision that this ribozyme can be engineered to function as an *in vivo* calcium sensor.

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264 A Architectural Diversity of PreQ1 Riboswitches

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Riboswitches are RNA structures that typically bind to small molecules and regulate gene expression. Riboswitches are placed into different classes based on their distinct secondary and tertiary structures and based on their ligands. In previous studies, we determined that a class of riboswitches for pre-queuosine$_1$ (PreQ$_1$) has representatives that conform to two different types based on the presence of unique sequence sub-domains (Roth et al. 2007). Subsequently, we identified a second class of PreQ$_1$ riboswitches that has no structural similarity to the first class (Meyer et al. 2008).

As DNA sequence databases continue to expand in data, additional searches for variants of the PreQ$_1$-I and PreQ$_1$-II riboswitch classes are yielding many more hits. Recently, we uncovered the presence of an additional type for each PreQ$_1$ riboswitch class. For PreQ$_1$-I riboswitches, we determined statistically that all three types of this class are distinct from each other. For PreQ$_1$-II riboswitches, the newly found type is able to discriminate more strongly against binding the immediate biosynthetic precursor of PreQ$_1$, called PreQ$_0$. We have also identified an entirely new class of PreQ$_1$ riboswitches, termed PreQ$_1$-III, which has no structural similarity to the other two classes. Based on this diverse collection of different PreQ$_1$-sensing riboswitches and other studies of PreQ$_1$, it is clear that numerous species of bacterial make extensive use of RNA to directly sense and respond to changing concentrations of this important modified nucleobase.

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265 B Theoretical Methods Suggest that Small Ribozymes may use Multichannel Mechanism During Catalysis

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Small self-cleaving ribozymes carry out site-specific cleavage and ligation of RNA substrates.[1] Experiments and biochemical studies identified nucleobases and hydrated magnesium ions as catalytically active species, but their exact roles in reaction mechanisms remain questionable.[2,3] We studied extensively two members of the group of self-cleavage ribozymes: hepatitis delta virus (HDV) and hairpin ribozymes.

X-ray structures of ribozymes have to be mutated or chemically modified in order to trap them in precursor-like states. We carried out all-atom molecular dynamics (MD) simulations in explicit solvent up to μs-time scales, which helped us to obtain native arrangement of the catalytically active sites. We tested the impact of different protonation states of key nucleobases on structural stability and compactness of active sites within bothribozymes. Finally, we used these MD simulations to generate geometries of the ribozymes in potentially reactive forms. These structures were further analyzed by the hybrid quantum-mechanical/molecular mechanical (QM/MM) calculations to decipher exact roles of chemical species (such as (de)protonated nucleobases and/or hydrated magnesium ion) involved in the catalysis. We used hybrid DFT functional MPW1K for the QMregion, which was carefully validated against chemically accurate CCSD(T)/CBS method.[4] We found that the MPW1K error in energies is less than 1 kcal/mol. The observed potential energy surfaces (PESs) of the sugar-phosphate backbone self-cleave reactions in both ribozymes are rather complex, therefore the transition states were localized using two-dimensional scans of PES.[5,6] In both ribozymes, we identified several reaction mechanisms having comparable free energy barriers (and thus comparable rate constants), which allowed us to suggest a hypothesis about multichannel mechanism of the sugar-phosphate backbone self-cleavage in these ribozymes.

This work was supported by the Operational Program Research and Development for Innovations - European Regional Development Fund (CZ.1.05/2.1.00/03.0058) and European Social Funds (CZ.1.07/2.3.00/20.0058, CZ.1.07/2.3.00/20.0017). Grant Agency of the Czech Republic (P208/12/G016, P208/12/1878) and NIH grant 2R01 GM062357 are also gratefully acknowledged.

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266 C Tuning the btuB riboswitch fold by chemically modifying its ligand coenzyme B12

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The focus of our research lies in the binding mechanism between the *btuB* riboswitch of *E. coli* and its ligand coenzyme B_{12} (AdoCbl).[1,2] One interesting fact about this interaction is that the *btuB* riboswitch is highly selective between some derivatives of the B_{12}-family, which however can strongly differ in their general structure.[2,3] In this regard, one of our early findings was that Adenosyl Factor A, which differs from AdoCbl in the shape and position of the lower group, binds and switches the riboswitch with nearly the same affinity and efficiency as the natural substrate. This surprising result lead to the question which are the structural requirements on the large and complex B_{12}-molecule to bind and switch the *btuB* riboswitch.

We have therefore synthesized three series of B_{12}-derivatives modified at the different functional sites (Figure 1). Modifications on the corrin ring sidechains include specific monoacids as well as secondary and tertiary amides. Modifications on the upper ligand include the two natural occurring CN- and Adenosyl-groups as well as synthetic moieties based on platinum(II)-linked nucleobases. A third group comprises derivatives with a peptide backbone replacing the sugar phospate at the lower loop. All these modifications were introduced to study the role of the H-bonding and electrostatic pattern of the *btuB*-B_{12} interaction.

To study the influence of these chemical modifications on the structural rearrangement of the riboswitch we applied in-line probing assays. Our experiments confirmed the importance of an upper moiety, preferentially being adenosyl, for a high affinity to the RNA. The presence of a secondary or tertiary amide group in the sidechains doesn't influence the *KD* value but leads to some differences in the structural switch of the RNA. Finally, the derivatives with the lower peptide loop show a strong dependence of the stereochemistry. In fact, only one isomer of this series was shown to switch the riboswitch, although with alterations and with a *KD* value in the micromolar range.

Acknowledgments. Financial support by the European Research Council (ERC starting grant 2010 259092-MIRNA to R.K.O.S.) and the University of Zurich is gratefully acknowledged.

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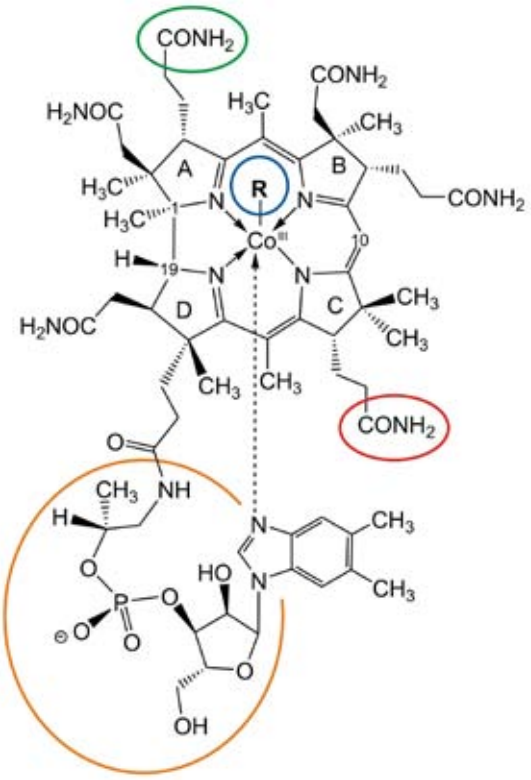

Figure 1: Shematic view of B_{12} with the moieties modified for this study indicated in color. Blue: upper ligand, orange: lower loop, green: sidecain b, red: sidechain e.

267 A A Riboswitch Class for the Bacterial Second Messenger c-di-AMP

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Riboswitches are small, structured RNA motifs commonly found in the 5'-untranslated regions of certain mRNAs. Each riboswitch class has evolved to selectively recognize a small molecule or ion and change the expression of associated genes. More than two dozen different riboswitch classes have been validated, including RNA motifs that respond to various ligands such as nucleobases, amino acids, coenzymes, second messengers, toxic anions, and other chemicals. The ligand identity of the majority of riboswitches can often be readily deduced by their genomic contexts, but for a few "orphan" riboswitch classes, ligand identification can be challenging. Some orphan riboswitch candidates are associated with many different genes, or with genes encoding proteins of unknown functions, which make ligand identification difficult.

A metabolite-binding riboswitch candidate called *ydaO* was discovered in bacteria nearly a decade ago, but its ligand has remained unknown. This predicted riboswitch class ranks among the top ten most common metabolite-sensing RNAs known, and it is associated with numerous genes for cell wall metabolism, osmotic stress response, and sporulation. We report that members of this noncoding RNA class selectively respond to the recently-discovered bacterial second messenger cyclic di-adenosine monophosphate (c-di-AMP). A representative RNA from *Bacillus subtilis* binds c-di-AMP with an affinity that is more than one million fold higher than for the c-di-AMP precursor ATP and for the bacterial second messenger c-di-GMP. Our findings resolve the mystery regarding the ligand for this extremely common riboswitch class, and expose a major portion of the super-regulon of genes that are controlled by the widespread bacterial second messenger c-di-AMP.

268 B Metal Ion Requirement and Substrate Specificity of Kinase Ribozyme K28(1-77)C

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The RNA world hypothesis posits that Ribonucleic Acids (RNA) played a major role as the repository for genetic material and as catalysts during the early evolution of life and metabolism. Phosphoryl transfer is one of the key reactions in metabolic pathways, signal transduction and gene regulation in all domains of contemporary life. In vitro selection of functional nucleic acids has identified multiple kinase ribozymes but much remains to be known about the mechanisms utilized by these ribozymes to carry catalysis. It is therefore important to understand the strategies used by kinase ribozymes to perform metabolically relevant reactions. Our lab selected ribozyme K28(1-77)C , a dual-site self(thio)phosphorylating kinase ribozyme that utilizes Cu2+ for catalysis. We used in-line cleavage assay to assess the role of Cu2+ during the self(thio)phosphorylation. Modulation of in-line cleavage was observed only when GTP(phosphoryl donor) or both GTP and Cu2+ were present while we saw no evidence of direct Cu2+-ribozyme interaction. Addition of competitor for Cu2+ resulted in the loss of in-line cleavage signal which is restored by supplementation with Cu2+. Self (thio) phosphorylation assays with competitors also showed similar results; loss of activity with competitor for Cu2+ and restoration with Cu2+supplementation. These experiments suggest that Cu2+-GTP complex is recognized by the ribozyme. Although 6 other families of ribozymes were isolated from the same selection, we found that only K28 (1-77)C is dependent upon Cu2+ for phosphoryl transfer catalysis.

To understand the substrate specificity for phosphoryl acceptor, we have assembled the ribozyme in a two-strand construct; a 22-nt substrate and a 44-nt catalytic strand. All kinase ribozymes studied to date have used the 2'OH or the 5'OH of ribose sugars as phosphoryl acceptors; however, we show here that the 5' substrate strand of the ribozyme can be made entirely of DNA. The DNA substrate gets phosphorylated even when the 5' end is blocked with biotin. We are proposing that the catalytic strand of the two stranded version of ribozyme K28 (1-77) C is the first kinase ribozyme that directly phosphorylates a nucleobase of its nucleic acid substrate. Atomic level mutations on a critical 5' triplet GGA suggest that 2-amino moiety in the two Guanosines plays important role during catalysis plausibly as hydrogen bond donors. We are currently studying the pH dependence of the reaction to identify potential role of Electron Donating Groups (EDGs) to activate the nucleophile for the reaction. Phosphoryl transfer on the nucleobase is an important step during the de novo biosynthesis of nucleotides such as ATP. Study of this ribozyme could help us understand mechanisms utilized by kinase ribozymes to catalyze biosynthetic reactions.

269 C Formation of a catalytic supramolecular RNA 1D-array through self-assembly of an engineered group I intron RNA enzyme

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Self-assembly of biopolymers plays fundamental roles in modern biological systems. DNA duplex structure is essential for storage and replication of genetic information and protein quaternary structure conducts elaborate functions (such as enzyme allosterism) and large complexes (such as virus capsids) of proteins. Self-assembly of polypeptides and nucleic acids are also attractive in the field of nanobio-technology, in which DNA is regarded as one of the most promising materials for designing 1D, 2D, and 3D nano-objects with defined sizes and shapes.

RNA has been considered as a candidate for nanobiotechnology material because of not only its similarity with DNA but also its functional roles (such as catalysts and receptors) in living cells. Artificial RNA design has produced polygonal and polyhedral RNA nano-objects composed of relatively short (ca. 25-100 nts) RNA oligonucleotides as monomer strands. On the other hand, biological evolution has generated complex RNA 3D structures with sophisticated functions, an extreme example of which is ribosome.

Amalgamation of artificial geometrical RNA nanostructures and naturally occurring RNA 3D structures would generate a new class of RNA nano-objects with enzyme-like functions. In this presentation, we wish to present our recent study to construct self-assembling 1D-nanoarray whose monomer unit is a group I intron RNA. Modular engineering of the *Tetrahymena* group I intron (ca. 400 nts) allowed us to convert its intramolecular interactions for the unimolecular self-folding into the intermolecular interactions for the multimolecular self-assembly, enabling the engineered intron to organize a head-to-tail 1D-assembly. The engineered intron exhibited the catalytic ability depending on the formation of the RNA 1D-array.

270 A A pinch model for activation of HDV-like ribozymes

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[1]University of California-Irvine

The HDV family of ribozymes are widely distributed in nature and their biological roles include processing of viral and retrotransposon transcripts and translation initiation of retrotransposon mRNAs. These ribozymes are characterized by their double-pseudoknot secondary structure. Many retrotransposon-associated ribozymes also contain large insertions predicted to form stable structures. We show that these peripheral domains activate the ribozymes by “pinching” two core helices together. A *trans*-cleaving construct derived from the *Drosophila* R2 ribozymes consisting of a ribozyme subunit and a long substrate strand that spans the core and peripheral domains exhibits a large activation barrier, supporting the pinch model. Our data point to a simple mechanism of activity modulation in HDV-like ribozymes (seeFig. 1).

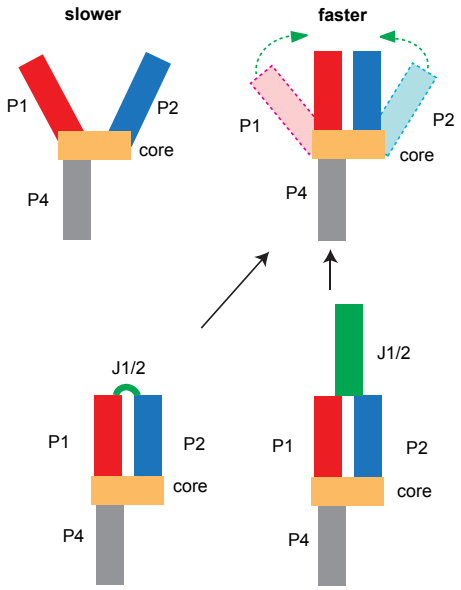

Figure 1

271 B The catalytic mechanism of a novel, widely disseminated small nucleolytic ribozyme

Timothy Wilson[1], *Tomas Fessl*[1], *David Lilley*[1]

[1]**University of Dundee**

The twister ribozyme, recently discovered in the Breaker laboratory, is a novel small nucleolytic ribozyme that is widely disseminated in the genomes of bacteria and eukarya (Roth and Breaker, personal communication). At its simplest, the ribozyme consists of one terminal and two internal loops joined by short helices and stabilized by two pseudoknots. Cleavage occurs in the internal loop distal to the terminal loop. Like other nucleolytic ribozymes, cleavage occurs via an internal phosphoester transfer reaction, resulting in 2'-3'-cyclic phosphate and 5'-OH termini. Metal ions do not appear to participate directly in the reaction as the ribozyme is active in a wide range of divalent and monovalent cations. The reaction is relatively independent of pH above pH 6, with activity declining with pH below this point.

We are investigating the importance of ten highly conserved nucleotides to the structure and activity of the ribozyme. All ten lie in the terminal and cleavage site loops, which are thought to interact to form the active ribozyme. Mutation reduces activity by one to three orders of magnitude under standard conditions and generally perturbs folding significantly as revealed by in-line probing. Two of these nucleotides are particularly interesting. Replacing the highly conserved C in the terminal loop with a U results in a 2000-fold loss in activity at pH 7, but the effect is ameliorated at high pH. Similarly, cleavage is 50-fold slower when the conserved G in the cleavage site loop is replaced by an A, with higher activity observed at lower pH. These effects are consistent with a catalytic mechanism in which the C and G participate in general acid-base catalysis and we are investigating this hypothesis through atomic mutagenesis and phosphorothiolate acid-rescue experiments. We will present our latest data and compare the proposed mechanism to those of other small nucleolytic ribozymes.

272 C Anion-p or cation-p interactions in RNA?

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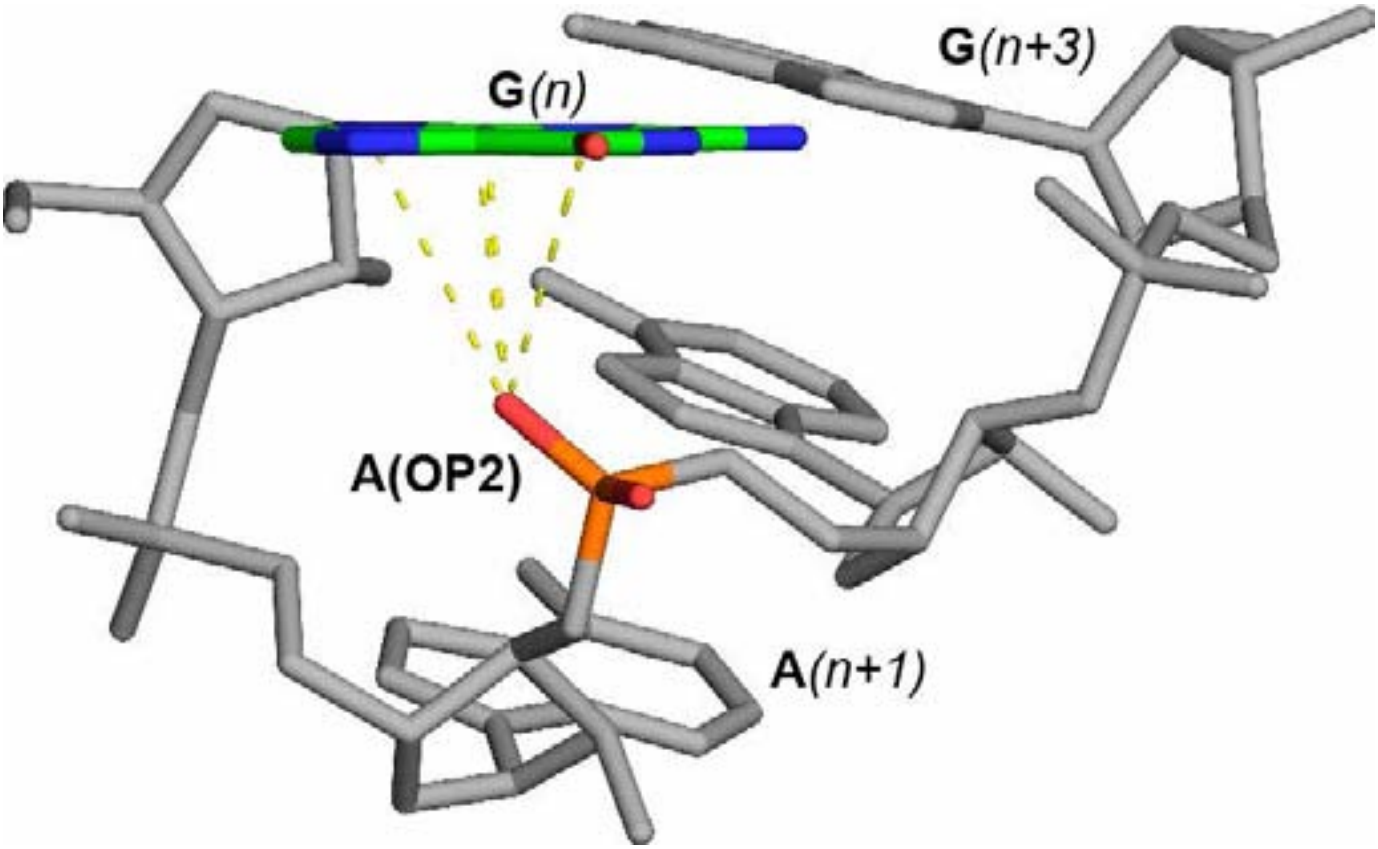
In order to better characterize RNA folding and RNA structure as well as RNA interactions with proteins and ligands, it is essential to refine our knowledge of the non-covalent interactions that are at play in these systems. Without doubt hydrogen-bonds are among the best known non-covalent interactions. But, next to them, a large diversity of non-covalent interactions exists. For instance, in the protein world, cation-π interactions, namely the stacking of cationic species over an aromatic group, became rapidly a necessary letter in the non-covalent interaction alphabet. Yet, despite their obvious aromatic character, no significant cation-π interactions were described so far for nucleic acid systems. Here we report that nucleic acid aromatic systems prefer to interact with anionic rather than cationic species.

Indeed, through the calculation of electrostatic potential surfaces, we were able to show that the protein and nucleic acid aromatic systems do not share the same characteristics. The formers are electron-rich systems able to establish cation-π contacts; the latters are electron-depleted systems that display a propensity to attract anions and establish anion-π interactions. This largely unnoticed dissimilarity between aromatic rings of both main biomolecular groups sheds new light on some of their essential properties. Through an exhaustive search of the PDB for anion-π interactions involving, among others, the DNA and RNA backbone phosphate groups, it is found that anion-π interactions are rare in DNA compared to RNA where they are mostly involved in sharp turns (75%) such as those found in tRNA anticodon loops and, more generally, in RNA tetraloops (see Figure 1 below that shows the stacking of a phosphate group under a guanine in a GAAA tetraloop). Besides, these interactions are also observed between sequence-distant residues in ribosomes and in crystal-lattice contacts. The relative rarity of these anion-π interactions outside of loops indicates that it is generally not prevailing over classical hydrogen bonds in the nucleic acid context but nevertheless vital for their folding, structure and function.



273 A The solution structure of Tetrahymena telomerase p65-RNA(TER)-TERT RBD determined by SAXS reveals contacts critical for particle stability

Andrea Berman[1], Anne Gooding[4], Robert Rambo[2], John Tainer[3], Thomas Cech[4]

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The *Tetrahymena* telomerase ribonucleoprotein particle catalyzes telomeric DNA synthesis at the end of eukaryotic chromosomes. Although it minimally contains a reverse transcriptase protein subunit (TERT) and a template-containing RNA (TER), the biogenesis of the enzyme complex requires the protein p65[1,2], which binds TER and induces a conformational change that facilitates the binding of TERT[3-5]. We used small-angle x-ray scattering (SAXS) to determine the low-resolution solution structures of p65 alone, a portion of the TER alone, p65 bound to TER and p65-TER in complex with the RNA binding domain (RBD) of TERT. The SAXS analysis has allowed us to confidently distinguish the RNA from the protein in the scattering density. This has significantly aided in modeling the SAXS envelopes with published crystal and NMR structures of the RNA and protein components, allowing us to understand the global arrangement of the minimal holoenzyme[2]. Our structure reveals an elongated arrangement of the domains of p65. The observation that p65 contacts both TER and TERT supports the hypothesis that p65 serves as a molecular buttress that stabilizes both the TER and TERT in their catalytically active conformations[6]. Comparison of SAXS models of p65 complexed with constructs of TER containing and lacking the pseudoknot indicates that the pseudoknot is required for the proper folding of TER. These data offer insight into how the three molecules in the minimal complex interact with each other to make a functional telomerase and provide additional evidence for why the pseudoknot is essential in catalysis[7].

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274 B Sizing large RNA molecules using single molecule fluorescence correlation spectroscopy

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Large RNA molecules (>1kb), encompassing mRNA, long non-coding RNAs (lncRNA) and genomes of RNA viruses, play important role in many biological processes. However, given their size and general propensity of ssRNA to form extensive secondary structure it is difficult to study their folding. Single molecule fluorescence correlation spectroscopy (smFCS) allows to determine hydrodynamic size of large RNA molecules at concentrations approximating infinite dilution conditions (<1 nM), thus minimizing artifacts attributed to non-specific interactions and aggregation. We have labelled representative RNAs at 3'-end with a single fluorophore and examined their hydrodynamic sizes under low-salt conditions, i.e. in 'good solvent', when electrostatic interactions dominate and prevent formation of tertiary structure. Under these conditions most RNAs adopt an ensemble of relatively compact conformations. The average hydrodynamic radii can be related to the ensemble of branched structures generated by Mfold, suggesting that under low salt conditions the size of large RNAs is determined by electrostatic repulsion between partially double stranded, branched segments. The size of most large RNA molecules decreases in the presence of physiological concentrations of polyvalent cations, reflecting further folding into compact tertiary structure. In contrast to mRNAs and viral genomes, lncRNAs do not undergo similar compaction, maintaining their rather elongated shape. Such distinct shape may be related to their function as molecular scaffolds in gene regulation.

275 C CompaRNA: a server for continuous benchmarking of automated methods for RNA structure prediction

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We have developed a benchmarking approach for the assessment of RNA secondary and tertiary structure prediction methods and implemented it in the CompaRNA web server. In the assessmend of secondary structure prediction methods, he performance of 28 single-sequence and 13 comparative methods has been evaluated on RNA sequences/structures released weekly by the Protein Data Bank. We have also calculated a static benchmark generated on RNA 2D structures derived from the RNAstrand database. Benchmarks on both data sets offer insight into the relative performance of RNA secondary structure prediction methods on RNAs of different size and with respect to different types of structure. The best comparative methods typically outperform the best single-sequence methods if an alignment of homologous RNA sequences is available. The initial tests on RNA 3D structure prediction methods provides additional insight into their strengths and weaknesses, and suggest strategies for the combination of the existing methods into meta-predictors that may aid in constructing superior models.

276 A Symmetry and asymmetry in the unwinding of nucleic acids

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The forming and melting of complementary base pairs in RNA and DNA duplexes are conformational transitions required to accomplish a plethora of biological functions. Using fully atomistic simulations we have shown that RNA unwinding occurs by a stepwise process in which the probability of unbinding of the base on the 5′ strand is significantly higher than that on the 3′ strand [Colizzi and Bussi JACS, 2012]. The asymmetry in the RNA unwinding dynamics is compliant with the mechanism of helicase activity shown by prototypical DEx(H/D) RNA helicases and could allow deciphering the basis of the evolutionary pressure responsible for the unwinding mechanism catalyzed by RNA-duplex processing enzymes. In this spirit and from a broader standpoint, here we use a topology-based coarse-grain model to compare and characterize the mechanism of unwinding for both DNA and RNA. The (a)symmetric behavior of the 3′- and 5′-strand could be related to the (bi)directionality observed in molecular machineries processing nucleic acids.

277 B A revised folding pathway of group II introns: Assigning specific structures to the individual FRET states

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*Sc.*ai5γ is a large (~900 nts), multi-domain group II intron RNA transcribed from the mitochondrial genome of *S. Cerevisiae*.[1] These RNAs catalyze their self-excision upon formation of long-range tertiary interactions. Their folding and related catalytic activity are influenced by co-factors such as metal ions (especially Mg^{2+}) and proteins. We have designed a shortened construct of *Sc.*ai5γ, D135-L14, labeled with the Cy3-Cy5 fluorophore pair and biotin for surface immobilization. This construct enables a detailed characterisation of the folding pathway by smFRET using TIRF microscopy, since it preserves the dynamics and the catalytic activity of the parent ribozyme.[2] Using smFRET spectroscopy, it becomes possible to record data from one single molecule isolated from its neighbors in real-time and the direct analysis of individual folding pathways of different molecules within a large ensemble. Previous studies revealed a linear three-state folding pathway from the unfolded to the native state devoid of kinetic traps.[2] The rate constants of those transitions depend on concentration and the type of metal ions (*i.e.* Mg^{2+} and Ca^{2+}).[2, 3] Our studies revealed an additional folding step. Furthermore we made three distinct mutants each carrying a different mutation that interrupts a specific tertiary interaction, known to be involved in crucial interdomain tertiary interactions (Figure 1). This enabled us to assign each FRET state to a specific folding intermediate. The construct carrying a tetra-loop mutation in Domain 5, involved in the ζ- ζ' interaction with D1 (D13mut5-L14), basically stops the folding process at the lowest FRET state. A 2-point mutation in Domain 3 aimed to interrupt the μ-μ' (D3-D5) docking (D15mut3-L14) does not have a drastic effect. In contrast, Domain 3 deletion (D15-L14) causes misfolding of the molecule to a more compact state (Figure 1). Moreover, the presence of the substrate affects the distribution of conformational intermediates and their dynamic equilibrium in the different mutants.

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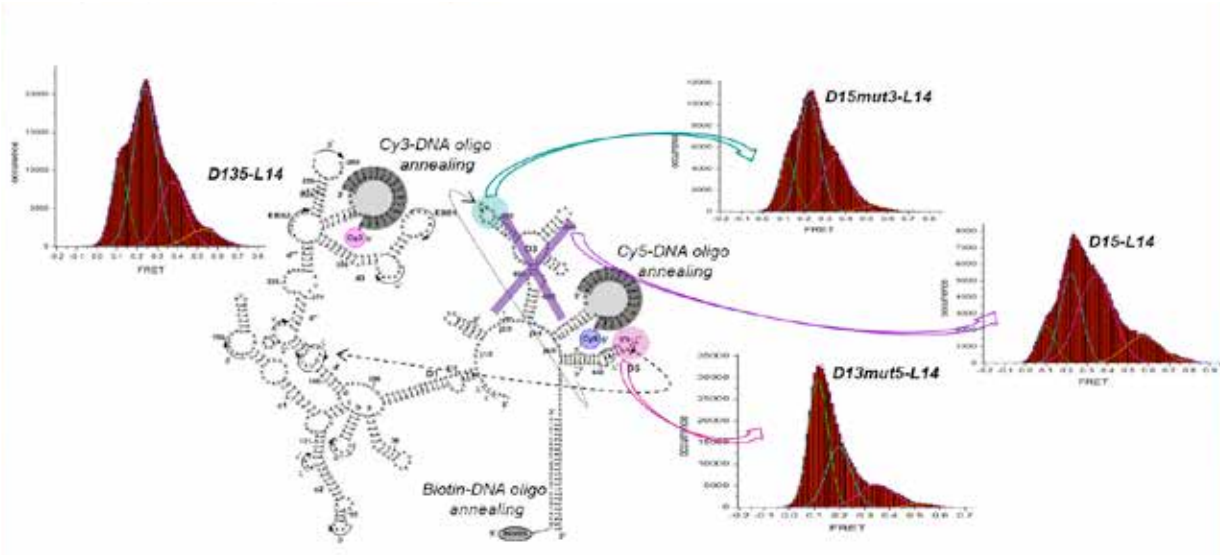

Figure 1. Secondary structure of labeled D135-L14 ribozyme (left). Mutations are indicated in green (D15mut3-L14), purple (D15-L14) and pink (D13mut5-L14). Histograms allow us to identify the number of conformations adopted by the molecule, the relative abundance of each FRET state and therefore the differences between the mutant constructs (right) and D135-L14 (left).

278 C Elucidation of viroids structure by SHAPE

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Viroids are single stranded, circular RNA with the size range of 246-400 nucleotides. They are the smallest known phytopathogens causing a wide array of symptoms. Viroids do not encode any pathogen-specific proteins and therefore both the secondary as well as the tertiary structures are of the upmost importance. In general, the secondary structures of viroids have been predicted based on their sequence using computer software programs, which have been shown to possess several important limitations. The predicted structure of a viroid needs to receive physical support prior to its use in the accurate interpretation of any mechanistic studies. Recently, we have adapted SHAPE probing coupled with fluorescence sequencing technics and computer-assisted structure prediction in order to fasten the structural determination of viroid species. As proof-of-concept, the secondary structure of the RNA strands of both the plus and minus polarities of the peach latent mosaic viroid has been determined. Both of these structures are in good agreement with the ones obtained using conventional SHAPE protocol. The same methodology has been applied to determine the secondary structure of both the strands (plus/minus) of several viroids of the *Avsunviroidae* family. This easy-to-use and fast protocol will be very useful to compare the structures of many viroid variants revealed from high-throughput sequencing data in a relatively short amount of time.

279 A Non-Canonical Base Pair Formation and Ion Binding in Small Bulged RNA

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Bulges are found in most small and large RNA. The presence of a bulge alters the A-form helical structure and allows the formation of many non-canonical base pairs. Thus unique conformations of the bulge region become sites for recognition and interactions with other biomolecules. We are comparing small bulged RNA to determine sequence and ionic conditions in which a particular non-canonical base pair forms and its contribution to RNA stability. For our studies, RNA constructs containing small symmetric or asymmetric bulges are derived from functionally interesting and well-characterized RNA. Thermodynamic data on small RNA constructs is collected in 1 M KCl buffers where charge-charge interactions are expected to be fully satisfied, based on polyelectrolyte theory of ion condensation. Comparative analysis of $?G^{o}_{37}$ data on multiple RNA constructs in 1 M KCl is utilized to measure the contribution (if any) of non-canonical base pairs. Measurements are also performed in 0-10 mM magnesium ions to determine the contributions of magnesium ions in stabilizing these constructs. The penalty for breaking an A-form helix in RNA can be several kcal/mol. Formation of strong non-canonical base pairs in the bulge, such as C+•C, provides significant stabilization and minimizes the cost of breaking the helical structure. In the presence of 10 mM of magnesium ions, RNA constructs are equally or more stable ($?G^{o}_{37}$) than in 1 M KCl. An additional 2-4 kcal/mol of free energy is available in 10 mM magnesium buffer for constructs in which magnesium interactions are significant. A-form helical constructs also show stabilization in magnesium ions depending on the sequence. Bulged containing DNA constructs examined were not additionally stabilized by magnesium ions over 1 M KCl. Thermodynamic analysis provides accurate and straightforward method to measure the strength of non-canonical base pairs and ion-binding in nucleic acids. This work is supported by NSF Grant MCB-0950582.

280 B The plasticity of a structural motif in RNA: structural polymorphism of a k-turn in response to its environment

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The k-turn is a widespread structural motif that introduces a tight kink into the helical axis of double-stranded RNA, and is frequently used to make tertiary interactions. The adenine bases of consecutive G•A pairs are directed towards the minor groove of the opposing helix, hydrogen bonding in a typical A-minor interaction. We find that the available structures of k-turns divide into two classes, depending on a key A-minor hydrogen bonding in the core of the structure. The two classes differ on whether N3 or N1 of the adenine at the 2b position accepts a hydrogen bond from the O2' at the -1n position. There is a coordinated structural change involving a number of hydrogen bonds between the two classes.

We show that Kt-7 can adopt either the N3 or N1 structures depending on environment. While it has the N1 structure in the ribosome, we find that crystal structures of the same sequence, either engineered into the SAM-I riboswitch or in complex with the L7Ae protein as a simple duplex, have the N3 structure. The change between the N1 and N3 structures results in a significant alteration in the trajectory of the helical arms, and in the rotational setting of the helical arms. This will be very significant for making tertiary contacts.

281 C Ligand selectivity of the neomycin RNA aptamer is highly influenced by its ionic surroundings

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Nucleic acid aptamers are frequently characterized as highly specific for their cognate targets, which implies precise molecular recognition between the aptamer and its target. However, as short oligonucleotides, aptamers are likely to be structurally flexible and more responsive to environmental changes adjusting to alternative conformations with altered specificity. To understand the role of aptamer structure in establishing ligand specificity, we examined an RNA aptamer reported as highly selective for neomycin-B over other aminoglycosides. Docking scores correlated well with the experimentally determined dissociation constants showing that the aptamer is more promiscuous than previously reported. The ionic surroundings strongly affected the ligand affinities for its aminoglycoside ligand and influenced the promiscuity of aminoglycoside binding. In parallel, the observation of a flexible pentaloop in molecular dynamics simulations led us to investigate the impact of this region on target selectivity. Studies with aptamer variants, including those with 2-aminopurine substitutions and altered bases, showed that the aptamer conformation was ligand-dependent and strongly affected by the ionic environment. The results of this study demonstrate that the structure of the neomycin aptamer pentaloop is highly malleable, impacted by the ionic environment and coordinating with the binding pocket for ligand incorporation. An impact of buffer composition was also observed on the conformation of the malachite green aptamer interacting with malachite green. Thus, buffer composition may also characterize the interaction of other aptamers with their ligands. These results support a view of aptamer conformations as malleable, responding to the ionic environment and also the ligand structure, with potential impacts on aptamer affinity and specificity.

282 A A predictive value optimizing the evaluation of biological RNA G-quadruplexes formation

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RNA G-quadruplexes are non-canonical structures involved in many important cellular processes such as translation regulation and mRNA polyadenylation. They are composed of stacks of at least two G-quartets, a co-planar arrangement of four guanines linked together by Hoogsteen base-pairs and stabilized by monovalent cations, usually K^+. Sequences agreeing to the algorithm G_x-N_{1-7}-G_x-N_{1-7}-G_x-N_{1-7}-G_x (where $x \geq 3$, N=A,U,G,C) are considered as potential candidates for G-quadruplexes (PG4). However, many sequences identified as forming G-quadruplexes with known biological functions do not correspond to this algorithm. Moreover, this algorithm led to the identification of a number of false positives, hence there is a need to consider other factors than the PG4 sequence only. For example, we previously reported that many false positive PG4s do not form G-quadruplexes because of the presence of multiple tracks of cytosines (C) in their genomic context competing to form Watson-Crick structures [1]. In order to determine the extent of the genomic context's influence on G-quadruplex formation, we selected 12 predicted PG4s found in the 5'- and 3'-untranslated region (UTR) of human mRNAs. *In vitro,* we have assessed the adoption of the G-quadruplex structure for the same PG4 with either 15 or 50 nucleotides of their upstream and downstream genomic context by in-line probing. We then sought to find a predictive value representative of the competitiveness of the genomic context. Several analyses have allowed the development of a new scoring system for the prediction of PG4 considering the sequence in the vicinity. This new system could be used in combination with the standard algorithm as a scoring-system to optimize RNA G-quadruplexes prediction.

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283 B Metal ions determine the kinetics and thermodynamics of single RNA-RNA associations according to the Irving-Williams series

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The formation and stability of a complex RNA architecture is to a large part dependent on base pairing, being the key to secondary and tertiary structure. Any such RNA-RNA interaction is only possible in the close proximity of cations, i.e. largely metal ions, to overcome charge repulsion of the negatively charged phosphate sugar backbone. Here we dissect the fundamental role of M^{2+} ions on the kinetics and thus also stability of RNA-RNA strand association. Here, we investigate the 5'-splicie site formation of group II introns representing a typical example of such a basic interaction. Group II introns are functional RNAs that catalyze their own excision, i.e. splicing [1]. Splice site recognition is provided by the association of the exon- and intron-binding sequences (EBS, IBS), which has been proposed to be directly dependent on divalent metal ions [2]. We now used single-molecule FRET [3] to systematically assess the influence of various divalent metal ions along the Irving-Williams Series on the EBS1*/IBS1* interaction (Figure 1): In the rigorous absence of M^{2+} ions, strand pairing is basically absent, i.e. the presence of M^{2+} is crucial for EBS1*/IBS1* interaction to occur to a significant extent. However, the inter-oligonucleotide affinity as well as docking/undocking kinetic varies by several orders of magnitude depending on the cation present. These observations can be rationalized by the intrinsic affinities of the individual metal ions towards the different coordinating atoms, e.g. ring nitrogens, carbonyl oxygens, and phosphate oxygens, of the RNA. This allowed us to dissect such a "simple" RNA-RNA strand association into subsequent small steps of a clearly M^{2+}-dependent EBS1*/IBS1* folding pathway. Our study thus not only validates smFRET for the systematic study of metal ion-mediated nucleic acid folding, but also shows that metal ion-dependent RNA folding can be explained by the coordination chemistry of the metal ion cofactor.

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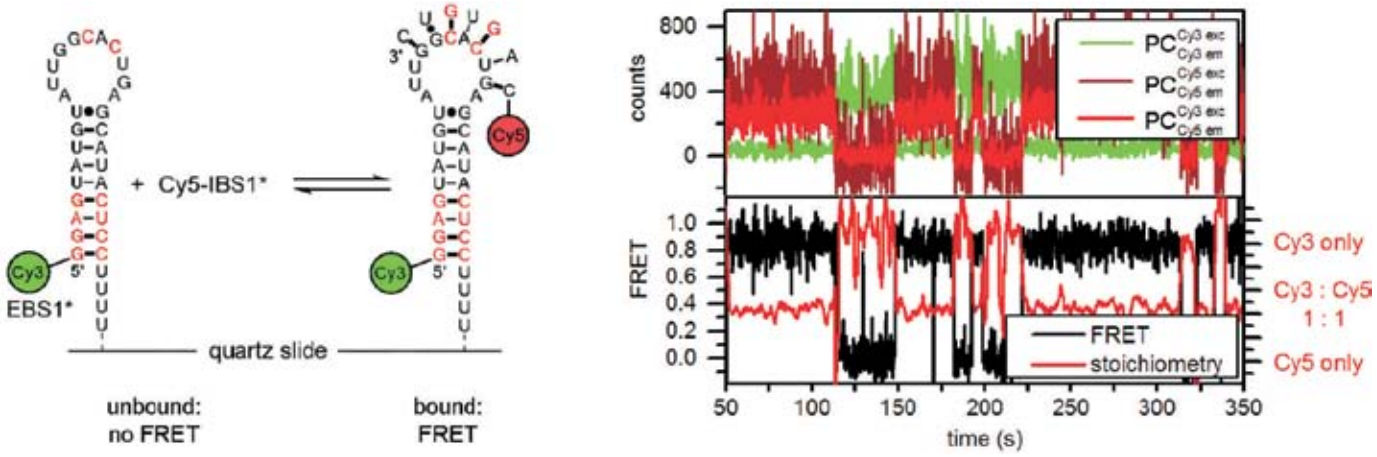

284 C Crowded Environments Compensate Destabilizing Mutations in the Azoarcus Ribozyme

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Using the *Azoarcus* ribozyme as a model system, our laboratory has previously shown that the native structure of the RNA is stabilized by the cooperative formation of tertiary interactions at different places in the RNA (1). On the other hand, molecular crowding stabilizes the RNA structure (2). Here, we show that crowded environments offset the destabilization due to RNA mutations. We created point mutations in tetraloops (A25U and A190U) or in the central triple helix (G125A) to disrupt tertiary interactions and destabilize the structure. Combinations of these mutations create RNA molecules carrying single, double or triple mutations. We followed the RNA folding process by native PAGE, small angle X-ray scattering (SAXS) and ribozyme activity in the presence of 18% PEG_{1000}. We found that PEG_{1000} reduced the average size of RNA and altered the distribution of unfolded structures at low magnesium concentrations. Furthermore, PEG_{1000} shifted the midpoints of the folding transitions to lower magnesium. In the presence of 18% PEG_{1000}, some mutants even show similar stability as the wild-type ribozyme. The magnitude of this compensation depends on the position of mutation. The results show that crowder molecules in solution compensate for the destabilization caused by mutations, and change the magnitude of cooperativity between tertiary interactions. Greater tolerance to mutation in physiological environments may allow for neutral drift among non-coding RNA sequences.

285 A A Universal RNA Structural Motif Docking the Elbow of tRNA in the Ribosome, RNAse P and T-box Leaders

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The structure and function of conserved motifs constituting the apex of Stem I in T-box mRNA leaders are investigated. We point out that this apex shares striking similarities with the L1 Stalk (helices 76-78) of the ribosome. A sequence and structural analysis of both elements shows that, similarly to the head of the L1 Stalk, the function of the apex of Stem I lies in the docking of tRNA through a stacking interaction with the conserved G19:C56 base pair platform. The inferred structure in the apex of Stem I consists of a module of two T-loops bound together head-to-tail, a module which is also present in the head of the L1 Stalk, but went unnoticed. Supporting the analysis, we show that a highly conserved structure in RNAse P formerly described as the J11/12-J12/11 module, which is precisely known to bind the elbow of tRNA, constitutes a third instance of this T-loop module. A structural analysis explains why six nucleotides constituting the core of this module are highly invariant among all three types of RNA. Our finding that major RNA partners of tRNA bind the elbow with a same RNA structure (Figure 1) suggests an explanation for the origin of the tRNA L-shape.

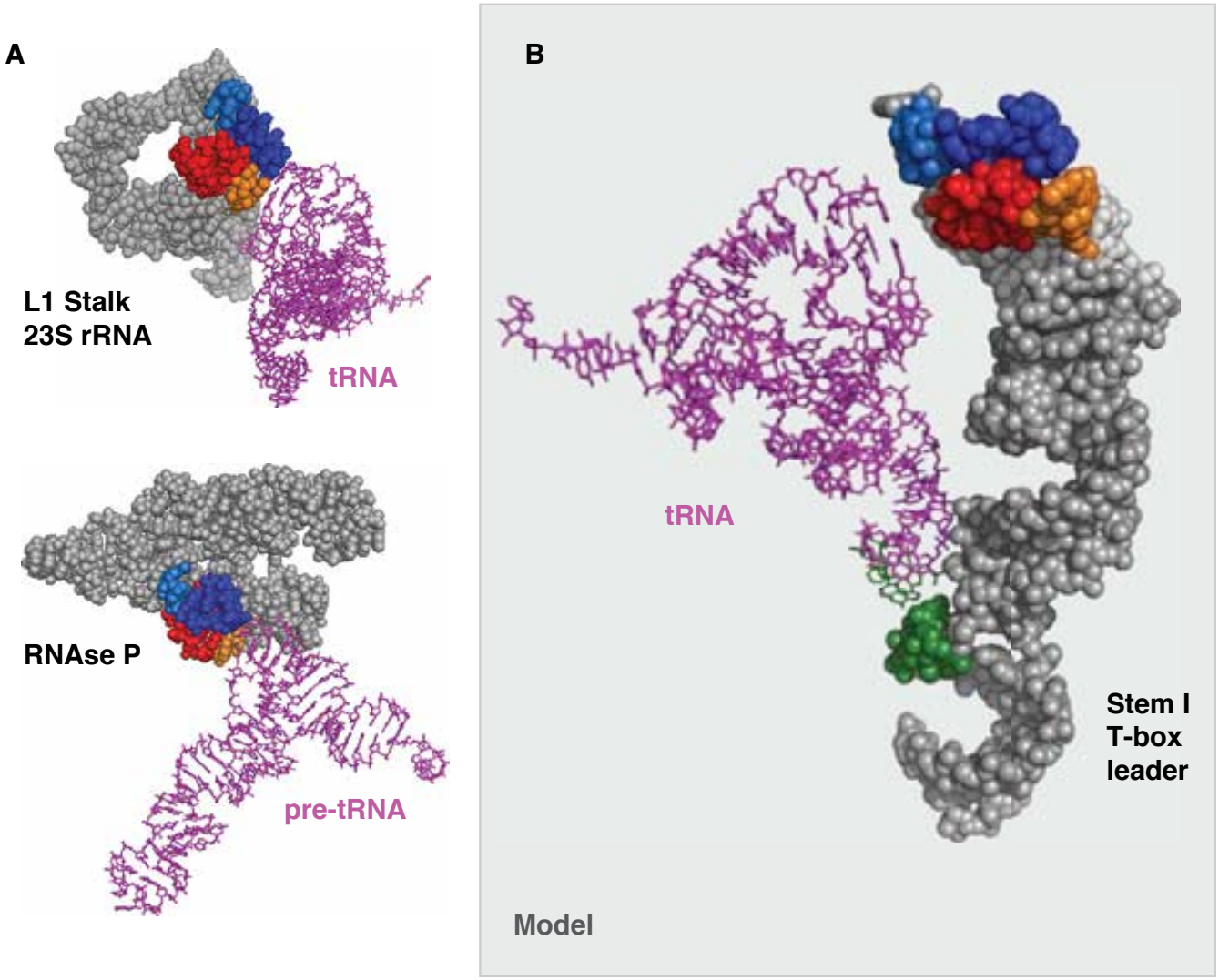

Figure 1:

Crystallographically established (a) and model (b) of tRNA – RNA binding in which a stacking interaction between the G19:C56 base pair platform defining the elbow of a tRNA and a platform of a head-to-tail double T-loop module is involved. (a) L1 Stalk – tRNA (pdb *1VSA*) and RNAse P – pre-tRNA (pdb *3Q1Q*) interactions. Only a fragment of RNAse P is shown. (b) Model of *B. subtilis ProI* Stem I – tRNA interaction.

286 B One-bead coarse grained models for RNA dynamics and folding

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The knowledge of RNA tertiary structure1 and its internal dynamics2 is essential for understanding key biological processes such as the regulation of mRNA translation. However, experimental studies of RNA structure and dynamics are expensive and troublesome. This is confirmed by a large discrepancy between the number of protein and RNA X-ray crystallography resolved structures in the Protein Data Bank. On February 19, 2013 there were more than 70 000 deposited protein X-ray structures and only 500 RNAs. In principle, computational approaches could affordably complement experiments. At this moment, however, the quality of RNA simulation methods and their predictive power needs improvement. One of the promising approaches is molecular dynamics with a coarse-grained representation of RNA. Nucleic acids are modeled as sets of beads and each bead corresponds to a single nucleotide. The time-dependent behavior of such beads is simulated by solving the classical equations of motions. This requires the formula for the potential energy function with parameters - the so called force field. Finding such a force field for various coarse-grained models of RNA is a long process, so a lot of effort has been put to find a universal force field that can be applied to all kinds of problems. However, in our opinion, the best results with a coarse-grained approach can only be obtained by carefully adapting the energy function to a particular need.

Therefore, we have created a methodological help for developers of coarse-grained models3,4. We have implemented a metaheuristic optimization algorithm (evolutionary algorithm) that finds a best set of parameters for a given one-bead RNA potential energy function. Using such an automatized routine, in short time, one can adapt a general model to a particular task. As an example, we will show the results of the same one-bead coarse-grained force field, with parameters optimized in the first case for the internal dynamics of a folded RNA helix (Figure 1A,B) and in the second case for the RNA structure prediction (Figure 1C,D). We will present the differences in the construction of the two models that are essential for their performance for a particular task. We will also show statistical studies performed on thousands of force field models that emphasize the importance of particular force field terms and their relative correlations. The results allow us to define the boundaries of one-bead representations in modeling various aspects of RNA structure and dynamics.

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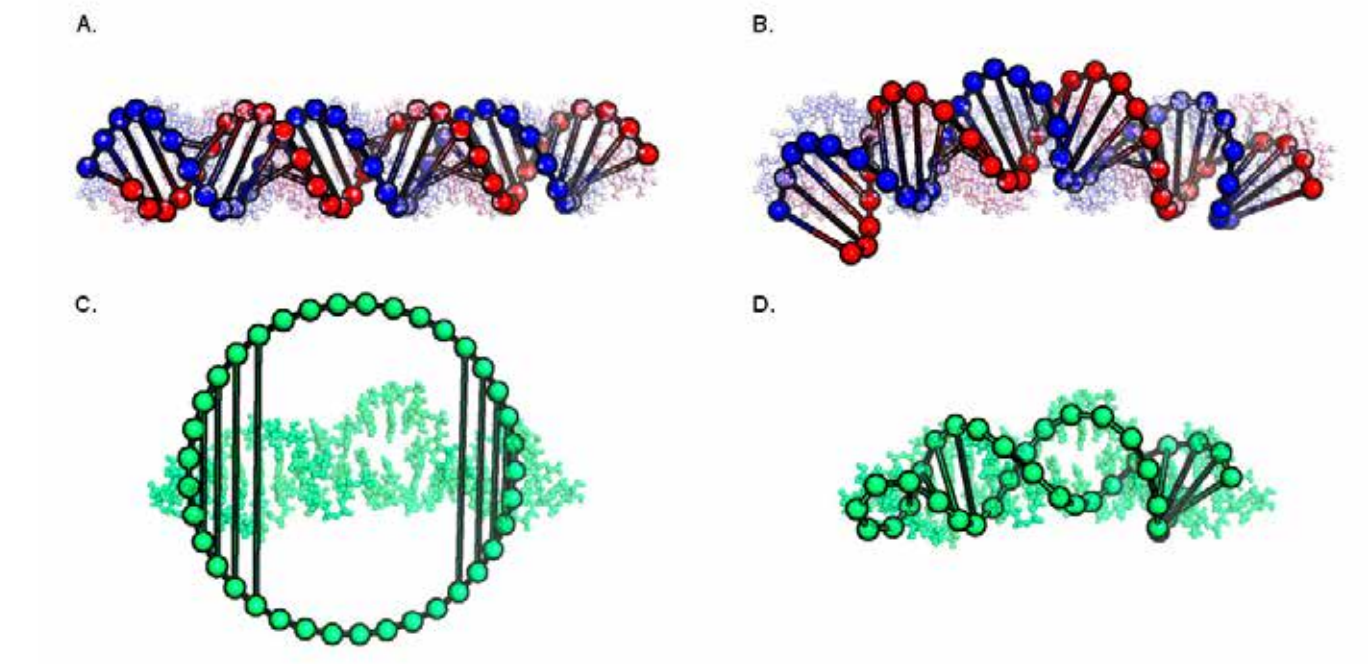

287 C Crystallization of the active form of the Lariat-Capping ribozyme.

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The lariat-capping ribozyme (LCrz) followed by an ORF encoding a homing endonuclease (HE) is embedded in the P2 stem of a regular group I intron named GIR2. This complex twin-ribozyme intron found in the SSU rRNA precursor of several eukaryotes has been mostly studied in *Didymium iridis*. In connection with the microorganism life cycle, three different intron processing pathways are observed. In the regular self-splicing pathway, GIR2 performs splicing to allow the LCrz branching reaction to proceed, leading to a 3' product with the first and the third nucleotide linked by a 2' 5'phosphodiester bound. This three nucleotides lariat caps the HE mRNA and replaces the conventional m^7G cap. LCrz shares high sequence and secondary structure similarities with group I introns, but distinct three-dimensional features. Thus, the LCrz constitutes an independent class of naturally occurring ribozymes. The LCrz branching reaction takes place in a genuine structural context despite the fact it resembles the first step of splicing by group II introns or the spliceosome.

Formerly known as the GIR1 branching ribozyme, it has been renamed regarding the 2' 5' branching reaction it catalyzes and its specific structural features revealed by our 2.5 Å crystal structure of the full-length DiLCrz (192 nucleotides) in the post-cleavage form. We are currently crystallizing the active form of the LCrz to better understand the chemical and structural basis of lariat formation. We now have data up to 4 Å and a readily interpretable electron density map. These data show that the unit cell of the active form of the full-length DiLCrz is shrunk along two dimensions as compared to the post-cleavage form indicating discrete conformational changes with respect to the post-catalytic state.

288 A Structural characterization of the yeast telomerase RNA core by SHAPE

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Telomerase is a ribonucleoprotein (RNP) that catalyzes the addition of telomere repeats to the ends of linear eukaryotic chromosomes. The core enzyme consists of telomerase reverse transcriptase (TERT) and telomerase RNA (TR). Our aim is to investigate the overall architecture of *S. cerevisiae* telomerase RNA (TLC1) to gain insights into the relationship between RNA structure and telomerase function. The large size of TLC1 (1157 nts) makes structural characterization difficult. To overcome this obstacle, we took advantage of an active 170-nt yeast telomerase RNA, Micro-T. This catalytic core RNA contains the conserved structural elements, including the 16-nt template sequence that is reverse transcribed, a catalytically important pseudoknot, and a poorly characterized protein-binding region. We find that Micro-T adopts fewer conformations compared to TLC1 *in vitro* by native gel electrophoresis, suggesting it should be more amenable to structural studies. We investigated the structure of this RNA using Selective 2'-Hydroxyl Acylation analyzed by Primer Extension (SHAPE), which interrogates local flexibility at each nucleotide position in a given RNA. Here we report the first application of SHAPE chemistry on yeast telomerase RNA. Our Micro-T SHAPE analysis supports the current secondary structure model for the TLC1 core. The template nucleotides and adjacent single-stranded region are highly SHAPE reactive, suggesting the RNA is in a biologically relevant conformation. Furthermore, our data indicate that the yeast pseudoknot is able to form in the absence of TERT. This is in contrast to the in-vitro folding of protein-free *Tetrahymena thermophila* TR, which does not form a pseudoknot or maintain an unpaired template. This suggests the intrinsic stability of the yeast pseudoknot is more similar to that of human TR. SHAPE will allow further examination of structure-function relationships in the telomerase RNP. For instance, in human telomerase evidence suggests that physical flexibility resulting from an unusual 5-nucleotide bulge within the pseudoknot is important for catalysis. We propose physical flexibility within the pseudoknot is a prominent feature of all telomerase RNAs. Although yeast does not contain a bulge at the corresponding position, it has an unusually long unpaired region, which we find to be reactive by SHAPE. We are now testing for conformational changes in telomerase RNA the presence of TERT. Results from these studies will help determine how yeast telomerase RNA and TERT interact and coordinate telomeric DNA synthesis.

289 B Molecular crowding accelerates ribozyme docking and catalysis

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All biological processes take place in highly crowded cellular environments. However, how molecular crowding agents affect the folding and catalytic properties of RNA molecules remains largely unknown. Here, we have combined single-molecule fluorescence resonance energy transfer (smFRET) and bulk cleavage assays to determine the effect of a molecular crowding agent (polyethylene glycol, PEG) in the folding and catalysis of a model RNA enzyme, the hairpin ribozyme. Our single-molecule data reveal that PEG favors highly the formation of the docked (active) structure by increasing the docking rate constant specifically with increasing PEG concentrations. Furthermore, in the presence of PEG, the concentration of Mg^{2+} ions required to induced the active state decreases by 10-fold, near the physiological range (~1 mM). Lastly, bulk cleavage assays show that the ribozyme's activity accelerates by ~10-fold in the presence of the crowding agent. Together, our data show that molecular crowding agents can affect both the dynamics and function of RNA enzymes, such as the hairpin ribozyme. We propose that crowding agents in the cell play an important role in stabilizing and accelerating the native structure of RNA enzymes *in vivo*.

290 C Imp3p unfolds conserved and stable stem structures in both U3 snoRNA and pre-rRNA to promote annealing

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Growth of eukaryotic cells depends on ribosome biogenesis and its dysfunction is closely linked to cancer progression. An essential step in making ribosomes is rapid hybridization between the pre-rRNA and the U3 snoRNA, which is required for the pre-rRNA cleavages that liberate the small subunit (SSU) precursor. One of the three prerequisite U3-pre-rRNA hybridization sites, designated the U3-18S duplex, is not observed in vitro in the absence of protein because the bases involved in hybridization in each RNA are buried in conserved and stable structures: box A/A' stem loop in U3 snoRNA and helix 1 (H1) in the 18S region of the pre-rRNA. Thus, energy to unwind these stems is needed to permit hybridization. Previously, we showed that Imp3p and Imp4p are required for U3-18S hybridization in vitro (Gerczei '09). However, the minimal 18S substrate used in that work was unable to form H1 and it was unknown whether protein binding exposes the box A/A' bases. Here, we employ larger pre-rRNA fragments more representative of in vivo substrates. By probing base accessibility with CMCT modification and backbone accessibility with RNase T1 our studies demonstrate that the larger substrates form the secondary structures observed in vivo and that binding of Imp3p alone provides sufficient energy to unfold the pre-rRNA H1 and the U3 box A/A' stem structure. Moreover, this protein dependent unfolding activity is required to observe hybridization. These stable RNA structures may serve as a switch to block U3-pre-rRNA interactions until recruitment of Imp3p, which is expected to happen late in assembly of the 90S preribosome. Imp3p recruitment promotes U3-18S hybridization, which in turn leads to recruitment of the endonucleases that release the SSU precursor.

Gerczei *et al* (2009) *JMB* 390, 991.

291 A The influence of metal ions on the structure of the CPEB3 ribozyme's P4 region

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The CPEB3 ribozyme is one of two small ribozymes that have been discovered in mammalian genomes (Salehi-Ashtiani et al. 2006; Teixeira et al. 2004). This small self-cleaving RNA is exclusively found in mammals with a highly conserved sequence and resides on an intron of the *cpeb3* gene (Salehi-Ashtiani et al. 2006). The function of the CPEB3 ribozyme is yet unknown, but its secondary structure and cleavage mechanism have been proposed to be highly similar to the one of the Hepatitis Delta Virus ribozyme (Salehi-Ashtiani et al. 2006), which uses Mg^{2+} ions for cleavage (Nakano et al. 2001).

As a first step in the investigation of the 3D structure of the CPEB3 ribozyme and of its interaction with metal ions, we have solved the solution structure of the P4 region of the ribozyme and investigated its metal-ion binding sites using NMR-spectroscopy. This small hairpin is closed by a UGGU tetraloop, which is stabilized by base stacking interactions and an internal hydrogen bond and which contains one inner-sphere Mg^{2+}-binding site. The tetraloop's structural features are very similar to the ones previously described for an AGUU tetraloop being an RNAse III recognition motif in yeast (Wu et al. 2001). A second Mg^{2+}-binding site is contained in the stem of P4, sequentially close to the proposed ribozyme active site. Comparison of the NMR spectra of the full-length CPEB3 ribozyme and the free P4 hairpin in the absence and in the presence of Mg^{2+}indicates that the structure and the Mg^{2+}-binding sites of the isolated P4 are identical or very similar to the ones found in the structural context of the full-length ribozyme, thus providing first information on the CPEB3 ribozyme's fold and metal-ion interaction sites.

Financial support by the Swiss National Science Foundation, the European Research Council and the University of Zurich is gratefully acknowledged.

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292 B Structural complexity of Dengue untranslated regions: the role of cis-acting RNA motifs and pseudoknot interaction in modulating functionality of the viral genome.

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Dengue virus (DENV) is a single-stranded positive-sense RNA virus belonging to the *Flaviviridae* family, whose members cause diseases of major health importance. The DENV genome encloses multiple *cis*-acting elements required for translation and replication. Previous studies indicated that a 719 nt subgenomic minigenome (DENV-MINI) is an efficient template for translation and (-) strand RNA synthesis *in vitro* (1, 2). We performed a detailed structural analysis of DENV-MINI RNA, combining chemical acylation techniques (SHAPE, aiSHAPE), Pb^{2+} ion-induced hydrolysis and site-directed mutagenesis. Our results highlight protein-independent 5'–3' terminal interactions involving hybridization between specific *cis*-acting RNA motifs. Probing analyses identified tandem dumbbell structures (DBs) within the 3' terminus spaced by single-stranded regions, and internal loops and hairpins with embedded GNRA-like motifs. Analysis of conserved motifs and top loops (TLs) of these dumbbells, and their predicted interactions with downstream pseudoknot (PK) regions, predicted an H-type pseudoknot involving TL1 of the 5' DB and the complementary region, PK2. Since disrupting the TL1/PK2 interaction, via "flipping" mutations of PK2, previously attenuated DENV replication, this pseudoknot may participate in regulation of RNA synthesis. Computer modeling implied that this motif might function as autonomous structural/regulatory element. In addition, our studies targeting elements of the 3' DB and its complementary region PK1 indicated that communication between 5'-3' terminal regions strongly depends on structure and sequence composition of the 5' cyclization region.

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293 C Self-dimerizing group I ribozymes as a new class of modular units for RNA synthetic biology

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Biopolymers are promising materials for nanotechnology. DNA has been used for the construction of chemically stable 1D, 2D and 3D nano-objects because self-assembly of DNA is easily programmable. On the other hand, polypeptides (proteins) form complex 3D structures and exhibit diverse functions.

From the viewpoint of a nano-biotechnology and synthetic biology, RNA is also an attractive biopolymer. Due to the structural similarity between RNA and DNA, design principles of DNA nano-objects can be applicable to the design of RNA nano-objects. Moreover, RNA is also able to perform protein-like functions such as catalysis and molecular recognition because RNA can also form complex 3D structures. Several RNA nano-structures such as polygons and polyhedrons have been reported but they were composed of relatively short and simple RNA units (ca. 25-100 nts). In contrast, functional RNAs in living cells such as ribosomes and self-splicing ribozymes are composed of 200-3000 nucleotides.

We have constructed a new class of nano-structures possessing catalytic activity derived from a group I intron ribozyme. Because RNA nano-structures possessing the enzyme-like functions has not been reported so far. We converted a group I intron ribozyme to self- or hetero-dimerizing RNA that exhibits enzyme function upon self-dimerization. Molecular design for the dimerization was carried out with modular engineering, by which intramolecular interactions of the parent ribozyme was redesigned to form intermolecular interactions for the dimerization. The dimerization proceeded highly effectively and the ribozyme activity was strongly depended on the RNA dimerization. The resulting dimeric ribozyme would be applicable to new device modules for RNA synthetic biology.

294 A Automated identification of RNA 3D modules with discriminative power in RNA structural alignments.

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The particular three-dimensional architecture, which enables structured RNAs to accomplish a variety of functions, is established by local or remote tertiary interactions. A set of such tertiary interactions enclosed by canonical base pairs is called an RNA 3D module. The steady growth of experimentally determined atomic resolution RNA structures (X-ray or NMR) have revealed different types of RNA 3D modules, such as kink-turns, C-loops and G-bulges. Even though they in generally only consist of a few nucleotides, they play a major role in mediating protein and ligand docking, directing the folding process or stabilizing the structural conformation of an RNA molecule. The RNA 3D modules have shown up over numerous structures as recurring building blocks, which often are conserved throughout all kingdoms of life. Thus, it is a general interest to match RNA 3D modules known from one molecule to another for which the 3D structure is not known or the RNA sequences are only partially known. Recent strategies in RNA structure prediction include to 'fill the gap' in 2D RNA structure by including 3D information into the structure prediction, such as probing data. The assignment of such 3D information to 2D structures can improve structure prediction, limits the number of false positives, assign functions to unknown molecules, help to find new modules as well as classifying transcripts as ncRNAs.

Here, we address the usage of 3D information, by merging RNA 3D modules in existing 2D structural alignments of RNA. To meet this challenge we have created a pipeline, called metaRNAmodules, which completely automates extracting of known and unknown modules from the FR3D database and subsequently map them to Rfam structural RNA alignments to obtain comparative information. Subsequently, the modules are turned into graph-based statistical models (using Bayesian networks) for the RMDetect program, which allows for test of their discriminative power using real and shuffled Rfam alignments. An initial extraction of 15290 RNA 3D modules in all PDB files results in 977 modules with clear discriminative power. Many of these modules describe only minor variants of each other. Indeed, mapping of the modules onto Rfam families results in 28 unique locations in 10 different families. Our pipeline is available as source code along with the generated RNA 3D modules at http://rth.dk/resources/mrm.

295 B The regulatory significance of RNA secondary structure in Arabidopsis

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Recent transcriptome-wide studies have illustrated the role of RNA secondary structure as a post-transcriptional *cis*-regulator that controls multiple steps of mRNA maturation and translation. We have focused our attention on uncovering the regulatory impact of secondary structure on the *Arabidopsis thaliana* transcriptome. Here, we present findings from these recent studies revealing that mRNA structure modulates translation. For instance, we find that RNA secondary structure sharply decreases across translation start and stop sites, while increasing folding has significant positive correlation with ribosome occupancy, perhaps due to ribosomal stalling. We also demonstrate a correspondence between mRNA and protein structure. Specifically, the portions of mRNAs encoding predicted protein domains are on average significantly more structured than portions encoding inter-domain regions. We then reveal that RNA folding significantly anticorrelates with transcript abundance, due in part to increased degradation and smRNA processing of highly structured protein-coding mRNAs. Finally, we uncover that transcripts with similar structure not only share similar regulation, but also encode proteins with coherent functions. In total, we demonstrate the global impact of structure on post-transcriptional regulation in *Arabidopsis*.

296 C Crystallographic Studies of the Complex between G-quadruplex RNA and the RGG Domain of Fragile X Mental Retardation Protein

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Fragile X syndrome is one of the leading causes of inherited mental retardation and autism. The syndrome is associated with the loss of functional Fragile X Mental Retardation Protein (FMRP). In neurons, FMRP binds to a subset of neuronal mRNAs, normally activated by specific receptors, and inhibits translation of these mRNAs. FMRP contains three canonical RNA-binding domains: two KH domains and an arginine-glycine rich (RGG) box. The RGG motif recognizes mRNAs that contain guanine-rich regions capable of forming guanine quartets. We have determined the crystal structure of the complex between the RGG peptide and a guanine-rich RNA at 2.8 Å resolution. The RNA structure revealed formation of three G-quartets assembled in a potassium-stabilized G-quadruplex and connected to a helical stem. The RGG peptide interacts predominantly with the stem and the adjacent junction through base-paring with guanine bases and the sugar-phosphate backbone. The structure explains previous biochemical observations and suggests the molecular principles of FMRP binding to natural mRNAs.

297 A How do platinum drugs interact with RNA?

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We are currently studying the influence of platinum drugs on RNA structure, with a special focus on the anticancer drug cisplatin and its derivatives. Such drugs are generally believed to mainly target DNA by preferentially binding to the N7 atoms of two neighbouring guanines.[1] Nevertheless, additional potential targets are likely to exist, including RNA.[2]

In fact, cisplatin was shown to inhibit RNA-dependent processes.[2] Moreover, in the last decades several biochemical studies have been reported confirming the interaction of platinum drugs with RNA.[2,3] However, still very little is known on RNA structural changes upon platinum binding. The presence in RNA of loops, bulges and junctions, eventually important for tertiary contacts, possibly opens up a multifaceted scenario for RNA-platinum drug interaction.

The goal of our project is to obtain structural information on how platinum drugs interact with RNA, to evaluate actual structural preferences in the binding. For this purpose, we use a 27nt RNA hairpin model system derived from the yeast mitochondrial group II intron *Sc*.ai5γ.[4] The structure of this construct in solution is known,[5] and it contains secondary structure motifs common in RNAs.[6] Different platination agents are used, including cisplatin and oxaliplatin. The platination products are characterised by several techniques, like mass spectrometry, UV spectroscopy and gel mobility shift assays. We are currently optimising large scale platination conditions to obtain higher amount of pure platinated sample to be used for structure evaluation by NMR spectroscopy.

Acknowledgements. Financial support by the Swiss National Science Foundation (*Ambizione* fellowship PZ00P2_136726 to DD), by the University of Zurich and within the COST Action CM1105 is gratefully acknowledged.

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298 B Synthesis of fluorophore- and spin-labeled RNA using deoxyribozymes

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Studying the multifaceted functions of non-coding RNAs by biophysical techniques such as electron paramagnetic resonance (EPR) spectroscopy and fluorescence measurements requires the site-specific installation of spectroscopic labels. Several synthetic approaches are known to directly incorporate modifications via solid-phase synthesis or install reporter groups post-synthetically on pre-functionalized RNA. The investigation of longer RNAs than routinely achievable by chemical synthesis demands the ligation of labeled RNA fragments, which is traditionally performed with (protein) enzymes T4 DNA ligase or T4 RNA ligase.

Here we report on an alternative approach for obtaining long labeled RNAs by employing DNA enzymes (deoxyribozymes) for the site-specific attachment of reporter groups, as well as for the efficient ligation of labeled RNA fragments. Deoxyribozymes are artificial single stranded DNA molecules that are identified by in vitro selection and serve as useful tools for practical applications. Synthetically beneficial DNA enzymes require only metal ions (usually Mg^{2+} or Mn^{2+}) as cofactors for their catalytic activity, function at neutral pH and can easily be isolated and reused for multiple reactions. For the site-specific, posttranscriptional labeling of RNA, we capitalized on a DNA enzyme that has been shown to accept GTP as substrate to attach a single nucleotide to the branch site adenosine of a model RNA.[1] We have optimized this reaction based on our recent finding of lanthanide-assisted acceleration of DNA-catalyzed RNA ligations. In this report, we demonstrate the attachment of chemically modified nucleotide analogs that introduce bioorthogonal functional groups or directly install detectable probes at desired target sites (see Figure). In a general sense, this deoxyribozyme combines in one molecule the functions of target-site selection and catalysis of the labeling reaction. Guidelines for the general application of the deoxyribozyme-based labeling strategy are developed and the approach is exemplified for the synthesis of spin-labeled and fluorescently labeled *S*-adenosylmethionine (SAM)-binding riboswitch RNAs.

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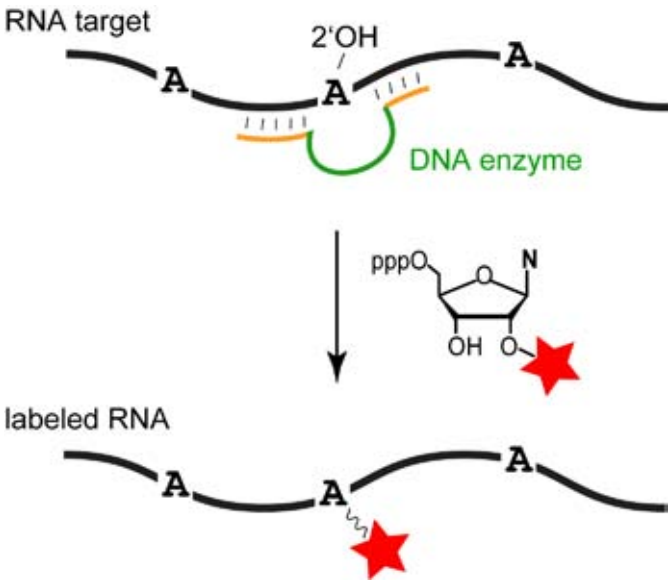

299 C Spin-labeled cytidine nucleotides in long synthetic RNAs: solid-phase synthesis, post-synthetic labeling, ligation and EPR-spectroscopic characterization

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Chemically stable nitroxide radicals that can be monitored by electron paramagnetic resonance (EPR) spectroscopy can provide information on structural and dynamic properties of functional RNAs, such as for example riboswitches. Since natural RNA is diamagnetic, the site-specific installation of nitroxide spin-labels is required. A number of post-synthetic strategies have been established for the attachment of nitroxide spin labels at various sites in pre-functionalized RNA, but advanced EPR techniques and instrumentation demand the development of new spin-labeling approaches for RNA. Challenges that need to be addressed include the synthesis of rigid spin labels and the preparation of long spin-labeled RNA by combined chemical and enzymatic strategies.

We highlight the synthesis and application of the nitroxide-containing nucleoside C¸m, reported as the first rigid spin label for paramagnetic modification of RNA by solid-phase synthesis[1,2] and the convertible nucleoside approach that enabled the direct attachment of TEMPO and proxyl spin labels at the exocyclic N^4-amino group of cytidine and 2'-*O*-methylcytidine nucleotides in RNA.[3,4] Recent results on the synthesis of long spin-labeled RNAs will be discussed, based on using deoxyribozymes as alternatives to protein enzymes for ligation of spin-labeled RNA. To obtain site-specifically labeled long riboswitch RNAs beyond the limit of solid-phase synthesis, we report the ligation of paramagnetic RNA using an in vitro selected deoxyribozyme as catalyst, and demonstrate the synthesis of TEMPO-labeled 53-nt SAM-III and 118-nt SAM-I riboswitch domains (SAM = *S*-adenosylmethionine).[4]

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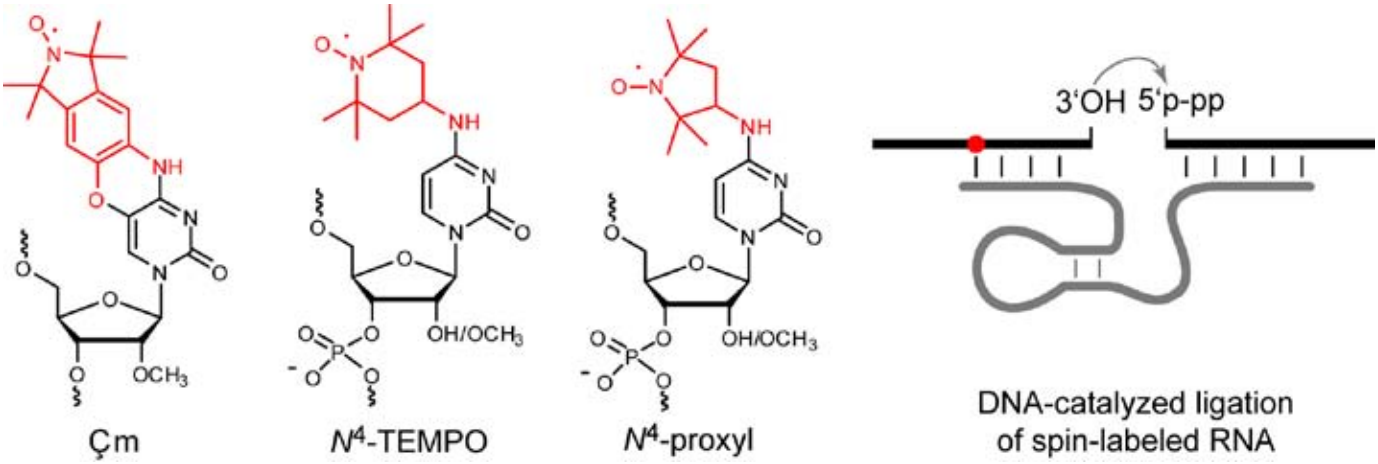

300 A Inhibition of Bcl-2 using photo-crosslinking antisense oligonucleotides

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[1]Kyoto Institute of Technology

Oligonucleotide analogues forming covalent bonds with complementary nucleotides in a sequence-specific manner under physiological conditions are of potential clinical and biological interest. In particular, photoresponsive oligonucleotide analogues which cross-link with complementary nucleotides using photo-irradiation as a trigger of the reaction have been developed to investigate and control gene functions without damaging living systems. These oligonucleotide analogues have photoresponsive moieties introduced on a site which does not affect duplex or triplex formation. The introduced photoresponsive moieties, which are non-reactive or less reactive toward bio-molecules in their original form, generate highly reactive species after photo-irradiation. We have reported that oligonucleotides having a psplaren derivative (Ps) at the 2'-O hydroxy group of adenosine (2'-Ps-eom) recognize one base difference in the target sequences under clinically relevant conditions. Using 2'-Ps-eom, we successfully achieved the inhibition of K-ras-immortalized cell proliferation (K12V) but not of Vco cells that contain the wild-type K-ras gene. Considering the potential benefits of photoresponsive cross-linking reagent-nucleotide conjugates, it is important to develop new photoresponsive groups that could give high yields and exhibit specific reactive characteristics to target nucleobases located in specific positions in the target sequence. In this study, we newly synthesized photo-cross-linking ODNs having a photoresponsive a-chloroaldehyde (PCA) group at the 5'-end of the ODN. The PCA group was comprised of an a-chloro bis(2-nitrobenzyl)acetal group, which was converted to an a-chloroaldehyde group after 1 min of UV irradiation. Photo-cross-linking studies revealed that the oligonucleotide conjugates underwent sequence-selective cross-linking to target nucleotides in a time-dependent manner under physiological conditions.

301 B Translational regulation by ligand-inducible formation of RNA pseudoknot

Saki Matsumoto[1], *Changfeng Hong*[1], *Asako Murata*[1], *Kazuhiko Nakatani*[1]
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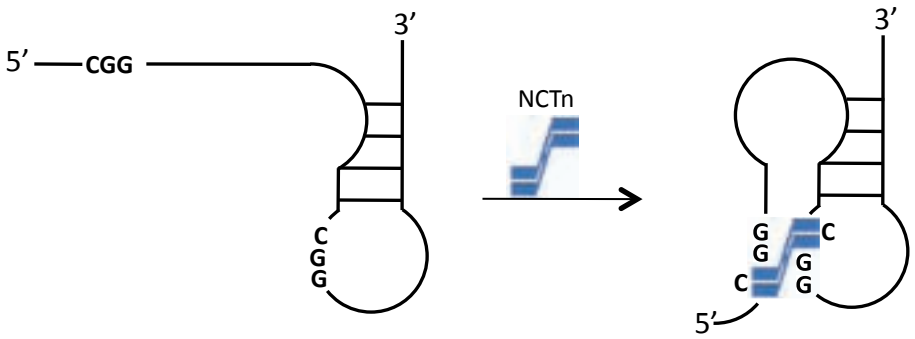
RNA secondary structures play crucial roles for noncoding RNAs in catalyzing biological reactions, controlling gene expression, responding to cellular signals, and so on. A small molecule that binds specifically to target RNA and induces a particular secondary structure might be a promising tool for gene regulation and gene therapy. We have developed a series of synthetic small molecules that can specifically bind to G-G mismatches in double-stranded DNA. Naphtyridine carbamate tetramer (NCTn), one of such mismatch binding molecules, consists of four naphthyridine units connected by flexible methylene linker -$(CH_2)_n$- and selectively binds to the CGG/CGG triad in dsDNA by forming hydrogen bonding with guanine bases. Our previous study has shown that NCTn can bind to $(CGG)_n$ sequence in single-stranded region of RNA and act as a molecular glue for promoting duplex formation from two single strand. Here we demonstrated that NCTn can induce a pseudoknot structure in RNA by the simultaneous binding between the loop region and the single-stranded tail as illustrated in Figure and furthermore, ligand-inducible formation of RNA pseudoknot can regulate translation of target gene by frame shifting.

We used VPK pseudoknot, which causes ribosomal frameshifting in mouse mammary tumor viruses, as the structural template for engineering to NCTn-inducible pseudoknots. Ribosomal frameshifting is the translational recoding mechanism used in many viruses, which is promoted by a heptanucleotide slippery sequence and an adjacent mRNA secondary structure, most often an mRNA pseudoknot. The ribosome is stalled at a pseudoknot and forced to shift one nucleotide backwards into an overlapping reading frame and to translate an entirely new sequence of amino acids. We designed NCTn-inducible frameshifting system by introducing a potential binding site (CGG/CGG triad) for NCTn in VPK sequence (mmVPK) located between the initiation codon and the firefly luciferase gene. The frameshifting efficiency increased by NCTn-induced pseudoknot folding can be detected by increase in luciferase activity. To evaluate the effect of NCTn on translation of the firefly luciferase gene, *in vitro* protein synthesis assay was performed. In the presence of NCT8, frameshifting efficiency gradually increased in a concentration-dependent manner, whereas only slight increase of frameshifting efficiency was observed without NCT8. Similar results were obtained for other NCTn ligands. These results suggested that NCTn could induce pseudoknot formation and regulate frameshifting efficiency. The detail of NCTn-induced frameshifting will be discussed.



302 C Targeting secondary structures of pre-miRNA by small molecules: Development of potential inhibitors of pre-miRNA processing

Asako Murata[1], Ayako Sugai[1], Takeo Fukuzumi[1], Shiori Umemoto[1], Chikara Dohno[1], Kazuhiko Nakatani[1]
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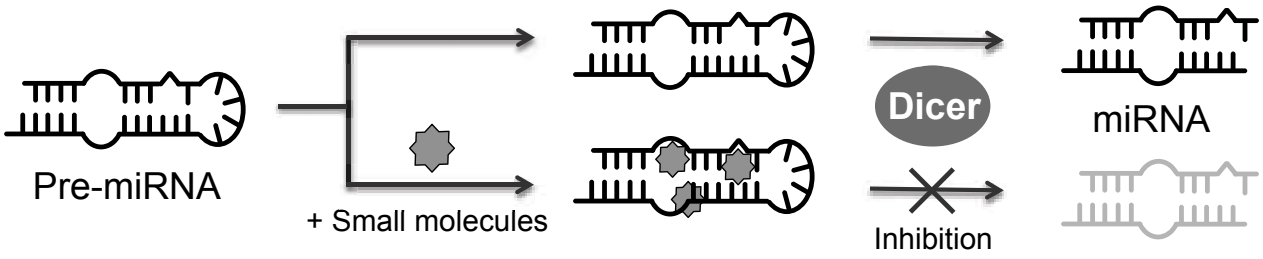
MicroRNAs (miRNA) are involved in many biological processes including development, differentiation and carcinogenesis through translational repression by binding to a target mRNA. Inhibition of miRNA pathways by altering miRNA expression and/or maturation in cells would modulate gene expression, and enable us to understand miRNA regulatory effects on various biological processes. Anti-miRNA oligonucleotide (antagomir) is the most readily available tool to knock-down the expression of an endogenous miRNA, and thereby perturb miRNA-mediated gene regulation in a sequence specific manner. In addition to the antagomir approach, a small molecule that bind to precursor-miRNA (pre-miRNA) and inhibit Dicer-catalyzed pre-miRNA processing will provide other options for modulating miRNA-mediated gene regulation. Several groups have shown that small molecules such as aminoglycosides, peptides, and peptoids would be potential inhibitors of Dicer-catalyzed pre-miRNA processing.

We have previously reported the synthesis and structure-activity relationships of xanthone and thioxanthone derivatives as the fluorescent indicators for detecting the interactions between RNA and small molecules [*J. Am. Chem. Soc.* 2010, 132, 3660., *Chem. Eur. J.* 2012, 18, 9999.]. Some of the 2,7-disubstituted xanthone and thioxanthone derivatives preferentially bind to certain secondary structures of RNA such as loops and bulges rather than double-stranded regions. Since most pre-miRNAs have such secondary structures, we explored a possibility of inhibitory activity of the xanthone and thioxanthone derivatives against the dicing reaction upon their binding to pre-miRNA. We herein report that an aminoalkoxy-substituted thioxanthone derivative interferes Dicer-mediated processing of pre-miRNA (the figure below). Chemically synthesized pre-miR-29a was digested by human recombinant dicer in the absence or presence of xanthone and thioxanthone deriviative, and the resulting products were analyzed by denaturing PAGE. X2SS, one of the thioxhanthone derivatives we synthesized, effectively suppressed the formation of both the intermediate and mature miR-29a, indicating the inhibitory effect of X2SS on pre-miR-29a processing. Analysis of the dicing reaction of pre-miR-29a mutant revealed that binding of X2SS close to a cleavage site is capable of interfering the processing of pre-miR-29a. Information about the interaction between these xanthone derivatives and pre-miRNAs will enable us to design and develop new small molecule-based inhibitors for miRNA pathway.



303 A Suppression of miR-29a maturation by ligand binding

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In recent years, the discovery of functional non-coding RNA (ncRNA) has raised the interest to modulate the function of ncRNA molecules. Small molecules that bind to specific secondary structural motifs in RNA will provide valuable tools for modulating and studying RNA function.

We have developed a series of naphthyridine derivatives that can bind to single nucleotide bulges in DNA duplexes. Among these derivatives, *N,N*-bis(3-aminopropyl)-2,7-diamino-1,8-naphthyridine (DANP) was found to bind to cytosine and guanine bulges in RNA duplexes. The stabilizing effect of DANP on the complex with bulged RNA duplexes, however, was rather smaller than that observed for bulged DNA duplexes. The results of molecular modeling of RNA-DANP complex indicate that stacking interaction of DANP with the neighboring bases in the RNA duplex would be insufficient compared with that of DNA-DANP complex, which explain the reduced ability of DANP for stabilizing the RNA-DANP complex. We therefore designed BzDANP that has the same hydrogen-bonding surface as that of DANP but has an expanded aromatic plane in order to examine the effect of the molecular size and the π-stacking with the neighboring base pairs on binding affinity with RNA duplexes.

In this study, we describe the synthesis and the evaluation of binding capability of BzDANP to RNA duplexes containing a single nucleotide bulge. BzDANP was synthesized from 2-amino-7-bromo-isoquinoline as a starting material in five steps. The binding ability of BzDANP to RNA duplexes containing a single bulge was evaluated by thermal melting studies. A significant increase in melting temperature with RNA duplexes containing a single bulge was observed in presence of the BzDANP, suggesting the increased stabilizing effect of BzDANP on bulged RNA duplex relative to DANP. Next we explored a potential inhibitory of BzDANP against the dicing reaction of precursor-miRNA (pre-miRNA) upon binding to its secondary structures. Pre-miR-29a has a cytosine bulge near the putative cleavage sites by Dicer, so we expected that the binding of BzDANP to the bulge would interfere the dicing reaction of pre-miR-29a. Chemically synthesized pre-miR-29a was digested with recombinant human Dicer and the reaction products were analyzed by denaturing PAGE. Dicer digested pre-miR-29a to produce multiple bands on the gel when stained with SYBER GOLD. The rapidly migrating bands were obviously mature miR-29a and star miR-29a, and the others are likely intermediates where anick was made at either strand of the stem. The band intensity of both mature miR-29a and intermediates were decreased with increasing concentration of BzDANP, suggesting the inhibitory effect of BzDANP on pre-miR-29a processing.

304 B Chemical Synthesis of 3′-Aminoacyl-tRNA Mimics to Investigate Antibiotic Induced Ribosome Stalling

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Nascent proteins leave the ribosome through the peptide exit tunnel. For decades it has been thought that this tunnel plays a passive role in protein synthesis. Yet there are interactions between the nascent peptide and the ribosome that can lead to ribosome stalling. Such translation arrest is used for regulation of gene expression. Recent studies showed that the nature of the donor and acceptor substrates for the peptidyl transfer reaction plays an important role in the mechanism of programmed translation arrest [1]. To clarify whether charge or size of the aminoacyl moiety of the substrates is important for translation arrest, amino acids – natural and unnatural – covalently bound to RNA via hydrolysis-resistant linkages are required. Starting from orthogonally protected 3'-deoxy-3'-azido adenosine we synthesize functionalized solid supports for automated oligonucleotide-peptide synthesis [2]. With those in hand, we have efficient access to 3'-deoxy-3'-amide-linked aminoacyl-tRNA mimics. These conjugates represent important substrates for ribosomal stalling assays to shed light on the mechanism of translation arrest.

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305 C Coumarin fluorochrome binds to Rev responsible element RNA with extremely large absorption shift

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Since the discovery of a role for RNA in modulating gene expression, functional RNA has become an attractive drug target. However, there is not much report of the synthetic molecules that bind to RNA. Because of the complicated conformation of RNA, it is difficult to design binding molecules only by the conventional molecular design. The development of ligands that bind selectively to a particular structure and sequence of RNA is useful to understand and modulate the function of RNA. In this study, we focused on the design and synthesis of novel ccoumarin derivatives that can bind to particular secondary structures of RNA. It is well known that C7-substituted coumarin derivatives with electron-donating group at the C7 position show strong fluorescence. In addition, further substitution with a electron-accepting group at the C3 position change their electron spectrum, which are characterized by such as red shift of absorption maximum and increase of fluorescence intensity. Considering these characters of coumarin, we envisioned that modification of coumarin at C3 and C7 positions enable us to observe binding of coumarin derivatives to RNA as a change in electronic spectrum.

We designed and synthesized a series of coumarin derivatives, in which piperazinyl group at the C7 position is introduced as a electron-donating group. The piperazinyl group would facilitate binding to RNA, due to its positive charge. Various functional groups, such as acetyl and benzoyl group, pyridine analogues, benzimidazole analogues, and 1,8-naphthyridine analogues is introduced to coumarin at the C3 position. Binding affinity of these coumarin derivatives to RNA were investigated by the absorption and fluorescence spectra in the presence of RNA. We used Rev responsible element (RRE), as the model RNA that consist of an internal loop and a terminal loop, and double-strand regions. 7-Piperadinylcoumarin, a control compound that does not have any functional group at the C3 position, showed only a small change of electronic spectrum when titrated with RRE. In contrast, apparent large red shift of absorption maximum from 398 nm to 460 nm was observed in the coumarin derivative having 2'-aminonaphthyridine at the C3 position (TT7) with one equivalent of RRE. To investigate the binding site of TT7 to RRE, we prepared three RRE-mutants. RRE-mutant 1 lacked both the internal and hairpin loops of RRE, RRE-mutant 2 lacked the hairpin loop, and RRE-mutants 3 lacked the internal loop. Large red shift of absorption maximum was also observed when TT7 was titrated with RRE-mutant 2 or 3. These results suggested that TT7 bound selectively to the hairpin loop and changed to absorption spectrum.

306 A Versatile phosphoramidation reactions for effective labeling and conjugation of nucleic acids

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Chemical conjugations of post-synthetic nucleic acids with macromolecules or smaller tag molecules are common approaches to study nucleic acids in chemistry and biology, and to exploit nucleic acids for medical applications. For example, conjugations of nucleic acids such as oligonucleotide siRNA and miRNA with peptides to acquire peptide-oligonucleotide conjugates (POCs) is especially useful to circumvent cell delivery and specificity problems of RNA as therapeutic agents. Recently, my laboratory has developed an aqueous-phase two-step nucleic acid phosphoramidation method for effective POC synthesis (Wang *et al.*, 2010,*Bioconjugate Chem.* 21, 1642–1655; Wang *et al.*, 2012, *Bioconjugate Chem*., 23, 2417-2433). Here we discuss the chemistry of nucleic acid phosphoramidation reactions and applications of the reactions for post-synthetic RNA conjugation and labeling essential to chemical and biological studies of RNA. When coupling with bio-orthogonal reactions such as click reactions, phosphoramidation reactions could be an even more powerful approach for nucleic acid conjugation and labeling. We are expecting that phosphoramidation reactions will have broader applications in conjugation and labeling of nucleic acids useful in fundamental research and in clinic.

307 B CACNA2D4: a novel paradigm for the application of antisense-mediated gene therapy to the cure of retinal dystrophies

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Retinal dystrophies are a large set of genetic diseases that lead to partial or complete blindness as a consequence of retinal degeneration. No available cures have been reported so far. Antisense RNA-based correction approaches have important advantages over the extensively investigated gene therapy approaches. In particular, they would allow expression at the natural site and under physiological circumstances. The use of appropriately engineered small nuclear RNA-U1 (U1snRNA) can induce therapeutic "exon skipping" and restore gene expression impaired by different types of mutations. We are currently developing a U1snRNA based therapeutic approach for the cure of Autosomal Recessive Cone Dystrophy 4 (RCD4, OMIM #608171), caused by a mutation in the L-type calcium channel accessory subunit CACNA2D4. We will test the feasibility of this approach on *in vitro* (minigene systems, patients derived cells) and *in vivo* (mutant Cacna2d4^{S4804X} mice) models. The results of our work will foster current knowledge on RNA-based therapies, on their safety and on their effectiveness in the treatment of retinal dystrophies.

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308 C ANTISENSE RNA-INDUCED EXON-SKIPPING FOR THE GENE THERAPY OF FRONTOTEMPORAL DEMENTIA AND PARKINSONISM ASSOCIATED WITH CHROMOSOME 17 (FTDP-17).

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Tau, encoded by a single gene on chromosome 17p21, is a cytoskeletal component expressed in the central nervous system (CNS), with a role in neurogenesis, axonal maintenance and axonal transport. A number of neurodegenerative diseases, including FTDP-17 (rare autosomal dominant condition), are characterized by intra-neuronal accumulation of the tau protein.

The genetic linkage between tau protein and FTDP-17 seems to be, for about half of the cases of FTDP-17, mutations affecting the alternative splicing of exon 10 (E10) of the tau mRNA. The aberrant inclusion of E10 in the tau mRNA, in fact, leads to the aggregation of tau as Neurofibrillary Tangles (NFTs) in brain

The project explores the feasibility of an antisense (as-) RNA-based gene therapy to correct tau splicing in FTDP-17.

We first tested whether it was possible to modulate E10 alternative splicing by the usage of Antisense Oligo-Nucleotides (AONs) that mask specific sites regulating splicing pathways.

RT-PCR and Western blot analyses showed that the transfection of specific AONs is able to alter the splicing behaviour of tau E10 in the rat endogenous transcript (PC-12 cell lines), with variable efficiencies depending on the concentration of the AONs and on the targeted sequence. The results were confirmed by transfection of Scramble Control Oligonucleotides that had no effects on E10 inclusion at the same concentration.

Based on these results, we constructed Adeno-Associated Viral (AAV) vectors coding for specific as-RNAs. We embedded the as-RNA sequences in chimeric U snRNA vectors whose promoters themselves lead to long-term as-RNA expression. We tested whether, the splicing behaviour of tau is corrected in endogenous rat mRNA by these chimeric antisense sn-RNAs.

To evaluate the effects of AONs/Chimeric Antisense-snRNA on the human tau pre-mRNA, we constructed a minigene reporter system, containing luciferase and that recapitulates to a large extent the behaviour of E10 in the context of the full-length tau gene. We carried out co-transfection into HeLa cells and evaluated the induction of E10 skipping by Luciferase Expression Assay, Real Time-PCR and Western blot analyses. Further work will be directed to test the therapeutic efficacy of the AONs and AAV-vectored as-RNAs in the animal model of FTDP-17 (T-279 mouse).

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309 A Messenger RNA as a novel therapeutic approach

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Moderna is pioneering messenger RNA Therapeutics™, a novel biotherapeutic modality with the unprecedented capability of stimulating the body's natural ability to produce therapeutic proteins. If successful in human clinical trials, this advance will usher in the first entirely new way of making therapeutic proteins since the development of recombinant technology more than 30 years ago, with dramatic implications for both patients and industry. Moderna is using this approach to develop first-ever treatments for a wide range of diseases that cannot be addressed today using existing technologies, and to drastically reduce the time and expense associated with creating therapeutic proteins using recombinant technologies.

Any protein biologic, antibody or vaccine can be rapidly made using mRNA. The technology is "cell-free", and alleviates the need for laborious and expensive *in vitro* tissue culture or protein manufacture and purification. A new messenger RNA can be developed very quickly, in only a matter of hours (when producing a known mRNA) to weeks (when going from concept to new drug), thereby saving precious time and R&D resources. Moderna has conducted proof of concept studies in numerous preclinical models, including non-human primates and has demonstrated the ability to induce *in vivo* production of dozens of intracellular and secreted therapeutic proteins through intramuscular, subcutaneous or intravenous administration. Pre-clinical programs have been established focused on four key therapeutic areas: oncology supportive care, inherited genetic disorders, hemophilia and diabetes. Results will be discussed.

310 B Identification of novel anti-obesity genes in primary human adipocytes using RNAi screening

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Obesity is an epidemic health problem worldwide. It is characterized by excess fat deposit in the adipose tissue. An increase in adiposity is associated with a constellation of disorders known as the metabolic syndrome including insulin resistance, type 2 diabetes, hypertension and atherogenic dyslipidemia that contribute to the increased risk for cardiovascular morbidity & mortality. A multidisciplinary research effort involving a combination of clinical, biochemical and omics approaches appears mandatory to increase knowledge in the complexity of biological traits, processes associated with obesity and to identify novel targets for drug discovery.

We sought to identify novel genes implicated in adipose fat reduction by affecting triglyceride hydrolysis and/or increasing the metabolic capacity of the adipocytes. These gene products directly or components in the signaling pathway(s) of these gene products can be used as drug targets for the development of novel targeted anti-obesity therapies.

We employed loss-of-function RNAi genetic screen using an adenoviral shRNA library to identify genes involved in the reduction of adipose mass. The adenoviral shRNA library was generated against a set of target human genes known to be modulated in human overweight, obese or metabolic syndrome subjects. shRNA adenoviral transductions were performed in primary human sub-cutaneous adipocytes and five different read-outs (lipolysis, lipid droplet formation, adiponectin secretion, mitochondrial function and gene expression) were monitored. This was followed by confirmation of specific shRNAs to induce the desired phenotype by in-depth experimental analysis. Furthermore, "hits" were substantiated by correlation with the clinical parameters. This strategy allowed for the identification of several new genes involved in adipose functions along with some genes reported previously in published studies. We have reason to believe that some of these newly discovered genes may represent potential novel anti-obesity drug targets.

311 C Inhibition of the HIV -1 virus RRE-Rev interaction by small molecule Rev mimics. A new synthetic scaffold for specific recognition of RNA structure.

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Functional and structured RNA motifs are not easily targeted by antisense agents and have the advantage of their high sequence and/or three-dimensional structure conservation. However, leaving aside natural products binding to sites within bacterial ribosomal RNA, the development of small RNA-binding agents has been hampered by the difficulties posed by these structures, which have limited physicochemical diversity and are often flexible. In order for this approach to be successful, it is essential to identify novel chemical scaffolds capable of specifically recognizing these motifs1. The Rev Recognition Element (RRE) is a strongly conserved 350 -nucleotide structure located in the *env* gene of human immunodeficiency virus type-1 (HIV-1) RNA. Within subdomain IIB of the RRE, the unusually widened major groove of a large 5:6 internal loop forms a high-affinity complex with the arginine-rich α-helix of the virally-encoded protein Rev, Rev34 -50. This interaction is essential for virus viability, as it triggers a cascade of events allowing the transport of unspliced or incompletely spliced viral RNA molecules to the cytoplasm of the infected cell. The RRE-Rev system offers unique advantages for drug design: the three-dimensional structures of the RRE-Rev34-50 complex and of unbound RRE and Rev are known. Making use of these advantages, we used structure-based methods to design small molecule mimics of Rev with a novel chemical scaffold. A set of these ligands was subsequently synthesized and tested *in vitro* and *in vivo*. Surface plasmon resonance and isothermal titration calorimetry experiments indicated that the compounds bound specifically to the RRE with an affinity of 5 μM. Nuclear magnetic resonance spectroscopy analyses showed that they occupied the binding site of Rev34 -50 in the major groove of the IIB loop, inducing an RNA conformational change strickingly similar to that detected upon Rev34-50 binding. Fluorescence polarization experiments indicated that these terphenyls were capable of inhibiting the RRE-Rev34 -50 interaction *in vitro.* Cellular assays demonstrated that the most potent inhibitors blocked HIV -1 replication *in vivo* and exerted this effect post- transcriptionally, as expected2. Most of the small RNA-binding agents described so far are related to peptides or antibiotics, or were discovered by screening. To our knowledge, this is the first time that a new organic scaffold with RNA-based activity has been designed *de novo.*

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312 A RNA aptamer C3 is a non-ATP site inhibitor of the MAP kinase ERK2

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Mitogen-activated protein kinases (MAPK) play a central role during signal transduction in eukaryotes. The ERK (extracellular signal-regulated kinase) MAP kinase pathway is of profound importance for cellular processes, such as proliferation and differentiation.

Since aberrant ERK signaling is a potent driver of oncogenesis in most human cancers, much attention has been focused on the development of inhibitors that target this pathway. The major class of available MAP kinase inhibitors is represented by so-called ATP analogues that inhibit phosphorylation of substrates and downstream signaling by competing with ATP in cells. Unfortunately, ATP-analogues often display poor specificity because the ATP-site is highly conserved amongst kinases. Thus, there is a high demand for the development of novel MAP kinase signaling inhibitors that enable selective inhibition by targeting kinase domains and individual sub-domains specifically.

Besides low-molecular weight ligands and antibodies, aptamers represent a promising class of molecules that can be applied extra- and intracellularly as high affine and specific inhibitors of biomolecules. Aptamers are short, single stranded nucleic acids that fold into a well-defined three-dimensional structure upon which they bind to a specific target molecule.

By performing an *in vitro* selection approach, we identified a RNA aptamer, namely C3, which bound to the MAP kinases ERK1 and ERK2 with high affinity and, furthermore, revealed high specificity, since no binding to other members of the MAP kinase superfamily or a panel of 10 other kinases was detected. More importantly, we were able to show that this aptamer interacts with ERK2 in an ATP-independent manner.

Aptamer C3 was identified as an inhibitor with an alternative and novel mode of action in respect of kinase activity perturbation. We could detect that the putative binding site of C3 on ERK2 includes the so-called "F-recruitment site" docking domain. By targeting a domain on ERK2 that is involved in regulating interactions with activators and substrate proteins, we were able to potently inhibit the activation of ERK2 by its activating kinase MEK1 *in vitro*. Future studies will be performed to investigate the aptamers potential to selectively block interactions of ERK2 with substrates.

Taken together, our data indicates that aptamer C3 binds to a physiologically relevant side on ERK and therefore could pave the way for the development of alternative acting and selective kinase inhibitors.

313 B mRNA transfection - a transient transgene expression in human mesenchymal stem cells as an attractive tool for regenerative medicine

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Introduction: Adult human bone marrow-derived mesenchymal stem cells (hMSCs) display a variety of beneficial properties. In stroke, therapeutic effects have been reported after the systemic delivery of hMSCs. It seems that a minimally invasive, intra-arterial route is an attractive method for stem cell transplantation to the injured brain. However, hMSCs lack the intrinsic mechanisms that make possible homing of the cells from the circulatory system to the area of infarction. It has been shown that genetic engineering can be effectively used for overexpression of certain molecules responsible for adhesion and transendothelial migration of systemically delivered cells. It is hypothesized that transient expression of integrins may be sufficient for promoting diapedesis and cell homing to the brain after intra-arterial delivery. Since hMSCs known for being extremely difficult to transfect using DNA plasmid vector, we investigated an mRNA transfection method for high efficiency expression of transgenes in hMSCs.

Methods: Human mesenchymal stem cells (hMSCs, PT-2501, Lonza) were cultured in a humidified atmosphere at 37°C and 5% CO_2 in an appropriate medium MSCBM (PT-3238, Lonza) supplemented with 10% MCGS (PT-4106E, Lonza), L-glutamine (PT-4107E, Lonza), and gentamicin sulfate (GA-1000, PT-4504E, Lonza). Cells were maintained in 75 cm^2 flasks but for transgene induction experiments were transferred to 24-well plates and seeded at a density of 15,000 cells/well. Plasmid DNA-eGFP (BD Biosciences) at a dose of 0.5 and 1.0 µg/well, and mRNA-eGFP (StemGent) at doses of 0.12, 0.25, and 0.5 µg/well were used. Four transfection agents, The Lipofectamine® 2000 (Invitrogen), TransIT-2020 (Mirus), TransIT ® - mRNA (Mirus) and Stemfect™ RNA Transfection Kit (StemGent) were tested. The transfection efficiency was assessed over 21 days using GFP fluorescent signal detection by confocal microscopy.

Results: Consistently with previous reports the efficiency for pDNA-eGFP transfections of hMSCs was very low, less than 1%. In contrast, mRNA-eGFP transfection resulted in an efficiency exceeding 95% in all of the tested conditions. mRNA-eGFP dose of 0.5µg/well and the use of Lipofectamine® 2000 was the most effective method for transgene expression, lasting up to three weeks.

Conclusions: The mRNA transfection is an attractive tool for inducing transient expression of transgenes in otherwise difficult to transfect hMSCs. Due to its non-integration nature mRNA is highly desirable gene delivery technique for clinical applications. This engineering method for hMSCs may open new opportunities in regenerative medicine.

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314 C Combined systemic and local morpholino treatment rescues the phenotype of the SMA Delta 7 mouse model

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Spinal muscular atrophy (SMA) is a childhood fatal motor neuron disease caused by mutations in the Survival Motor Neuron 1 (SMN1) gene, currently without effective treatment. One possible therapeutic approach is the use of antisense oligonucleotides (ASOs) to redirect the splicing of a paralogous gene, SMN2, to increase the production of functional SMN protein. A range of ASOs with different chemical properties is suitable for these applications, including a morpholino (MO) variant, which has a particularly excellent safety, and efficacy profile. We used a 25-nt MO oligomer sequence against the ISS-N1 region of SMN2 (HSMN2Ex7D(-10-34)) with superior efficacy to previously described sequences also in transgenic SMA Δ7 mice. The combined local and systemic administration of MO (bare or conjugated to octa-guanidine) is necessary to increase full-length SMN expression, leading to robust neuropathological features improvement and survival rescue. Additionally, several snRNA levels that are dysregulated in SMA mice could be restored by MO treatment. These results demonstrate that MO therapy is efficacious and can result in phenotypic rescue. These data provide important insights for the development of therapeutic strategies in SMA patients.

315 A RNA-i Therapy for Fronto Temporal Dementia and Parkinsonism Linked to Chromosome 17

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Abnormalities of microtubule associated protein tau have been shown to be linked to pathogenesis of neurodegenerative disease collectively termed as "Tauopathies. Gene mutations in tau lead to perturbation of gene splicing and tau fibrillization leading to formation of tau aggregates. A missense mutation in exon 10 at codon 279, results in an asparagine to lysine substitution (N279K). This impinges alternative splicing of exon 10 of the tau mRNA, and amends the normal ratio of 4Rtau/3Rtau,This in turn leads to an increased expression of 4R tau causing agglomeration of tau proteins.

RNA interference has proven to be an efficient strategy for silencing mutant alleles of dominant disease genes as in Alzheimer's disease, Machado-Joseph disease, Spinocerebellar ataxia type 3 and tau mutation (V337M) that causes fronto-temporal dementia.

This project explores the feasibility of a siRNA-based gene therapy to enable post-transcriptional gene silencing of Exon10 in FTDP-17. A panel of siRNAs targeting Tau exon 10 have been synthesised, which will be further tested upon PC12 cells and Ren cells. The outcome of RNA interference will be tested by both RT-PCR and western blot analysis. Based on these results, the small interfering RNA sequences will be embedded in siRNA expressing vector (psiUx) relying on strong and ubiquitous pol II dependent promoter of human U1 small nuclear RNA (U1 snRNA) gene. Allele specific silencing effects of these constructs will be monitored and analysed in PC12 cells. The effect on human tau pre-mRNA, will be monitored via a mini-gene reporter system, recapitulating to a large extent the behaviour of exon 10 in the context of tau gene.

Further work will be directed to test the therapeutic efficacy of the AAV-vectored sh-RNAs in the animal model of FTDP-17 (T-279 mouse) which recapitulates the disease from a histopathological and behavioural point of view. Preliminary data on the behavioural analysis will be presented.

316 B Studies on structure-function relationships of the snoRNP assembly machinery

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As well pre-rRNA processing as pre-mRNA splicing depends upon activity of ribonucleoprotein particles. These RNPs are generated by association of sets of proteins onto small non coding RNAs, such as snoRNAs, scaRNAs or UsnRNAs. Work from the recent years demonstrated that like spliceosomal UsnRNP assembly, the assembly of snoRNPs which are involved in pre-rRNA processing and that of scaRNPs that are involved in UsnRNA modification depend upon cellular factors required to increase the efficiency and accuracy of assembly. We and others have identified an important factor of the snoRNP assembly machinery, the Rsa1(yeast)/NUFIP(human) protein. This plateform protein associates with nascent RNPs by binding to the snoRNP RNA primary binding protein (Snu13 in yeast, 15.5K in human). Rsa1/NUFIP is able to bind to several other snoRNP core proteins and can recruit the R2TP complex a co-chaperon of the Hsp90 protein. The yeast R2TP complex contains proteins: Tah1, Pih1, Rvb1 and Rvb2. Tah1 forms a heterodimer with Pih1 and binds to Hsp90 by interaction with the Hsp90 C-terminal region. The idea is that by its numerous interactions with the core proteins and cellular factors, Rsa1 favors the specific recruitment of snoRNP proteins on the snoRNA and their remodeling, leading to stable mature snoRNPs. We focused our effort on understanding the mechanism of action and architecture of the C/D box snoRNP assembly machinery. We used yeast genetic approaches to determine the order of action of the cellular factors and identify their domains of interaction. In parallel, we developed structure-function analyses on components of the snoRNP assembly machinery based on NMR and other biophysical-chemical approaches. Studies on the R2TP-Hsp90 are as much important as this complex is involved in other assembly processes, in particular RNA polymerase assembly.

Although they play similar functions in snoRNP assembly, proteins Rsa1 and NUFIP show limited sequence homology. Only one 31 amino acid long sequence is strongly conserved. We showed that this peptide is crucial for the Rsa1p/NUFIP activity in box C/D snoRNP assembly. We identified an essential sub-fragment and solved its 3D structure by NMR. Then, by using the known 3D structure of yeast Snu13, we built a 3D model of the Rsa1-Snu13 interaction. We validated this model by biophysical methods and functional assays in yeast. In parallel, we purified Tah1 at a high degree of purity and established the 3D structure of free Tah1 and Tah1 bound to Hsp90. Our data explain how Tah1 specifically recognizes Hsp90. We characterized the interacting domains of Tah1p and Pih1p. We showed that Rsa1 bound to Snu13p can interact with another assembly factor Hit1 and also characterized the interacting domains. We will present the present stage of our functional and structural studies and their implication in terms of mechanism of action of the snoRNP assembly machinery.

317 C Lateral tRNA gene transfer in Methanobrevibacter ruminantium

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The increase of the available microbial genomes has enabled thorough comparative studies across multiple species. Substantial differences in gene content among closely related species in addition to the similarities found among distant species provide evidence of genetic information exchange. One well-known example is *Thermotoga maritima*, a thermophilic bacteria reported to contain 24% of genes that are homologs from archaea [1]. While multiple possible mechanisms may involve in the process of lateral gene transfer, most research have been solely focused on examining the origin of protein-coding genes. The extent of non-coding RNA gene transfer across species remains unclear.

During our study of *Methanobrevibacter ruminantium*, an archaeon that was isolated from bovine rumen fluid, we identified 59 tRNA genes as compared to 36 in its close relative, *Methanobrevibacter smithii*. A detailed inspection shows that 20 of these tRNA genes have a second copy with different sequence but same anticodon. While a small number of archaeal tRNAs has two copies in a genome, over 90% of them are single-copy genes. More unexpectedly, a copy of these 20 tRNA genes was found in a cluster within a region of 4000 bp. This represents about four times the size of the largest archaeal tRNA gene cluster. In addition, a tRNA-ArgACG that has never been found in archaea [2] was also identified in this cluster. Another round of gene prediction using bacterial tRNA model suggests that these 21 tRNA genes may have a bacterial origin. The 13% of bacterial homologs reported in a previous study [3] and some found upstream of the tRNA cluster further confirm the foreign insertion of this region.

Lateral gene transfer has been considered as a powerful evolutionary force for microbial genomes. However, the reasons and the biological significance of this event are not fully understood. Our finding demonstrates the first example of tRNA gene transfer between archaea and bacteria and highlights opportunities for new RNA biology.

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318 A Mitochondrial poly(A) polymerase is involved in tRNA editing

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Transcription of the mitochondrial genome results in long polycistronic precursors, which are processed mainly by endonucleolytic release of the tRNAs that are interspersed between rRNAs and mRNAs. Interestingly, in most metazoan mitochondrial genomes, genes for some tRNAs overlap with downstream genes encoded on the same strand: in the case of human mitochondria the genes for tRNATyr and tRNACys share one nucleotide. The processing of the precursor transcript releases a complete tRNACys whereas tRNATyr lacks the 3' terminal nucleotide, the so-called discriminator 1. This nucleotide has to be added before the tRNA can be matured by addition of the CCA end and subsequently aminoacylated. The enzyme responsible for this editing reaction has not been identified so far. Since the overlapping nucleotide is an adenosine, we tested whether the mitochondrial poly(A) polymerase (PAPD1) is involved in the reaction. We show that the tRNATyr lacking the discriminator is a substrate for PAPD1 *in vitro*. *In vivo*, knock-down of PAPD1 leads not only to a decrease of oligoadenylated tRNA species, but also to a decrease of mature tRNATyr carrying both the correct discriminator as well as the CCA end, whereas the number of tRNA species lacking the discriminator and the CCA end was increased correspondingly. Therefore, we suggest that PAPD1 is the enzyme responsible for the addition of the discriminator base.

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319 B High throughput quantification of tRNA function reveals unexpected interactions between tRNA residues

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Evolution has fine-tuned tRNAs to allow for their efficient and accurate participation in translation, and much has been learned about the structural and functional roles played by many specific residues. However, it is not known precisely how each residue and modification contributes to the structure and function of tRNAs; nor is it known how much tolerance tRNAs have to mutation. Here we report a high throughput method to comprehensively quantify the effects of every possible single mutation and of many combinations of mutations on the function of an individual yeast suppressor tRNA, $SUP4_{ochre}$ (*SUP4oc*). Application of this method resulted in the identification of several unexpected interactions in tRNA.

We generated a library of 95,000 *SUP4oc* variants in which all residues (except for anticodon residues N_{34}-N_{36} and residue N_{37}) contain random mutations at a 3% frequency. To quantify individual tRNA activity, we transformed the library into a strain carrying a *SUP4oc*-suppressable codon in *GFP*, whose expression can be compared to a control *RFP* (Dean, K. M. and Grayhack, E. J. (2012) *RNA* 18:2335-2344). Then, we sorted the *SUP4oc* transformants by fluorescence-activated cell sorting to separate cells into bins based on GFP/RFP expression, and analyzed the bins by recovering the *SUP4oc* genes and deep sequencing. A measure of the function of each variant tRNA is derived from the fractional representation of the sequence reads of the variant in each bin (Fowler, D. M., et al., (2010) *Nature Meth.* 7:741-746). Individual analysis of 35 variants shows that experimentally determined GFP/RFP values measured by flow cytometry correlate well with quantification of function by deep sequencing.

These experiments yielded surprising results. First, we detected *GFP* suppression in 137 of 213 possible single mutant *SUP4oc* variants, 75 of which display more than 50% of wild-type activity. This result suggests that there is remarkable tolerance in the tRNA sequences that are acceptable for function. Second, we found a number of double mutant variants that are unexpectedly active given the activities of their constituent single mutants, and whose function is not easily explained by simple compensatory effects of residues that interact. We individually reconstructed and tested 12 such double mutant variants, and found that 11 of them recapitulated the data from deep sequencing. We will discuss possible explanations for these unexpectedly active variants, as well as our progress using this methodology to explore other important questions in tRNA biology, including the comprehensive analysis of determinants for the rapid tRNA decay pathway.

320 C Gene expression analysis of nematode-specific tRNAs (nev-tRNAs) that can decode an alternative genetic code

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Transfer RNAs (tRNAs) are small RNA molecules that play a crucial role in protein biosynthesis as the links between the codons and the amino acids. Although translational fidelity is essential for protein and cell integrity, which is achieved by accurate tRNA aminoacylation, we have previously found unexpected nematode tRNAs (nev-tRNAs) that possess a glycine or isoleucine anticodon but can be charged with leucine (1). An *in vitro* translation analysis showed that nev-tRNAs can be incorporated into eukaryotic ribosomes and participate in protein biosynthesis, indicating that nev-tRNAs decode an alternative genetic code for leucine at least *in vitro*.

To understand the biological function of these unusual tRNAs, we first performed gene expression analyses of nev-tRNAs in *Caenorhabditis elegans* and *C. brenneri*, which has more number of nev-tRNAs than any other nematodes. The expression of nev-$tRNA^{Gly}$ (CCC) and nev-$tRNA^{Ile}$ (UAU) genes in mixed stage (egg, larvae 1-4, adult) of both species was detected by RT-PCR and northern blot analysis, but the levels are quite lower than those of general tRNAs. Further expression analysis during nematodes development and the results showed that the expression levels in egg and adult stages are higher than those of other stages. Taken together, our findings suggest that the expression of nev-tRNAs might be generally down-regulated, but stimulated in specific conditions and play a certain role, for example, in development. Now, we are constructing an over-expression system of heat-inducible nev-tRNA genes in *C. elegans* for multi-omics approach. Possible functions of this RNA molecule are discussed.

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321 A Structure and Kinetic Mechanism of Protein-only RNase P from A. thaliana

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Ribonuclease P (RNase P) catalyzes the maturation of the 5′ end of tRNA precursors. Typically these enzymes are ribonucleoproteins with a conserved RNA component responsible for catalysis. However, protein-only RNase P (PRORP) enzymes process precursor tRNAs in human mitochondria and in all tRNA-using compartments of *Arabidopsis thaliana*. PRORP enzymes are nuclear encoded and conserved among many eukaryotes. Here we report the crystal structure of PRORP1 from A. thaliana at 1.75 Å resolution, revealing a prototypical metallonuclease domain tethered to a pentatricopeptide repeat (PPR) domain by a structural zinc-binding domain. The metallonuclease domain is a novel high-resolution structure of a Nedd4-BP1, YacP Nucleases (NYN) domain that is a member of the PIN domain-like fold superfamily, including the FLAP nuclease family. In order to understand how this new family of metallonucleases function a kinetic mechanism is required. A minimal kinetic mechanism was derived from measuring steady-state, single-turnover, and binding kinetics. These data suggest a two-step binding mechanism, followed by cleavage of the phosphodiester bond with a maximal rate constant of 4 min^{-1}. Product release is faster than phosphodiester bond hydrolysis and is not the rate-limiting step under steady-state conditions. These studies allow for a molecular-level comparison of the catalytic strategies used by the only known naturally evolved protein and RNA-based catalysts that perform the same biological function, pre-tRNA maturation, thereby providing insight into the differences between the prebiotic RNA world and the present protein-dominated world.

322 B Biochemical Characterization of Archaeal RNase E-like Protein, FAU-1 in *Pyrococcus furiosus*

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Ribosomal RNAs (rRNAs) are important non-coding RNAs in all organisms. It is reported that precursor rRNAs (pre-rRNAs) are processed to mature rRNAs by various ribonucleases (RNases). RNase E is one of well-known endoribonucleases to process a pre-5S rRNA in *Escherichia coli*. However, in archaea, a processing enzyme of pre-5S rRNA has not been clearly understood yet. Previously, we identified an RNA-binding protein called FAU-1 that consists of 472 amino acids residues in the hyperthermophilic archaeon *Pyrococcus furiosus*. We showed that the N-terminal half of the FAU-1 had a degree of similarity (25%) with RNase E from *E. coli* (Kanai *et al*., 2003). In the current research, we detected an endoribonuclease activity of the FAU-1 protein.

First, the recombinant FAU-1 protein with a His_6 tag sequence was induced in *E. coli*. Then, we purified the FAU-1 to near homogeneity by a His-affinity column chromatography, followed by a RESOURCE-Q ion exchange column chromatography. Using the purified protein, it was tested whether the FAU-1 was able to process *P. furiosus* pre-5S rRNA *in vitro*. As a result, accumulation of the FAU-1-dependent cleavage was observed by Northern blot analysis, and the cleavage site can be mapped at approximately 10 bases upstream of the 5'-end of mature *P. furiosus* 5S rRNA. A cleavage site specificity by the FAU-1 was also examined using a short RNA probe (73 nt) partially representing the *P. furiosus* pre-5S rRNA. The result showed that FAU-1 preferentially cleaved the AU-rich sequences of the RNA probe. Next, to confirm that FAU-1 actually possessed the RNase activity, we constructed a plasmid encoding mutant FAU-1 lacking 170-189 amino acid residues that showed a high degree of amino acid similarity among closely related proteins in archaeal species. Consequently, the mutant protein markedly reduced the FAU-1-dependent cleavage against the pre-5S rRNA, showing that the FAU-1 is an endoribonuclease and possibly involved in the pre-5S rRNA processing.

In previous study, it is reported that 5' end of the pre-5S rRNA is processed by tRNase Z in archaeon *Haloferax volcanii*, and transfer RNA (tRNA)-like structure in the 5' end of pre-5S rRNA is necessary to recognize by tRNase Z (Hölzle *et al.*, 2008). However, such tRNA-like structure has not been found in most of archaeal pre-5S rRNAs. Our results suggest that the FAU-1 could provide an alternative pathway to process the pre-5S rRNA in archaea.

323 C Strong anion exchange fast performance liquid chromatography as a versatile tool for preparation and purification of RNA produced by in vitro transcription

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In vitro transcription is a common technique for producing large quantities of RNAthat requires exquisite control of a number of variables including template preparations, enzyme selection, transcription reaction conditions and purification protocols.

Here we demonstrate the use of strong anion exchange fast performance liquid chromatography (FPLC) as a simple, fast and robust technique for RNA production by *in vitro* transcription. With this method, we purified short transcription templates from unreacted reagents in large quantities; only minor changes to the protocol were necessary to readily obtain nuclease free pyrophosphatase. In addition, *in vitro* transcription reactions were monitored by strong anion exchange FPLC to enable facile optimization of reaction conditions. Transcribed tRNA was purified by strong anion exchange FPLC and the functionality of purified tRNA was confirmed by enzymatic assay. Every procedure described here required only minimum sample manipulation and was completed within 30 minutes.

324 A Identification of proteins co-purifying with the yeast RNA exosome and their effect on the complex stabilization

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The eukaryotic RNA exosome is a conserved multiprotein complex essential for processing, degradation, and quality control of a wide variety of RNAs. The complex is formed by a nine-subunit core that associates with two hydrolytic 3'-5' exoribonucleases. The involvement of the exosome in different RNA processing pathways suggests that protein cofactors are required for directing the complex to its substrates. We have previously characterized the functions of nuclear proteins that interact with the exosome and modulate its function in the pre-rRNA processing pathway, and are currently interested in identifying other proteins that might associate with the exosome and influence its assembly, stability and activity. Therefore, we have carried out protein co-immunoprecipitation experiments by means of the TAP method, followed by anion-exchange or size-exclusion chromatography. According to mass spectrometry analyses, the exosome core subunits were co-purified with the tagged subunits. These purified complexes are being used in complex stability analyses and RNA degradation assays to evaluate their *in vitro* activity. In addition, we are testing other purification conditions to investigate whether amino acid substitutions in one of the core subunits affect the ability of the exosome to interact with distinct cofactors.

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325 B tRNAscan-SE and GtRNAdb: Improving Detection and Functional Prediction Based on Genomic Context, Structure, and Expression of Transfer RNAs

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Transfer RNAs (tRNAs) are the largest, most complex non-coding RNA family that is universal to all living things. tRNAs are central to the flow of genetic information from messenger RNAs to proteins, and as ancient molecules, have acquired or retained a variety of other distinct functions in the cell (1, 2). The breadth and complexity of these functions are still coming into focus, but new hints are emerging from the study of genomic context, atypical structural variants, and differential expression/ChIP-seq patterns observed for various subsets of tRNAs. While there are now other useful, specialized tRNA detection methods available, tRNAscan-SE (3) and the Genomic tRNA Database (4) continue to be the most common source of gene predictions. Over the past decade, we have collected a large number of feature requests and algorithmic improvements that we are now actively implementing. These improvements will enhance the ability of tRNAscan-SE to more accurately identify and classify both typical and atypical tRNAs, identify partial tRNAs, and estimate biological relevance based on structural features, evolutionary conservation, and gene expression data. The GtRNAdb is being redesigned to integrate gene expression and other functional data, and automatically identify inconsistencies or unexpected features of full-genome gene sets. These analyses have already identified numerous outliers meriting closer study in vertebrate and microbial genomes. We present a variety of new observations from our work, including in-depth analyses of the human tRNA gene set.

We highly encourage tRNA researchers to contribute additional feature requests for tRNAscan-SE, as well as gene expression, population SNP variants, modification, and tRNA editing data to incorporate into the improved GtRNAdb (lowe@soe.ucsc.edu).

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3. Lowe, T.M. and Eddy, S.R. (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res*, 25, 955-964.
4. Chan, P.P. and Lowe, T.M. (2009) GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Res*, 37, D93-97.

326 C RIP-seq analysis of eukaryotic Sm proteins reveals interactions between snRNPs and mature mRNAs

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Sm proteins are a family of highly conserved RNA-binding factors present in all three domains of life. Sm proteins usually form hexameric or heptameric rings and associate with a variety of RNAs, and these RNP complexes perform many important roles in RNA metabolism. In bacteria, the Sm ortholog (called Hfq) associates with small (s)RNAs to regulate target mRNA stability and translation. In eukaryotes, Sm proteins bind small nuclear RNAs (snRNAs) to form snRNPs, which are basic components of the pre-mRNA splicing machinery. However, little is known about other functions of Sm proteins in eukaryotic cells, given their divergent roles in bacteria and archaea. Our lab recently found that Sm proteins are required for germ cell specification in *Drosophila*. This discovery led us to hypothesize that Sm proteins play important, but so far unrecognized, roles in RNA metabolism and fundamental cellular processes in eukaryotes.

To test this hypothesis, we developed a strategy to discover novel RNPs by deep sequencing immunopurified RNAs (RIP-seq) that associate with several distinct Sm proteins in *Drosophila* ovaries. Using this approach, we discovered, in addition to the known snRNAs, a highly reproducible subset of Sm-associated mRNAs and several novel, unannotated non-coding RNAs. One of the newly identified ncRNA is a bona fide snRNA, with a clearly recognizable Sm binding site and an snRNA-like secondary structure. We showed that the association between mRNAs and Sm proteins is independent of splicing, and distinct from the LSm1-7-mRNA interaction, which regulates mRNA degradation. Many of the Sm-associated mRNAs encode mitochondrial and ribosomal/translation-related proteins. We further verified these Sm-associated RNAs using RT-PCR on several different tissue/cell types.

Several lines of evidence suggest that Sm proteins associate with mRNAs indirectly. To characterize these RNP complexes, we performed immunoprecipitations with an anti-TMG cap antibody. Together with publicly available RIP-seq data targeting U1-70K (a U1 snRNP protein), we showed that Sm-associated mRNAs also co-purify with snRNAs and snRNP-specific proteins. Correspondingly, we identified potential snRNA base pairing interactions within these Sm-associated mRNAs. These data suggest that snRNPs interact with mature mRNAs directly through base pairing, raising an interesting similarity to the prokaryotic Sm (Hfq)-containing sRNPs.

To test whether this snRNP-mature mRNA interaction is conserved in evolution, we performed Sm RIP-seq in human HeLa cells. In addition to the spliceosomal snRNAs, we identified one of the histone mRNAs as highly enriched, confirming a previous report on U2 snRNP - histone mRNA interaction (Friend et al., 2007, Mol. Cell). We also identified many other mature human mRNAs associated with Sm proteins, suggesting that the snRNP-mature mRNA interaction is conserved in eukaryotes.

327 A Absence of a Universal Element for tRNAHis Identity in Eucarya

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Stringent discrimination between tRNA substrates by cognate aminoacyl-tRNA synthetase (aaRS) is essential for high fidelity gene expression. Identity elements within tRNAs aid this discrimination at a kinetic level. The identity element for histidyl tRNAs ($tRNA^{His}$), an extra guanosine at its 5'-end (G_{-1}), is essential for recognition and efficient histidylation by HisRS and its occurrence is conserved across bacteria, archaea and eukarya. In eukaryotes, the G_{-1} is acquired post-transcriptionally by an unusual 3'-5' addition reaction catalyzed by the essential Thg1 enzyme which was first identified in *S. cerevisiae*. Due to the critical nature of the G_{-1} identity element, the presence of a Thg1 gene is widespread in eukaryotes and until recently, no examples of eukaryotic histidyl-tRNAs that lack the essential G_{-1} had been identified.

We recently investigated several eukaryotes, including *Acanthamoeba castellanii* and *Typanosoma brucei* that lack any identifiable Thg1 homolog. Sequencing of tRNAHis isolated from these organisms revealed that they lack the universally conserved G-1 residue and thus appear to be exceptions to the general rule for tRNAHis identity. Due to the previously well-characterized dependence on the G-1 residue for recognition by HisRS, we tested the effect of G-1 on aminoacylation activity catalyzed by purified *A. castellanii* HisRS (AcHisRS) and *T. brucei* HisRS (TbHisRS) and observed that the presence of G-1 is not required for efficient histidylation by AcHisRS and TbHisRS. Yeast genetic complementation and steady state kinetic assays confirm that the recognition of tRNAHis by AcaHisRS at the cellular and kinetic level is truly independent of G-1 suggesting an alternate mechanism for tRNAHis recognition in comparison to the other eukaryotic HisRS. Hence, here we report the first instances of a tRNAHis/HisRS pair which do not conform to previously accepted dogma for tRNAHis identity and thus set the stage for further investigations of the co-evolution of this G-1 residue for tRNAHis identity, role of Thg1 enzymes in tRNAHis maturation and mechanisms for substrate recognition by HisRS.

328 B Thiolation of specific tRNAs by URM1 is required for efficient translation a subset of proteins by promoting ribosomal A-site binding

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Faithful protein synthesis relies on correct and efficient recognition of codons by tRNA molecules. To ensure this key role, tRNA molecules undergo many different modifications. In particular the wobble position, nucleotide 34, of the anti-codon is a hot spot for modifications and is thought to be functionally important for translation efficiency and fidelity. The wobble Uracil of $tRNA^{LYS}_{UUU}$, $tRNA^{GLU}_{UUC}$, and $tRNA^{GLN}_{UUG}$ is universally modified to 5-methyl-2-thio derivatives. Specifically, in eukaryotes these tRNAs bear the 5-methoxycarbonyl-methyl-2-thio modification (mcm^5s^2). Previous *in vitro* studies have implicated this modification in modulating the wobble capacity of these tRNAs, however the exact *in vivo* function of this modification has been largely unexplored. In *Saccharomyces cerevisiae*, the *URM1*-pathway is responsible for tRNA thiolation at the Uracil-34 and is important for resistance to various stresses such as nutrient starvation and oxidative agents.

In this study, we show that thiolation is not required for general translation but rather regulates translation of a subset of mRNAs. We performed a proteome wide analysis to identify changes in protein abundance induced by lack of thiolation. Subsequent bioinformatic analysis revealed that tRNA thiolation is required for the efficient translation of a subset of mRNAs rich in the cognate AAA, CAA, and GAA codons. Further analysis of selected candidates revealed that the decreases in protein abundance were not mediated by differential transcription nor degradation and therefore likely results from differential translation. Codon usage and translation regulation was assessed *in vivo* using a codon-specific fluorescent translation reporter in single cells. Indeed, we found that expression of translation reporters enriched for AAA, CAA or GAA codons was impaired in cells lacking *URM1*, while reporters enriched for the synonymous AAG codons showed wild-type expression levels. Furthermore, *in vitro* studies using native tRNAs from yeast showed that thiolation enhances ribosomal A-site binding and thereby increases peptide bond formation rates.

Taken together our data, show that tRNA thiolation at the wobble position is important to control expression of a subset of mRNAs rich in AAA, CAA, and GAA codons and that this is mediated by increased A-site binding to the ribosome. We suggest a model in which tRNA modification regulates expression of a subset of mRNAs enriched for specific codons under stress conditions.

329 C A comprehensive analysis of the natural variation of tRNA modification in two Saccharomyces species

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Transfer RNA (tRNA) is essential for protein synthesis by linking messenger RNA (mRNA) codons to the respective peptide sequence of proteins. Notably, tRNA molecules are decorated by a plethora of chemical modifications of their nucleobases or sugar backbone. Many of these RNA modifications are conserved throughout evolution, implying their importance. However, little is known about their *in vivo* function and whether RNA modifications are regulated. Furthermore, to date there has not been a single comprehensive study that analyzes the variation of tRNA modification in any species at the population level.

During evolution, yeast has adapted to grow in many ecological niches enabling it to cope with a wide range of external stress factors. This has led to a specific adaptation of cellular metabolism to very specific types of stress in different yeast strains. To elucidate how tRNA modification systems have evolved to modulate cellular adaptation to stress, we set out to analyze modified tRNA nucleosides in a large set of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* strains isolated from various environmental habitats. By means of two-dimensional thin layer chromatography (TLC) we have performed tentative characterization of basal level tRNA modification at logarithmic growth conditions for a total of 73 *Saccharomyces* strains.

Our preliminary screen shows variance in tRNA modification, allowing us to cluster strains according to their tRNA modification levels. We observed considerable variability of tRNA modification levels in *S. cerevisiae*strains. Surprisingly, this variability was much lower in *S. paradoxus* although the genetic diversity of the strains used in our analysis was larger than that of *S. cerevisiae*. Furthermore, mapping the strains according to single nucleotide polymorphisms (SNPs) in tRNA modification genes yields a similar clustering. This might imply, that key SNPs are essential in modulating basal level tRNA modification.

We are currently verifying our analysis using high performance liquid chromatography (HPLC) coupled with mass spectrometry to define the first comprehensive, quantitative high-resolution inventory of tRNA modification in representative *Saccharomyces* populations. Utilizing this approach will allow us to generate the first species wide analysis of tRNA modification.

330 A A Protease that Cleaves the C-terminal Domain of the RtcB-type RNA Ligase in the Hyperthermophilic Archaeon Pyrococcus furiosus

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RNA ligase is an enzyme that can catalyze the ligation of RNA molecules through phosphodiester bond formation. So far, three heat-stable RNA ligases are known in the hyperthermophilic archaeon, *P. furiosus*. The three enzymes are: (1) a putative 2', 5' RNA ligase (PF0027 protein), (2) an RtcB-type RNA ligase (PF1615 protein) and (3) a T4-type RNA ligase (PF0353 protein). The RtcB-type RNA ligase is reported as the missing component in tRNA maturation and the enzyme is able to ligate spliced tRNA halves into mature-sized tRNAs (Englert, M. *et al. PNAS* 2011).

Using purified recombinant RtcB RNA ligase as a substrate, we detected a protease activity in the *Pyrococcus* whole-cell extract that specifically cleaved the C-terminal portion (approximately 5 kDa) of the RtcB RNA ligase. No cleavage of the other two RNA ligases was observed. Protease inhibitors suggested that the responsible enzyme was a chymotrypsin-like serine protease, and gel filtration analysis of the whole cell extract showed that the protease activity was eluted at approximately 400 kDa. Moreover, gel filtration combined with western blotting analysis showed that *Pyrococcus* RtcB ligase formed a protein complex in the cell, and Blue-native PAGE analysis also suggested that the purified *Pyrococcus* RtcB RNA ligase formed a homo-oligomer complex. Since the RtcB RNA ligase lacking the C-terminal portion was able to form the homo-oligomer complex, the C-terminal portion was suggested to be not involved in the oligomerization activity. Meanwhile, the expression of the recombinant RtcB RNA ligase lacking the C-terminal portion became lower in*Escherichia coli* and the purified enzyme lost the RNA ligation activity. Our results suggest that a protease in *Pyrococcus* can cleave the RtcB RNA ligase and regulate its activity.

331 B Human mitochondrial RNase P and its multiple faces

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The mitochondrial genome is transcribed as long polycistronic precursor RNAs, encoding a complete set of tRNAs interspersed among rRNAs and coding RNAs. The processing of the tRNAs determines the concomitant release of all the RNAs required for mitochondrial protein synthesis. We have previously identified the human mitochondrial RNase P, the endonuclease responsible for 5' processing of tRNAs, which consists of three protein subunits. Moreover, we have shown that two of these subunits of RNase P constitute the methyltransferase responsible for methylation of purines at position 9, a modification supposed to be crucial for the proper folding of tRNAs. The human mitochondrial enzyme is unusual because of its ability to methylate both A and G at position 9 of tRNAs. Furthermore, in contrast to related methyltransferases, it includes as subunit a short-chain dehydrogenase, involved in the degradation of fatty and amino acids and thus with no obvious connection to tRNA maturation and function. The tRNA cleavage, tRNA methylation and dehydrogenase activities, physically associated in human mitochondrial RNase P complex, are nevertheless uncoupled and independent from each other. Thus, human mitochondrial RNase P is a multifunctional complex gathering diverse enzymatic activities related to tRNA maturation and beyond

332 C Playing RNase P evolution: replacing a complex ribonucleoprotein enzyme with a single protein

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RNase P is the endonuclease that removes 5' extensions from tRNA precursors. Despite their uniform and rather simple functional role, members of the RNase P enzyme family are of exceptional diversity. In their more ancient and widespread form, RNase P enzymes are ribonucleoproteins (RNP) based on a structurally conserved RNA molecule forming their catalytic core. In bacteria, a single small protein associates with the catalytic RNA; in the nucleus of some Eukarya (e.g., animals and fungi), however, the RNP reaches considerable complexity by including a set of up to 10 different proteins. A fundamentally different form of RNase P is composed of a single 60-kDa protein termed "proteinaceous" RNase P (PRORP) and is restricted to Eukarya. First found in mitochondria and chloroplasts, PRORPs were recently also found to be responsible for nuclear tRNA maturation in plants and trypanosomatids. In contrast to the complexity of the RNP form of nuclear RNase P, PRORPs have generally remained "simple", with the notable exception of animal mitochondrial RNase P, which is a multi-enzyme assemblage.

The evolutionary constraints and driving forces underlying this bewildering diversity of RNase P enzymes remain largely obscure. Increased enzyme versatility with respect to substrate range was suggested to be a possible reason for the complexity of the nuclear RNP-form of the enzyme. However, the finding that some organisms use a single protein for nuclear RNase P function challenges this view and suggests that either a single protein is as versatile as a complex RNP, or the spectrum of biological functions of the two enzyme forms in their host organisms is different.

By genome engineering, we replaced yeast nuclear RNase P with PRORP3 derived from *A. thaliana*. A thorough phenotypical characterization of different RNase P-swapped strains did not reveal any fundamental difference in the ability to grow under diverse environmental conditions or in their overall fitness. This indicates that a single protein is able to fully fill the biological role of nuclear RNase P in yeast, which naturally is a 10-component RNP. Molecular analyses moreover revealed normal levels of tRNAs and no evidence for alterations in a variety of suggested non-tRNA substrates that were previously reported to accumulate in an RNase P-deficiency model. Our results suggest that there is no inherent need for or advantage of RNA-based catalysis or enzyme complexity to cope with (nuclear) RNase P function.

333 A tRNA maturation abnormalities connected to stress conditions and transcription deregulation

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The normal processing order for transfer RNA (tRNA) in yeast generally starts with cleavage of 5' leader sequence from primary tRNA transcripts; this is then followed by removal of the 3' trailer sequence and, finally, by pre-tRNA splicing. We identified an unanticpated tRNA species which migrated slower than the species commonly assumed to be the primary transcript. This aberrant RNA was observed in wild type strain when the cells were propagated on nonfermentable carbon source or at elevated temperature; the aberrant tRNA was also detected in *rex1Δ* strain which is defective in the 3'-5' exoribonuclease that participates in 3' end processing. Preliminary sequencing indicates that the aberrant tRNA species from wild type cells results from maturation defects at the 3' end rather than from Pol III transcription termination defects. Additional studies show that there is diversification of this processing step among the different tRNA families. We also studied tRNA processing in cells lacking Maf1, the general negative regulator of Pol III transcription. Interestingly, when*maf1Δ* cells were grown on nonfermentable carbon sources, they generated end-processed intron-containing tRNAs species which migrated faster than pre-tRNAs from wild type cells. The migration of the aberrant tRNAs and other intermediates detected from *maf1Δ* cells suggests that there is/are alternative pathway(s) for tRNA maturation or degradation in the absence of Maf1. We are in the process of conducting large scale sequencing of tRNAs isolated from *maf1Δ* cells.

334 B A Genome-wide Analysis to Identify Novel Genes Involved in tRNA Metabolism and Subcellular Trafficking

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tRNAs are major components of the cell's protein synthesis machinery. In addition to this essential role in gene expression, they also contribute to other diverse functions including protein degradation, apoptosis, cellular response to stress, and tumorigenesis. tRNAs are transcribed in the nucleus. After the removal of the 5' and 3' ends and the addition of CCA and some modifications, tRNAs are exported to the cytoplasm where they complete their biogenesis and fulfill their functions. In both yeast and vertebrate cells, the subcellular movement of tRNAs involves the initial export of tRNAs from the nucleus to the cytoplasm, retrograde nuclear import of cytoplasmic tRNAs, and re-export of the imported tRNAs back to the cytoplasm. Although tRNAs have been studied for decades, some major players in tRNA metabolism and subcellular movement remain unknown. For example, there is an unknown nuclear export pathway for intron-containing tRNAs in yeast. The overall aim of my research is to identify and characterize all the missing gene products involved in tRNA biology, using yeast, *S. cerevisiae*, as a model organism. My strategy is to conduct a genome-wide assessment of the impact of every gene upon tRNAs utilizing the yeast deletion and temperature-sensitive (ts) collections. To conduct this screen in a timely fashion, I've developed a rapid method for genome-wide analysis of small RNAs from strains in the mutant collections. This method implements three optimized techniques: a procedure for growing small yeast cultures in 96-deepwell plates, a fast procedure for small RNA isolation from the plates, and a sensitive nonradioactive Northern method for RNA detection (Wu *et al.*, in press). To date, 3168 mutants in the deletion and ts collections have been analyzed; several candidates that affect tRNA biology have been identified and verified. For example, deletion of *GLN3* causes defects in the early steps of tRNA biogenesis. We hypothesize that Gln3, a well-characterized nitrogen-responsive transcription factor, plays a novel role in pre-tRNAs processing. This study will uncover important factors that function in tRNA metabolism and intracellular trafficking, which will contribute to a better understanding of the complexity of tRNA biology.

335 C Expanded Function of Trans-Editing Domains To Edit Non-canonical Amino Acids Prevents Errors In Translation

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Aminoacyl-tRNA synthetases (ARSs) activate specific amino acids and attach them to cognate tRNAs for use in protein synthesis. ARSs often misactivate isosteric standard and non-proteinaceous amino acids, potentially resulting in errors in translation that could be detrimental to cell survival. In the case of prolyl-tRNA synthetases (ProRS), smaller Ala and similar sized Cys are misactivated together with non-standard amino acids 4-hydroxyproline, azetidine, and α-aminobutyrate (Abu). Thus, due to ProRS promiscuity, editing mechanisms have evolved to ensure fidelity in Pro codon translation. In many bacterial systems, a so-called "triple-sieve" editing mechanism is employed, which consists of the ProRS active site that discriminates amino acids based primarily on volume and size (i.e., the "coarse sieve"), the ProRS editing domain (INS) that hydrolyzes Ala-tRNAPro in *cis* (i.e., the "fine sieve"), and a single-domain INS homolog YbaK that clears Cys-tRNAPro in *trans*. The latter occurs via a novel mechanism involving substrate sulfhydryl side-chain chemistry (i.e., the "chemical sieve"). Although these mechanisms clear standard non-cognate amino acids, how non-canonical amino acids are prevented from misincorporation is unclear. Moreover, many different combinations of INS-like*cis* and *trans*-editing domains exist in bacteria. For example, the metabolically versatile bacterium *Rhodopseudomonas palustris* (Rp) encodes a ProRS containing a truncated INS domain that we have shown is catalytically inactive, in addition to two distinct, INS homologs YbaK and ProXp-x. The function of the latter is unknown. In this work, Rp ProXp-x was cloned and purified and shown to have only weak activity in preventing mischarging of Ala-tRNAPro and Cys-tRNAPro *in vitro*. Comparison of known crystal structures reveals that the putative substrate-binding pocket of ProXp-x is larger than that of INS, which suggests substrates larger than Ala are preferred. Indeed, we demonstrate here that ProXp-x shows robust editing of the non-canonical amino acid Abu, a metabolite involved in catabolic and metabolic pathways of numerous amino acids to which ARSs are exposed. Rp ProRS specificity for activation of Pro over Abu is only about 1,000:1, which strongly suggests that editing is required *in vivo*. Furthermore, Abu is mischarged onto tRNAs not only by ProRS, but also by ValRS and IleRS and ProXp-x also displays robust editing of Abu-tRNAVal. Taken together, these data suggest that Abu-tRNA editing by the *trans*-editing factor ProXp-x is likely to be a critical checkpoint to ensure high fidelity in codon translation.

336 A Characterization of Rbtf1 and its role in ribosome synthesis

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Ribosome biogenesis is essential for the translational capacity of a cell and its regulation is tightly linked to the rate of cellular growth. Synthesis of ribosomes is one of the most energy consuming cellular processes, as these macromolecular machineries are present in high abundance and built up of a complex structure of RNA and proteins. Ribosome biogenesis includes transcription of rRNA, expression of ribosomal proteins and the activity of ribosomal maturation factors, which are processing rRNA and assembling ribosomal particles. In human cells, the regulatory network upstream of ribosome synthesis appears very complex; yet little is known as to how the various nutrient and growth factor induced signaling pathways cooperate to regulate ribosome synthesis.

We have recently performed a genome-wide siRNA screen to identify the cellular repertoire of factors involved in human 40S subunit biogenesis. The screen identified a number of candidate proteins that control ribosome synthesis, including the uncharacterized, putative transcription factor Rbtf1. We show that Rbtf1 is essential for both 40S and 60S subunit production. Rbtf1 is a nucleoplasmic protein, which is not required for rRNA transcription by RNA polymerase I, but for nuclear maturation of ribosomal subunits. Based on these data, we are testing how Rbtf1 influences ribosome synthesis by its potential function as a transcription factor and how Rbtf1 could link upstream signaling pathways to ribosome biogenesis.

337 B When p53 senses faulty ribosomes: Induction of Tp53 correlates with enhanced expression of c-Myc target nucleolar proteins in Rpl11-deficient zebrafish

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Nucleolar proteins play critical role in rRNA processing and pre-ribosomal assembly within the nucleolus. Impaired ribosome biogenesis causes nucleolar stress that triggers a p53 signaling pathway. Studies in cell lines have identified RPL11 as a key mediator in this pathway because of its ability to directly interact with MDM2, the negative regulator of p53. Interestingly, RPL11 has also been shown to control the transcriptional activity of c-Myc, an oncoprotein that positively regulates ribosome biogenesis. Contrary to the cell-line based results, we have previously demonstrated that loss of Rpl11 activates the Tp53 pathway in zebrafish. To gain further insight into the mechanism of Tp53 induction in response to Rpl11 deficiency, we analyzed the level of c-Myc and several c-Myc target nucleolar proteins in Rpl11-deficient zebrafish. Quantitative RT-PCR revealed 3-5 fold upregulation of c-Myc and the majority of its target nucleolar proteins, including those that bind to MDM2 or modify p53, in Rpl11-deficent zebrafish. *In situ* hybridization showed an enrichment of nucleolar protein transcripts in the head region of the Rpl11-deficient zebrafish, where morphological abnormalities and *tp53* localization were more pronounced. Furthermore, a time-course analysis indicated that the overexpression of nucleolar proteins coincided with the activation of Tp53 response in the Rpl11-deficient zebrafish. Rpl11 deficiency also led to defective rRNA processing and decreased abundance of functional ribosomes in zebrafish, indicating an impaired ribosome biogenesis. Many nucleolar proteins analyzed in this study are known p53 modulators. Therefore our results suggest a model in which increased c-Myc activity upon Rpl11 loss-of-function stimulates synthesis of nucleolar proteins, which, in turn, may trigger a p53 response.

338 C Exploring translation in S. pombe using ribosomal profiling

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Ribosomal profiling is a powerful technique to measure translation genome-wide in growing cells. By comparing ribosome protected fragments to mRNA abundance, translation efficiency can be calculated for every translated mRNA. *Schizosaccharomyces pombe* has been extensively characterized at the mRNA level; however, much less is known about translational control. To address this question we have performed ribosomal profiling of a wild type *S. pombe* strain. Analysis of our data has allowed the identification of translation start sites, uORFs, potential frameshifting events and translation of annotated non-coding RNAs. In addition, we have estimated translational efficiencies and identified subsets of poorly-translated genes that may be subject to translational repression. We are currently performing ribosomal profiling of *S. pombe* cells during meiotic differentiation and under stress conditions to investigate how translation is regulated in response to environmental and developmental cues.

339 A Escherichia coli ribosomal protein S1 unfolds structured mRNAs onto the ribosome for active translation initiation

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Many bacterial mRNAs adopt structures in their 5' untranslated regions that modulate the accessibility of the 30S ribosomal subunit. Structured mRNAs interact with the 30S in a two-step pathway where the docking of a folded mRNA precedes an accommodation step. Here, we demonstrate that ribosomal protein S1 endows the 30S with an RNA chaperone activity that is essential for the docking, the unfolding of structured mRNAs, and the correct positioning of the initiation codon inside the decoding channel. The first three OB-fold domains of S1 retain all the activities of the protein on the 30S subunit. We show that S1 is not required for all mRNAs and acts differently on mRNAs according to the signals present at their 5' ends. Therefore, S1 confers activities to the ribosome that are adapted for a given mRNA creating heterogenous populations of ribosomal initiation complexes to selectively translate unstructured and structured mRNAs.

340 B Antibiotic-mediated frameshift during translation of the leader ORF reveals a new principle of gene regulation

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The antibiotic-inducible gene *ermC* encodes an rRNA methyltransferase that renders bacteria resistant to macrolide antibiotics. The *ermC* gene is preceded by a 19 codon-long regulatory ORF. In the absence of antibiotic, the secondary structure of the intergenic region occludes the ribosome binding site and the initiation codon of the methyltransferase gene impeding its expression. The currently known classic mechanism of antibiotic-mediated induction of *ermC* expression operates via nascent peptide dependent ribosome stalling. A ribosome with bound erythromycin, an inducing macrolide antibiotic, stalls at a strategic location of the*ermCL* ORF and triggers mRNA isomerization. The conformational switch frees the *ermC* initiation region, allowing a drug-free ribosome to initiate expression of the resistance gene. Ribosome stalling critically depends on the sequence of the leader peptide and on the structure of the inducing macrolide antibiotic which binds to the ribosomal exit tunnel. Interestingly, the new generation of macrolides, the ketolides, which also bind in the exit tunnel, are unable to direct ribosome stalling at *ermCL*. Yet, puzzlingly, ketolide telithromycin can induce expression of *ermC*. We have unraveled the mechanism of ketolide-mediated induction of *ermC*. The novel mechanism uncovers a novel principle of gene regulation based on readthrough of the stop codon of the regulatory gene and reveals the previously unknown ability of macrolide drugs to cause translational frameshifting.

The mechanism of ketolide-mediated induction of *ermC* operates via antibiotic-induced -1 frameshift that takes place at the two last sense codons of the *ermCL* leader ORF. The N-terminal amino acid sequence of the ErmCL peptide allows it to thread through the antibiotic obstructed exit tunnel without displacing the drug. Consequently, the ribosomes with bound antibiotic reach the end of the ORF. The 'slippery sequence' encompassing the two last sense codons of *ermCL* and the presence of the antibiotic in the tunnel are the prerequisites for the freameshift to occur. After switching to the -1 frame, ribosomes can translate the entire intergenic region without encountering any stop codons until they reach the beginning of the *ermC* gene. Unfolding of the mRNA structure by translating ribosomes or re-initiation of translation likely facilitate expression of the resistance cistron. Our findings reveal a new paradigm of gene regulation by programmed frameshift at the regulatory ORF and open new venues for development of superior antibiotics.

341 C High-resolution cryo-electron microscopy structure of the Trypanosoma brucei ribosome

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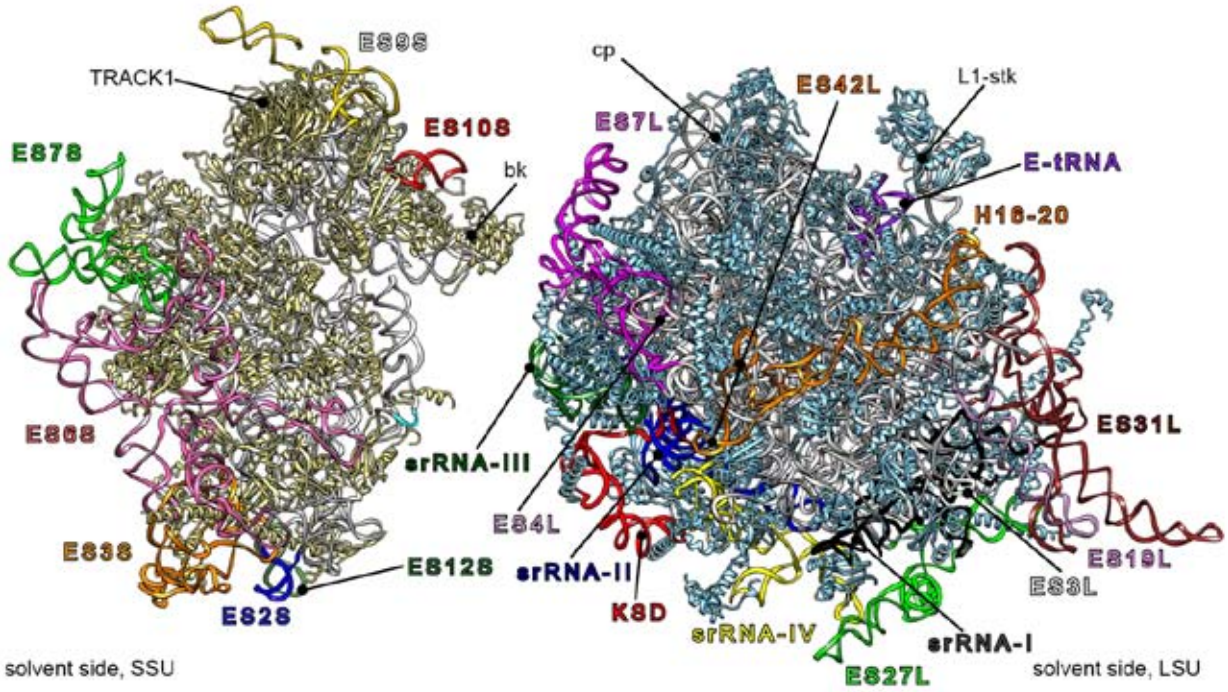
Ribosomes, the protein factories of living cells, translate genetic information carried by messenger RNAs into proteins, and are thus involved in virtually all aspects of cellular development and maintenance. The few available structures of the eukaryotic ribosome reveal that it is more complex than its prokaryotic counterpart owing mainly to the presence of eukaryote-specific ribosomal proteins and additional ribosomal RNA insertions, called expansion segments. The structures also differ among species, partly in the size and arrangement of these expansion segments. Such differences are extreme in kinetoplastids. Here we present a high-resolution (~5A) cryo-electron microscopy structure of the ribosome of *Trypanosoma brucei* (figure1 below), the parasite that is transmitted by the tsetse fly and that causes African sleeping sickness. The atomic model reveals the unique features of this ribosome (figure2 below), characterized mainly by the presence of unusually large expansion segments and ribosomal protein extensions leading to the formation of four additional inter-subunit bridges. We also find additional rRNA insertions, including one large rRNA domain that is not found in other eukaryotes. Furthermore, the structure reveals the five cleavage sites of the kinetoplastid large ribosomal subunit rRNA chain, which is known to be cleaved uniquely into six pieces, and suggests that the cleavage is important for the maintenance of the *T. brucei* ribosome in the observed structure. We discuss several possible implications of the large rRNA expansion segments for the translation regulation process and we show a possible link between the protein translation initiation process and expansion segments 6 and 7 on the small ribosomal subunit. The structure could serve as a basis for future experiments aimed at understanding the functional importance of these kinetoplastid-specific ribosomal features in protein translation regulation, an essential step towards finding effective and safe kinetoplastid-specific drugs.

1. Hashem *et al*., *Nature* 494, 385–389 (2013).



342 A Cryo-EM structure of the mammalian ribosomal 43S preinitiation complex

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Protein translation initiation in eukaryotes is a very complex process that involves a dozen of initiation factors and begins by the assembly of the 43S preinitiation complex. The process starts by the formation of the ternary complex (TC) from Met-tRNA$_i^{Met}$, eukaryotic initiation factor 2 (eIF2) and GTP. Then the TC along with eukaryotic initiation factors 3, 1 and 1A (eIF3, eIF1 and eIF1A respectively) cooperatively bind to the small ribosomal subunit subunit yielding the 43S preinitiation complex that is ready to bind the messenger RNA (mRNA) and scan for the initiation codon. Scanning on structured mRNAs in higher eukaryotes requires in addition the presence of DHX29, a DExH-box protein that also binds directly to the 40S subunit. The assembly of most of these factors on the 40S subunit, and the structure of the 43S complex more generally, remained so far elusive to structural studies and has been subject to numerous speculations. Here, we present the first cryo-electron microscopy structure of the mammalian DHX29-bound 43S complex (figure1 below). It reveals the assembly of the 43S complex and the binding sites of its different initiation factors including eIF3. Our structure reveals that eIF2 interacts with the 40S subunit via its alpha subunit and supports Met-tRNA$_i^{Met}$ in a novel eukaryotic P/I orientation (eP/I). The structural core of eIF3 resides on the back of the 40S subunit establishing a rather reduced interaction surface with the latter through two principal points of contact only, whereas DHX29 binds around helix 16. Although the assignment of eIF3 subunits is still controversial, our structure localizes at least three peripheral subunit of eIF3, thus representing the most complete structure of eIF3 to date. In addition, we discuss eIF3 binding on the 40S subunit in the context of different relevant complexes such as 40S-HCV IRES complex and the small ribosomal subunit from ribosomes of kinetoplatids, presenting extraordinarily large expansion segments. In conclusion, our structure provides insights into eukaryote-specific aspects of translation, including the mechanism of action of different initiation factors such as DHX29.

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3. Siridechadilok et al. Structural Roles for Human Translation Factor eIF3 in Initiation of Protein Synthesis. *Science* 2005
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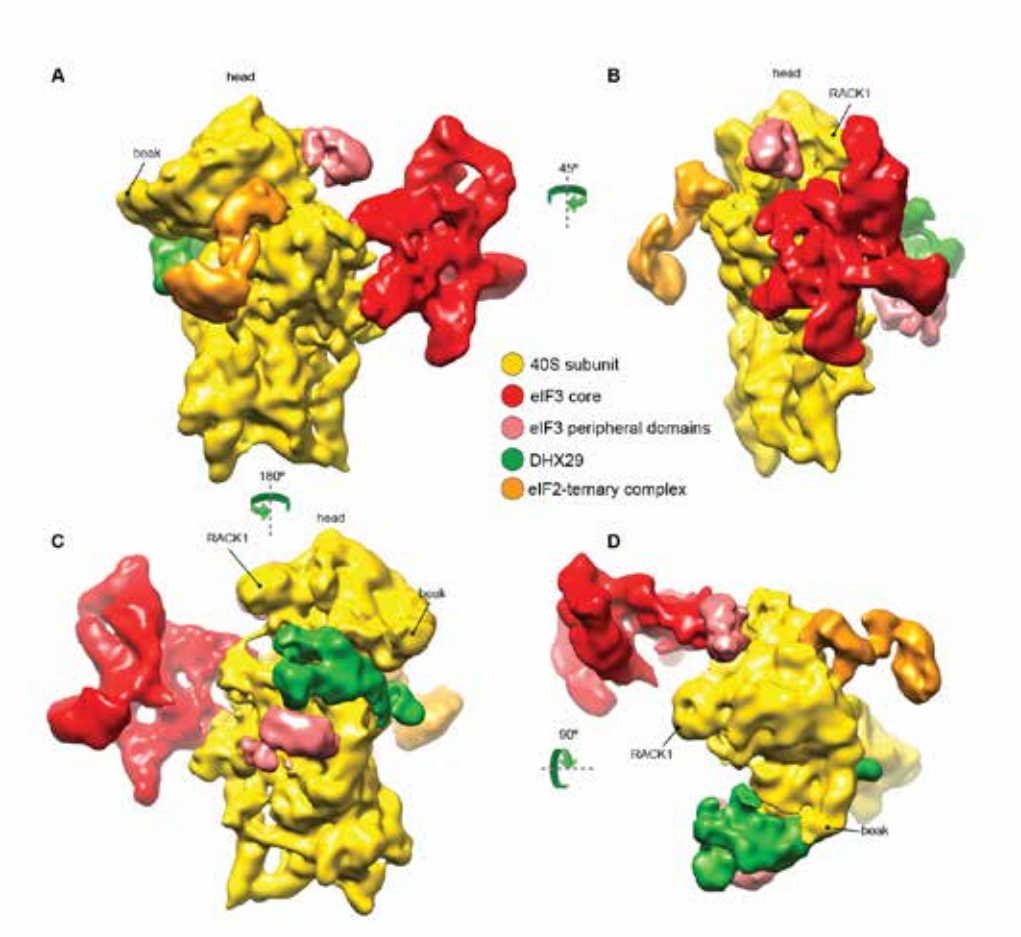

343 B Alu RNPs target 40S ribosomal subunits to repress translation initiation

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In primate cells, non-coding cytoplasmic *Alu* RNAs transcribed from the repetitive *Alu* elements form complexes, referred to as *Alu* RNPs, with the protein heterodimer SRP9/14. SRP9/14 is also a part of the signal recognition particle (SRP), a ribonucleoprotein complex, which plays a key role in protein translocation into the endoplasmic reticulum. The SRP RNA gene is the phylogenetic precursor of the *Alu* elements. We found that biochemically purified *Alu* RNPs inhibit translation initiation in rabbit reticulocyte lysate on capped and non-capped mRNAs in a dose-dependent manner whereas the protein and the RNA moieties alone had no effect. The identity of the RNA moiety in the *Alu* RNP was important for function. RNP particles assembled on *Alu* RNA derived from the SRP RNA gene failed to inhibit initiation. Those containing RNAs from the old*Alu* J and the younger *Alu* Sx families of *Alu* elements produced an intermediated phenotype whereas RNPs assembled on *Alu* RNA from the youngest Y family were the most efficient in translation inhibition indicating that this function was acquired during evolution. In the protein moiety of the *Alu* RNP, the presence of a positively charged domain composed of the C-terminal pentapeptide in SRP14 and three lysines in SRP9 was required to inhibit translation initiation. To investigate the mechanism of inhibition, we studied the effect of *Alu* RNPs on the *in vitro* assembly of different translation complexes. We found that *Alu* RNPs interfere with 48S complex formation by inhibiting the recruitment of the mRNA to the 43S complex. Moreover, *Alu* RNP repressed ribosomal complex assembly on the cricket paralysis virus IRES which occurs independently of any initiation factors, suggesting that *Alu* RNPs has a direct effect on 40S subunit. Consistently, we found SRP9/14 to be bound to 40S subunits upon their incubation with *Alu* RNPs. Our results describe a novel translational control mechanism in which *Alu* RNA ensures functional binding of SRP9/14 to the 40S ribosome and thereby prevents cap-dependent as well as IRES-mediated ribosome recruitment to the mRNA. This pathway might be activated in response to viral infection to interfere with IRES-dependent translation initiation.

344 C Interactions of modified oligonucleotides with RNA of the prokaryotic and eukaryotic decoding site

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Many known antibiotics that hinder protein synthesis in bacteria, target various functional regions of ribosomal RNA (rRNA). In principle, rRNA function can also be inhibited in a sequence-specific manner by using short oligonucleotides hybridizing with functional rRNA motifs. Indeed, some modified oligonucleotides were shown to hybridize with the RNA of bacterial ribosomes and inhibit the translation process[1]. Typically, the oligonucleotides are designed based solely on the sequence of rRNA, and the secondary and tertiary structures of the targeted rRNA motif are not taken into account. However, to increase the binding affinities of such oligonucleotides their interactions with RNA need to be fully elucidated, taking into account thermodynamic stability, structural and dynamical properties of the RNA target.

We have studied the binding of three S-DNA and 2'O-methyl-RNA decamers complementary to the models of prokaryotic and eukaryotic rRNA decoding sites (A-sites). We have used a structural model of the bacterial ribosomal target: the HX RNA construct[2] (PDB: 3LOA), which contains a fragment of the helix h44 of 16S rRNA.

The thermodynamics of binding was determined by the UV-monitored thermal denaturations and isothermal titration calorimetry (ITC) experiments. We have studied the specificity of the oligonucleotides, by testing the effect of one, two or three mismatches on the binding process. We have also performed 300 ns explicit solvent molecular dynamics (MD) simulations of both rRNA models to characterize the flexibility of the targeted rRNA structures. The fluctuations of the nucleotides indicate which fragments of the target are more susceptible to strand invasion by oligonucleotides. We compare the solution binding experimental studies with the computational predictions derived from the analysis of the MD trajectories. We focus on the relationship between the flexibility of the target fragment and the binding energy between the oligonucleotide and the rRNA.

In addition, we have compared the results obtained for model structures with *in vitro* studies employing whole ribosomal subunits in a cell-free transcription/translation system. We are looking for correlations between the physical parameters of binding of the oligonucleotides with their inhibitory efficiency on bacterial translation. Detailed study of the interaction between modified oligonucleotides and rRNA model structures is crucial for understanding the mechanisms of action of the therapeutic antibacterial oligonucleotide-containing compounds.

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345 A The intimate connection of RNA granules with human 4Es changes with use of different protein variants

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Three different eukaryotic translation initiation factors belonging to the 4E family are present in human cells. First, eIF4E1, which directs ribosome to the mRNA cap structure by recognizing it via residues Trp56 and Trp102. 4E1 initiates translation via interaction with the scaffolding protein eIF4G and is tightly controlled by 4E-binding proteins 4E-BP1,2,3. Second, eIF4E2, which possesses characteristic substitutions of Trp56 and Trp43 for tyrosines that cause weaker cap binding. In contrast to 4E1, 4E2 does not bind the scaffolding protein 4G, however, it is still able to bind 4E-BPs. Therefore, it is assumably unable to initiate translation and a regulatory role has been assigned to it instead. It establishes anterior-posterior body axis in *Drosophila* embryo by inhibiting caudal and hunchback mRNAs translation and drives embryonic patterning by inhibiting HoxB4 and HoxB8 mRNAs in mouse oocytes. Recently, 4E2 has been shown to play important role in a non-canonical translation initiation during cellular response to hypoxia. Last, 4E3 has the Trp 43 and 56 substituted for Cys or Tyr, which weakens its cap binding even more than that of 4E2. Contrary to 4E2, 4E3 is able to bind 4G but not 4E-BPs and its direct role in 5' mRNA cap recognition in translation initiation thus cannot be excluded. Each of the 4E1,2,3 proteins is present in human cells as several predicted variants arising from alternative mRNA splicing and alterations in transcription starts. We confirmed existence of some of them by cloning their cDNAs from human leukemic cells. To investigate subcellular distributions of the 4E1,2,3 protein variants corresponding to cloned cDNAs, we produced them as N-terminal GFP fusions in the human osteosarcoma cell line (U2OS). To our knowledge, subcellular localizations of only 4E1 and mouse 4E2 have been published till now. Contrary to strictly cytoplasmic distribution of mouse 4E2, we observed nuclear-cytoplasmic distribution of all the human 4E1,2,3 variants tested. Localizations of all the proteins were verified using antibodies against endogenous proteins. We were further interested whether all of the 4E1,2,3 variants form a part of RNA granules and thus could be involved in more general processes associated with mRNA turnover during normal conditions as well as under stresses. We found that during heat shock and oxidative stress both of the 4E1 variants tested localized to P-bodies (PB) and stress granules (SG). Contrary to that, all of the three 4E2 variants localized to PB only and 4E3 was not detected in any of those structures. Moreover, careful quantitative analysis of these data suggested different abilities to associate with PB between different protein variants of both 4E1 and 4E2 translation initiation factors. Our results thus indicate importance of alternative splicing for functional diversity of translation initiation factors belonging to the 4E family.

346 B Crystal structures of the protozoal cytoplasmic A site in complex with aminoglycosides

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Parasitic infections recognized as neglected tropical diseases are a source of concern for several regions of the world. Aminoglycosides are potent antimicrobial agents, which have been extensively studied by biochemical and structural studies in prokaryotes. However, the molecular mechanism of their potential antiprotozoal activity is less well understood.

In the present study, we have determined crystal structures of the protozoal cytoplasmic A site (aminoacyl-tRNA decoding site) in complex with aminoglycosides possessing a 6'-hydroxy group on ring I. They specifically bind within the deep/major groove of the protozoal cytoplasmic A site, where they displace two universally conserved adenines A1492 and A1493 (according to the numbering used in E. coli 16S rRNA), and stabilize the state called "on". Since two bulged-out adenines are involved in contacts with mRNA-tRNA complex, the binding of aminoglycosides to the protozoal cytoplasmic A site may lead to reduced discrimination of cognate tRNAs versus near-cognate tRNAs, thereby compromising translation fidelity.

Ring I of aminoglycosides with a 6'-hydroxy group stack on G1491, which is highly conserved in bacterial and protozoal species. In addition, ring I can form a pseudo pair with G1408 through two hydrogen bonds and one C-H...O bond. It is obvious from structural modeling that aminoglycosides with a 6'-ammonium group on ring I, such as gentamicin and kanamycin, cannot make the identical pseudo pair, because the ammonium group repels N1-H or N2-H of G1408. In fact, aminoglycosides with a 6'-ammonium group do not display antiprotozoal activity.

It is noteworthy that the secondary structure of the protozoal cytoplasmic A site is highly analogous to that of the bacterial A site with an A1408G mutation, which is the most prevalent antibiotic-resistant mutation found in clinical isolates. As evidence of this, the mutation is known to confer high-level resistance to aminoglycosides with a 6'-ammonium group but moderate resistance to those with a 6'-hydroxy group.

The structure information obtained in this study would contribute towards the structure-based design of next-generation aminoglycosides with high activities against parasitic protozoa and antibiotic-resistant bacteria.

347 C Rrp5 binds the pre-rRNA at multiple sites and is required for ribosomal processing and assembly

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The large, highly conserved ribosome synthesis factor Rrp5 has 12 S1 RNA binding domains and 7 TRP protein interaction domains, and is required for the early events in both 40S and 60S ribosomal subunit synthesis. In vivo complementation experiments show that the CTD of Rrp5 is required for pre-rRNA cleavage at sites A0-A2 on the pathway of 18S rRNA synthesis, whereas the NTD is required for A3 cleavage on the pathway of 5.8S/25S rRNA synthesis. Using the CRAC UV-crosslinking technique we identified multiple Rrp5 binding sites along the yeast pre-rRNA. The strongest interactions were seen between sequences flanking A2 and the CTD and between sequences flanking A3 and the NTD. The CTD also crosslinked to the U3, U14 and snR10 snoRNAs that are required for normal cleavage at sites A0-A2. In contrast, the NTD crosslinked to the RNA component of RNase MRP, which cleaves site A3. Mathematical modeling of kinetic labeling data and EM analyses both indicate that co-transcriptional cleavage at sites A0-A2 is not stochastic, but occurs during a window when the transcribing polymerase is ~1.5kb 3' to cleavage site A2 and within the 25S rRNA. Notably, a binding site for the Rrp5 CTD, which is required for A0-A2 cleavage, was found in the 25S rRNA region, suggesting that transcription through this Rrp5 binding site in 25S rRNA might set the timing for A2 cleavage. Dramatic support for a specific site in 25S was provided by crosslinking and ligation of hybrids (CLASH), which showed that the Rrp5 binding site in 25S base-pairs to the sequencing flanking cleavage sites A2. We propose that this long-range interaction, facilitated by Rrp5, plays a key role in coordinating the timing of pre-ribosomal assembly and processing.

348 A Function of Fap7 in the maturation of the ribosome small subunit

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Over 200 pre-ribosomal factors are involved in the maturation of ribosomes. Most of these factors are essential to cell survival, but their precise function remains elusive. One of the last steps of maturation of the ribosome small subunit is the cleavage of 20S pre-rRNA in 18S rRNA in the cytoplasm. This cleavage is carried out by the endonuclease Nob1 and also requires the presence of other factors such as the methyltransferase Dim1, and a plethora of NTPases including the Rio protein kinases, Prp43 and its cofactor Pfa1, the Ltv1 GTPase and the Fap7 NTPase.

The function of Fap7 is especially intriguing since the human homologue bears adenylate activity, an enzymatic activity not usually found during ribonucleoprotein biogenesis. In addition, the function of Fap7 is intimately linked to its interaction with the Rps14 ribosomal protein. The Rps14 C-terminal domain is essential for D-site cleavage and is located in proximity to the 18S rRNA 3'-extremity in the mature ribosome. The deletion of this protein causes the 5q syndrome that is phenotypically close to Diamond Blackfan anemia. The link between the enzymatic activity of Fap7 and its role in ribosome biogenesis remains enigmatic.

We have conducted functional and structural characterisation of the Fap7 protein alone and in complex with Rps14 and nucleotides. Using a combination of structural studies by X-ray crystallography, small angle X-ray scattering (SAXS) in solution, enzymatic studies on purified proteins, and *in vitro* D site cleavage reaction assays on purified pre-ribosomes, we were able to uncover the function of Fap7 within pre-40S ribosomes. We show that the Fap7/Rps14 interaction is involved in a major conformational change at the heart of the pre-ribosomes and that this structural rearrangement is necessary to expose the D-site for cleavage by the endonuclease Nob1. The link between the enzymatic activity and the conformational switch both before and after cleavage is currently been investigated.

349 B Molecular characterization of ribosomal E-site function

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During the elongation cycle of protein biosynthesis, tRNAs travel through the ribosome by consecutive binding to the three ribosomal binding sites (A-, P-, and E-sites). While the A and P sites have been functionally well characterized in the past, the contribution of the E site to translation is still poorly understood in molecular terms. Footprinting and crystallographic studies indicated an interaction of A76 of E-tRNA with the nucleobase of the universally conserved 23S rRNA residue C2394.

We use the 'atomic mutagenesis' approach,[a] which allows site-specific manipulation of functional rRNA groups in the context of reconstituted ribosomes, to identify critical interaction partners of E-tRNA with the 23S rRNA. The functional significance of E-site 23S rRNA nucleotides for protein synthesis was tested by performing *in vitro* translation reactions with chemically engineered ribosomes carrying either a deoxy-residue or an abasic nucleoside analog at position C2394, U1851, A2422, or G2421. Unexpectedly the removal of the nucleobase or the ribose 2'-OH at C2394 had no effect on protein synthesis. Also modifications at U1851 or A2422 had no inhibitory effect. However, removing the nucleobase at G2421 completely inhibited in vitro translation. G2421 forms a base pair with C2395 and it was shown that A76 of E-tRNA stacks on the nucleobase of G2421. Subsequent standard mutagenesis highlighted the importance of a classical Watson-Crick 2421-2395 base pair regardless of the involved nucleobase identities. Ongoing work focuses on the importance of this universally conserved base pair in the 50S E-site on E-tRNA binding, reading frame maintenance and translation accuracy.

[a] Erlacher et al. (2011), Nat. Prot., 6:580-592.

350 C Evolutionary decline for a nuclear-encoded human mitochondrial aminoacyl-tRNA synthetase

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Mammalian mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) are nuclear-encoded and fulfill their activity in mitochondria. Their genes differ from the ones of cytosolic-adressed aaRSs. Many mammalian mt-aaRSs are of bacterial type and share structural domains with homologous bacterial enzymes of the same specificity, such as human mt aspartyl-tRNA synthetase (AspRS). Despite the fact that the mt-AspRS and AspRS from*E.coli* have a common ancestor numerous functional idosyncrasies/discrepancies were reported for the human mitochondrial enzyme.

In order to enlarge the knowledge on mt-AspRS, the present study investigated a serendipitously amplified PCR fragment, which corresponds to an alternative spliced transcript. This isoform lacks the exon 13 coding for a region in the "Bacterial Insertion Domain" (BID). We showed that the alternative transcript was present in all tested tissues; co-existed with the full-length form, possesses a 5'-and 3'-UTRs, a poly-A tail and was bound to polysomes. The corresponding protein was hard to express *in vitro* and was not detectable *in cellulo* or *in vivo*, strongly suggesting a decreased stability. In addition, bioinformatic analysis revealed that the mean acid identities percentages of BID were divergent in opisthokont and protist sequences and distinguished them from the bacterial and viridiplantae ones. This suggests a loss of evolutionary pressure for this domain in non-viridiplantae AspRSs of mitochondrial location.

Altogether, the combination of released selective pressure with the occurrence of alternative splicing suggests that the new isoform serves as evolutionary playground towards a possible reshaping of the BID. This ongoing decline of the BID underlines a relaxation of the mt enzyme.

351 A The communication between ribosome biogenesis and cell cycle machinery

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Ribosome, the universal machine for decoding the genetic code into polypeptides, is a complex of RNAs and proteins that comprises over 50% of the cell mass in rapidly growing cells. Thus ribosome biosynthesis is required to support the growth and proliferation of cells and consumes a huge amount of cellular energy and other resources. Disruption of normal ribosome biogenesis affects cellular growth and can result in cell abnormalities in proliferative cells. In humans these abnormalities are classified as 'ribosomopathies' and includes Diamond Black Fan Anemia, Shwachman Diamond syndrome, and increased propensity for some cancers. Our lab is using budding yeast to establish basic parameters for the connection between disruption of ribosome biosynthesis and cell cycle progression. So far, we have studied cell cycle progression, cell morphology and bud site selection after the repression of the synthesis of 54 of the 79 ribosomal proteins.

Interestingly, repression of the synthesis of specific ribosomal proteins generates different responses, including arrest (or delay) in G1 or G2/M stage of the cell cycle. Cell morphology includes cells with elongated buds, cells with more than one bud, and increased size of mother cells. Furthermore, repression of synthesis of new ribosomal proteins destabilized the existing pool of ribosomes. Since responses specific to the repression of individual ribosomal protein genes results in a variety of phenotypes, these effects do not originate from just reduced protein synthesis capacity. We are currently investigating possible explanations, including abnormal localization and stability of the yeast cell cycle-dependent kinase Cdc28 and other cell cycle proteins and their mRNAs.

352 B Effect of tRNA core modifications and a D-loop sequence element on ribosomal decoding

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Unmodified tRNAs are capable of adopting the folded tertiary structure characteristic of native tRNAs, although they are less stable and more dynamic. While unmodified tRNAs are often active enzyme substrates, their activity on ribosomes may be compromised. tRNAs lacking all or some of the modifications in the anticodon stem loop (ASL) show weaker ribosome binding, slower rates of dipeptide bond formation and increased misreading than their modified forms.(1) Here we focus on the decoding properties of modified and unmodified *E. coli* tRNA Ala(GGC), tRNA Gly(GCC) and tRNA Val(GAC), which are the only three *E. coli* elongator tRNAs that naturally lack ASL modifications, permitting the combined effects of the less studied modifications in the core of tRNA to be evaluated.

Using ribosome decoding assays in a "high fidelity" buffer at 25?C, we confirm the results of our earlier experiments in RBB buffer indicating that the unmodified forms of all three of these tRNAs show identical rates of peptide bond formation (kpep) as their native counterparts. However, at elevated temperatures (37?C and 42?C), native tRNA Ala and tRNA Gly show substantially faster kpep values than at 25?C while the unmodified tRNAs remain slower. In-line probing experiments show that at 37?C the tRNA tertiary structure is partially disrupted when the modifications are absent, but not when the modifications are present. This conclusion is consistent with earlier studies indicating a stabilizing effect of the T54 and ψ55 modifications (2).

Interestingly, the kpep value of unmodified tRNA Val(GAC) remains equal to that of native tRNA Val(GAC) at all temperatures, suggesting that this tRNA does not need modifications to maintain its tertiary structure in the conditions tested. By measuring kpep with a set of unmodified tRNA Val/ tRNA Gly chimeras, the source of the increased kpep activity at higher temperatures was found to entirely be due to the presence of U20.1, which only occurs in tRNA Val. Removing U20.1 from unmodified tRNA Val makes it less active at 37?C, while adding U20.1 to tRNA Ala makes it more active at 37?C. In-line probing experiments confirm that the presence of U20.1 stabilizes the folded tertiary structure of tRNA.

A residue at position 20.1 (usually a U) is present in 40% of class 1 bacterial tRNAs. Although most tRNA species either have 20.1 or do not, a few such as tRNA Val(GAC) contain 20.1 in some bacteria and not in others. U20.1 is expected to be modified to D in many organisms. We are interested in understanding whether this residue acts as an independent stability element, why it is only present in certain tRNAs and how it acts to stabilize tRNA structure.

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353 C Non-ribosomal Interaction Partners of Ribosomal Protein S3

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The ribosomal protein S3 (Rps3) is an essential component of 40S ribosomal subunits. Rps3 contains a substantial amount of positively charged amino acids which facilitate its interactions with the negatively charged ribosomal RNA. Like other ribosomal proteins, Rps3 is prone to aggregation, which may be due to interactions of these regions with negatively charged components of the cytoplasm.

S. cerevisiae Rps3 engages in an interaction with the small ankyrin-repeat protein Yar1. Yar1, a 22 kDa protein, is hypothesized to contain two ankyrin-repeats, which are common protein-protein interaction motives. Yar1 is thought to bind and maintain the solubility of free Rps3 in the cytoplasm and deliver it to pre-ribosomes in the nucleus.

Yeast Rps3 shares 66 % identity and 78 % similarity with its human counterpart. We have been able to show using a yeast-2-hybrid analysis that human Rps3 interacts with the yeast ankyrin-repeat protein Yar1. As Rps3 is highly conserved from yeast to humans and ribosomal proteins undergo rapid turnover when not ribosomal bound, we are investigating whether a similar interaction may occur in human cells. Consequently, we are analyzing an ankyrin-repeat protein candidate which interacts with and may stabilize free human Rps3. These data will be presented here.

354 A Sequence-specific targeting of bacterial ribosomal RNA as a way to look for inhibition pockets

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Many antibiotics inhibit protein synthesis in bacteria by interacting with their ribosomal RNA [1]. Unfortunately, bacteria have developed various resistance mechanisms to fight back existing drugs. Also, in most cases, the known antibiotics lack specificity toward pathogenic bacteria. Designing new scaffolds or modifying the known compounds has been of moderate success and most importantly will not eliminate the cross-resistance of related antibiotics. To design effective inhibitors of bacterial translation one has to explore new sites and possibly unknown inhibition mechanisms.

We have been looking for good inhibition sites by rational screening of the ribosomal RNA with short modified oligonucleotides as probes. The aim is to evaluate the effectiveness of these antisense single-stranded oligonucleotides in inhibiting translation by binding to ribosomal RNA in a sequence-dependent manner. The design of oligonucleotide sequences is based on the atomic-resolution structures of the ribosomes [2], bioinformatics analyses, and molecular dynamics simulations. Not only sequence complementarity but also the secondary and tertiary structure, flexibility and other physicochemical descriptors of ribosomal RNA and modified oligonucleotides are taken into account in the design process.

Next, we check the ability of such oligomers to inhibit protein synthesis *in vitro* in an optimized by us *E. coli* cell-free transcription/translation system with β-galactosidase as a reporter protein. The oligonucleotides that have been tested are 2'O-methyl-RNA, peptide nucleic acid and locked nucleic acid. For example, we have identified potential 2'O-methyl-RNA oligonucleotides (10-15 nucleotides long) that block the function of certain fragments of ribosomal inter-subunit bridges through steric hindrance, thereby halting protein synthesis. If the oligomer target site is in proximity to known antibiotic binding site we also check the synergistic (or antagonistic) effect of their combinations. To further investigate the mode of binding and confirm strand invasion of oligonucleotides we perform fluorescence spectroscopy, isothermal titration calorimetry and thermal denaturation studies on selected isolated ribosomal RNA fragments.

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355 B Molecular pathogenesis of ribosomopaties in zebrafish model for Diamond-Blackfan anemia

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Ribosome biogenesis in eukaryotes involves a coordinated participation of ribosomal RNAs (rRNAs), ribosomal proteins (RPs), and a large number of accessory factors that contribute to processing of precursor rRNAs and assembly of ribosomal subunits. Recently, mutations in genes involved in ribosome biogenesis were identified in patients with various diseases, suggesting impaired ribosome can cause tissue-specific failures (ribosomopathies) via an unknown pathway. Diamond-Blackfan anemia (DBA) is one of such disorders, characterized by diminished numbers of erythroid progenitors. The DBA has been attributed to the defect in RP genes, such as *RPS19* and nine other so far. However, it is still unclear how mutations in such ubiquitously expressed genes affect mainly erythropoiesis.

To investigate the molecular mechanism underlying DBA, we have developed a zebrafish model for the anemia by repressing the translation initiation of *rps19* mRNA with the Morpholino antisenseoligo. The knockdown embryos displayed a drastic reduction of red blood cells, whereas differentiation of other myeloid cells and endothelial cells seemed to be normal. The anemic phenotype was almost completely rescued by injection of synthesized *rps19* mRNA, but not by mutated mRNAs with patient-type mutations. The DBA model also showed developmental abnormalities in the head and tail regions due to increased cellular apoptosis. A simultaneous inhibition of *p53* gene rescued the morphological abnormalities, but did not alleviate the erythroid aplasia. This suggests that a tp53-independent but RPS19-dependent pathway could be responsible for defective erythropoiesis in DBA. To evaluate the impact of RPS19 deficiency on translation, we carried out a polysome profiling of our DBA model. The polysome patterns were similar between *rps19* morphants and control embryos, although the amount of the heavier fractions was less in the morphants. Next, we carried out an RNA-Seq analysis of polysomal mRNAs purified from these embryos. We found that the translational efficiencies of about 300 genes were significantly changed in the *rps19* morphants. These data will provide important information to understand the pathogenesis of DBA.

356 C Investigating the function of the RNA helicase Prp43 and its cofactor Pfa1 in 40S ribosomal subunit synthesis

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Ribosome biogenesis starts in the nucleus with the formation of pre-ribosomal subunits that undergo maturation steps in the nucleolus and in the cytoplasm after export through the nuclear pore complex. Cytoplasmic 40S maturation steps comprise structural changes and processing of the rRNA, but how these events play together in detail remains elusive.

The DEAH box RNA helicase Prp43 and its cofactor, the G-patch protein Pfa1 participate in multiple steps of ribosome biogenesis. Prp43 removes sno-RNAs from pre-ribosomal particles and is involved in rRNA processing events during both 60S and 40S subunit maturation. However, its exact functions at the different stages of ribosome synthesis are not known. Here we investigated the genetic network of Pfa1 and Prp43 with late pre-40S factors and ribosomal proteins in order to understand the 40S specific role of this enzyme.

357 A A New Role for NOT5 of the CCR4-NOT Complex in Connecting Transcription with Translation

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The Ccr4-Not complex contributes to both transcription and cytoplasmic mRNA decay and is found both in the cytoplasm and in the nucleus1, like the Rpb4 subunit of RNA polymerase II, recently reported to play a role in translation by interacting with the translation scaffold factor eiF32. In our work we show that Not5 is involved in the crosstalk between transcription and translation by contributing to the nuclear-cytoplasmic shuttling of Rpb4 and to the assembly of RNAPII on translating ribosomes. Indeed, first we found that the transctipion factor Rpb4 and translation factor eiF3 interactions are dependent upon the Not5 subunit of the Ccr4-Not complex. This can be explained by the fact that Not5 is required for the presence of Rpb4 in translating polysomes and in fact for the appropriate accumulation of Rpb4 in the cytoplasm under relevant conditions altogether. Second, besides Rpb4, several other polymerase subunits (Rpb1, Rpb2, Rpb9, Rpb11) are present in translating ribosome fractions, compatible with the publised data showing that assembly of the polymerase is cytoplasmic and with the new idea that this is co-translational. Not5 is not only required for Rpb4 cytoplasmic accumulation, but also for appropriate co-translational interaction of newly synthesiezd Rpb1 with its chaperones. Consequently RNA polymerase II is not appropriately assembled in cells lacking Not5, and subcomplexes containing various polymerase subunits accumulate. Hence Not5 of the Ccr4-Not complex is central to the integration of transcription and translation into a system that efficiently controls eukaryotic gene expression.

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358 B Ribosome biogenesis in plants and its impact on developmental processes

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Ribosomes are highly complex ribonucleoprotein particles responsible for the synthesis of all proteins. Eukaryotic ribosomes consist of 79 ribosomal proteins and four ribosomal RNA molecules. Three of the four ribosomal RNAs (rRNA) are transcribed as a polycistronic transcript that is processed by endo- and exnucleolytic digestions to release the mature rRNAs. The processing path is well understood in yeast, but there is only limiting information on rRNA processing in plants. Only recently, a number of cleavage sites were mapped using mutants of the Arabidopsis orthologs of yeast Rat1/Xrn2 and Mtr4. rRNA processing and folding are prerequisite for the assembly of eukaryotic ribosomes, which additionally requires up to 200 ribosome assembly cofactors and more than 75 snoRNAs. Interestingly, In the plant model organism *Arabidopsis thaliana*, 2/3 of the ribosome biogenesis cofactors, that are orthologs of the yeast cofactors, are found multiple times in the genome pinpointing to additional or redundant functions of these factors. Furthermore, the different orthologs might be required at distinct developmental stages or in different tissues and thus, they link ribosome biogenesis to growth and development.

Here, we analyzed the rRNA processing pathway in *Arabidopsis thaliana* by northern blotting of wild type RNA to first define this pathway in more detail. Additionally, to gain further insights into ribosome biogenesis in plants and its link to other processes, we studied two late cofactors in Arabidopsis thaliana - the pre-40S endonuclease Nob1 responsible for D-cleavage and the pre-60S GTPase Lsg1 involved in the release of Nmd3 from pre-60S. Both are characterized in unicellular organisms (yeast and archaea), but information for multicellular eukaryotes like plants are lacking. Knock down of the Nob1 ortholog in Arabidopsis (atNob1) results in an accumulation of all precursors leading to 18S rRNA with the strongest accumulation in the P-A_3 fragment. Furthermore, Nob1 links ribosome biogenesis to pollen and embryo development. For Lsg1p we find two orthologs in Arabidopsis (atLsg1-1 and atLsg1-2). Knockout of atLsg1-2 shows significant alterations in the processing pathway. Interestingly, atLsg1-2 links ribosome biogenesis to early leaf development. AtLsg1-1 appears to have lost its ribosome biogenesis function due to the lack of an important basic C-terminus, and we are currently investigating its alternative function.

359 C Going through the motions: Network analysis reveals conserved intramolecular communication pathways within EF-Tu responsible for ribosome dependent GTPase activation and nucleotide exchange

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During the elongation cycle of bacterial protein synthesis elongation factor (EF) Tu delivers aminoacyl(aa)-tRNAs to the ribosome in a GTP-dependent manner. EF-Tu functions as a checkpoint in translation as correct codon/anticodon base pairing is required to send an activating signal over more than 80Å from the decoding centre on the small (30S) ribosomal subunit to the G domain of EF-Tu bound to the 50S large ribosomal subunit. Following dissociation from the ribosome EF-Tu?•GDP is recycled into its GTP-bound form through the interaction with its nucleotide exchange factor EF-Ts. Both functions require the transmission of signals form the respective interaction sites across EF-Tu to facilitate either GTP hydrolysis or nucleotide release. Little is known about the dynamic features governing signal transmission within a highly conserved protein such as EF-Tu and its particular evolutionary constraints.

Molecular dynamics (MD) simulations of EF-Tu and variants with single amino acid substitutions were performed and subsequently interrogated using network analysis methods. Here we report an intramolecular communication network for EF-Tu representing a complex set of long-range signal transmission pathways. Using this network we identify communication pathways essential for efficient GTPase activation on the ribosome as well as EF-Ts-catalyzed nucleotide exchange. We find that single amino acid substitutions in EF-Tu can change the network organization dramatically and result in significantly reduced communication between domains across a set of universally conserved interdomain bridges. To validate our findings in vitro we have performed Michaelis-Menten analyses to study ribosome stimulated GTP hydrolysis of the EF-Tu•GTP binary complex. Aa-tRNA was omitted to isolate this signal pathway from any aa-tRNA contributions. Consistent with our network analysis, these results reveal that single amino acid substitutions in domain II reduce the stimulatory effect of the 70S ribosome by five-fold, while not affecting 50S-dependent stimulation. Furthermore, variants of EF-Tu that disrupt the conserved interdomain bridges reduce the stimulatory effect of the 70S ribosome to a similar extent. Analyses of the EF-Tu•EF-Ts binary complex revealed a different set of communication pathways promoting nucleotide exchange in EF-Tu which were validated using rapid-kinetics techniques to determine nucleotide binding properties of the respective EF-Tu variants.

360 A Transcription regulation by cAMP-CRP of the rmf gene for 100S ribosome formation

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In proteobacteria group gamma including Escherichia coli, protein synthesis is suppressed by the formation of 100S ribosomes under stress conditions such as nutrient starvation. The 100S ribosome, a dimer of 70S ribosomes, is formed after association of 70S ribosome monomer with “Ribosome Modulation Factor” (RMF) [1]. Upon entry into the stationary growth phase, the 70S ribosomes are converted into 100S dimers, which are functionally inactive in translation. An E.coli strain deficient in the rmf gene cannot form 100S ribosomes and its lifetime is shorter than that of the wild-type strain, indicating that the transformation of ribosomes is an important strategy for E.coli survival under stress conditions.This ribosomal resting state is called the hibernation stage [2]. At present, however, little is known regarding the regulation of stationary-phase-coupled RMF expression.

After Genomic SELEX screening in vitro of regulation targets of CRP (cAMP receptor protein), the global regulator of genes for carbon source utilization, the rmf gene was predicted to be under the direct control of cAMP-CRP [3]. In order to confirm the regulation in vivo of rmf by cAMP-CRP, we investigated the effects of cAMP and CRP for the expression of RMF and the formation of 100S ribosome by using the deletion mutants of cyaA and crp genes. The results altogether, indicated the decreases in RMF production and 100S ribosome formation in the absence of cAMP-CRP. We concluded that cAMP-CRP is involved in transcription regulation of the rmf gene and the formation of 100S ribosome [4]. In addition, we will discuss other factors involved in ribosome dimerization.

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361 B Towards understanding the inhibitory effects of codon-anticodon wobble base pairing on the ribosome

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Accurate and rapid translation of the genetic code into functional peptides is a defining feature of the ribosome and the translation factors. The choice of codon within an mRNA has long been known to affect both translational efficiency and accuracy. While the first two positions of the codon are strictly decoded using Watson-Crick base pairing, the third position is often decoded using wobble base pairing. The inosine:adenosine (I:A) wobble base pair, a purine:purine base pair, is perhaps one of the most unusual; for example, the arginine CGA codon is nearly universally decoded by a tRNA isoacceptor bearing an ICG anticodon. In*Saccharomyces cerevisiae*, wobble decoding of the CGA codons appears to have a strong inhibitory effect on translation (Letzrig et al., RNA, 2010). Interestingly, the inhibitory effect is much more pronounced with CGA pairs relative to single codons suggesting synergy between consecutive decoding events. Previously, we have shown that in *E. coli* the quality of the P site tRNA-mRNA interaction has a profound effect on the decoding process in the A site of the ribosome where mismatches in the P site trigger iterated miscoding and eventually lead to premature termination. Given the results in yeast with consecutive CGA codons, we wondered whether the I:A base pair is acting like a mismatch inducing a process similar to that seen with *E. coli*. To this end we constructed reporters bearing stretches of synonymous arginine codons and characterized their translational efficiencies. Similar to earlier findings (Letzrig et al., RNA, 2010), a stretch of four CGA codons was sufficient to inhibit the production of full-length protein product. We further found these truncated products to be stabilized by the addition of the proteasome inhibitor MG132 or in the absence of the ribosome-bound E3 ligase LTN1. Remarkably, similar to the mismatch response seen in *E. coli*, mass-spec analysis of the stabilized truncated products revealed that the C-terminus gets extended with uncoded amino acids following the terminal encoded arginines. These findings suggest that I:A pairing induces iterated miscoding possibly similar to that previously observed in *E. coli*. We are currently extending these studies to identify factors responsible for the recognition of I:A wobble on the ribosome.

362 C One messenger RNA and three initiation codons govern the synthesis of two human Glycyl-tRNA synthetases isoforms

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Human Glycyl-tRNA synthetase (GlyRS) is a housekeeping enzyme with a key role in protein synthesis, responsible for the charging of glycine on its cognate tRNA. In metazoans, there is a unique gene, GARS, which encodes both cytoplasmic and mitochondrial GlyRSs. At least 3 putative initiator AUG codons were found amongst the 300 first nucleotides of the mRNA: AUG#1 is not in frame and would initiate the translation of a 32 amino-acids peptide, AUG#2 and AUG#3 initiates the translation of the mitochondrial GlyRS precursor and the cytosolic form of GlyRS, respectively.

AUG codons were mutated, alone or in combination, and the expression efficiency as well as the subcellular localization (mitochondria, cytosol) of each GlyRS variant were determined. Our results show that, this mRNA codes mainly for the cytosolic form of GlyRS. This expression becomes mainly mitochondrial when AUG#1 is removed and exclusively mitochondrial when AUG#1 and AUG#3 are mutated together. On the contrary, the simultaneous presence of AUG#1 and AUG#2 does not allow any GlyRS translation. *In vitro* translation assays not only confirmed these results but also showed that AUG#1 is an efficient initiator codon (the design of a frame-shift mutant leads to the synthesis of an extra-long GlyRS).

Based on these observations, we hypothesized that the ribosome initiates translation at AUG#1, translates the 32 amino acid long peptide through AUG#2, terminates at the stop codon and reinitiates translation at AUG#3 to produce the cytosolic GlyRS. However, when mitochondrial GlyRS is needed, the ribosome reinitiate translation at AUG#2, suggesting that the peptide translation is prematurely terminated. Indeed, the introduction of a stop codon between both initiation sites (AUG#1 and AUG#2) allowed recovering the synthesis of the mitochondrial form of GlyRS.

The very short 5' UTR present in front of AUG#1 (only 15 nucleotides) as well as a dense structure characterized by a high GC content suggest the presence of a particular initiation mechanism. Moreover, the sequence of the short peptide (rich in Arginine and Proline residues) supports a regulatory mechanism based on the cellular level of Arg and Pro, which metabolism depends on mitochondria.

363 A A tale of two termini: profiling mRNA 5′-3′ interactions in vivo.

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Interactions between the 5' and 3' termini of mRNAs have long been postulated to occur in the cell. This "closed-loop" model of mRNA is attractive from a number of viewpoints: functionally, the closed loop explains the ability of 3' UTR features to modulate events occurring at the 5' end such as translation initiation, while mechanistically, the 5' cap structure is known to bind the eIF4F complex, which also interacts with the poly(A)-tail via PABP, conceptually forming a closed loop mRNP if all interactions occur simultaneously.

Here we introduce a novel assay to detect the closed-loop conformation of specific mRNAs *in vivo* for the first time. Using *Saccharomyces cerevisiae*, we demonstrate that the closed-loop is the predominant conformation found in mRNAs bound by eIF4F, but also introduce the possibility that this structure is not adopted by all mRNAs during normal growth. While the closed-loop conformation has been reconstituted *in vitro*by mixing eIF4F and PABP with mRNA, the situation may be different in the cell. Various *in vivo* examples are known where interactions between the eIF4F subunits and PABP come under regulatory intervention, either globally or in a transcript-specific manner, resulting in reduced translational efficiency. Thus, detecting altered closed-loop status represents a catch-all method of identifying whether mRNA is under translational regulation. We are investigating the global and transcript-specific regulation of the closed-loop conformation in response to environmental cues, which will identify regulatory mechanisms that have been expanded upon during the evolution of more complex organisms to rapidly fine-tune gene expression.

364 B Translation of the human erythropoietin transcript is regulated by an upstream open reading frame in response to hypoxia

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In mammalian cells, most translational regulation is exerted at the cap-translation initiation step, where the AUG codon is identified and decoded. However, when translation is inhibited by cell stress, alternative mechanisms of translation initiation act to maintain the synthesis of certain proteins required either for the stress response or to aid recovery from the stress stimulus. These pathways are evolutionarily conserved and have been shown to dramatically impact translation. In many cases, features in the 5' untranslated region (UTR) of these mRNAs are important for translational control. These include small structural elements, internal ribosome entry sites and regulatory upstream open reading frames (uORFs). uORFs modulate translation of the main ORF by decreasing the number and/or efficiency of scanning ribosomes to reinitiate at the start codon of the main ORF.

In its classical hormonal role, human erythropoietin (EPO) is a glycoprotein synthesized and released mainly from the kidney, which has a key role in hematopoiesis. However, recent studies have revealed that EPO is a multifunctional molecule produced and utilized by many tissues that rapidly responds to different cell stress stimuli and tissue injuries. Thus, it has the potential to be used as a therapeutic target/strategy for the treatment of several human disorders. Understanding the EPO translational control mechanisms will be valuable in the determination of these therapies. Knowing that human EPO transcript presents a 5'UTR with 181 nucleotides containing a 14-codon-uORF, conserved among different species, which might indicate its role in translational regulation, we aimed to prove this hypothesis.

To explore the role of the *EPO* uORF in controlling translation, we cloned the EPO 5'UTR, with the intact or disrupted uORF, fused to the firefly luciferase reporter cistron. HepG2, HEK293 and REPC cells were transiently transfected with these constructs. Luciferase activity was measured by luminometry and the corresponding mRNA levels were quantified by real-time RT-PCR to obtain translation efficiencies. Results have shown that the uORF can decrease the main ORF translation efficiency in about 3-fold. In addition, results support the conclusion that reinitiation, and in less extent leaky scanning, are responsible for the main ORF translation. Furthermore, this repression is released under hypoxia, specifically in REPC renal cells, via eIF2a phosphorylation. These findings provide a framework for understanding that production of high levels of EPO induced by hypoxia is also regulated at the translational level.

365 C Nanos recruits the CCR4-NOT complex to induce degradation of mRNA targets

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The Nanos protein is a non-specific RNA-binding protein, which together with Pumilio represses translation of the maternally deposited *hunchback* (*hb*) mRNA and plays a major role in the establishment of the anterior-posterior body axis in *Drosophila melanogaster*. Previous studies have indicated that the *hb* 3' UTR is sufficient for translational repression and that reporter mRNAs containing this 3' UTR are translationally repressed and deadenylated in a Nanos- and Pumilio-dependent manner. However, the exact molecular mechanism of deadenylase complex recruitment to the mRNA reporter has so far remained elusive. Here we investigated the role of Nanos in the regulation of the *hb* 3' UTR. The Nanos protein contains an N-terminal region predicted to be unstructured and two conserved C-terminal CCHC type zinc finger motifs. Using coimmunoprecipitation assays in *Drosophila* S2 cells, we show that Nanos interacts with both the CCR4-NOT and PAN2-PAN3 deadenylase complexes. Furthermore, we mapped the deadenylase-binding region to a fragment within the unstructured part of the protein. An *in vitro* pull-down experiment revealed that Nanos interacts directly with the NOT module of the CCR4-NOT complex. We also show that the deadenylase-binding region of Nanos is required to elicit mRNA degradation via the 5'-to-3' decay pathway. In addition, our results show that tethered Nanos can promote both translational repression and mRNA degradation independently of Pumilio. Interestingly, the three human Nanos proteins, Nanos 1-3, also interact with the CCR4-NOT deadenylase complexes in human cells. Similar to *Dm* Nanos, human Nanos 1-3 promote the degradation of bound mRNAs. We conclude that Nanos proteins can recruit the CCR4-NOT complex to mRNA targets independently of Pumilio and that this activity is conserved amongst Nanos orthologs.

366 A Study of PTBP1-RRM12 in complex with an RNA-stemloop of the EMCV-IRES

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Internal ribosomal entry sites (IRESes) are large RNA structures present in the 5' untranslated region (5' UTR) of many viral and some cellular mRNAs(1). Unlike canonical translation initiation, IRES mediated translation initiation is 5' cap independent but often requires cellular IRES trans-acting factors (ITAFs). The polypyrimidine tract binding protein 1 (PTBP1) is among the most frequently found ITAFs and might modulate the IRES activity by stabilization of the appropriate IRES structure(2).

PTBP1 harbors four RNA recognition motifs (RRMs) of which the 2 N-terminal RRMs, RRM1 and RRM2, act independently whereas the 2 C-terminal RRMs, RRM3 and RRM4, interact with each other, thereby orienting their RNA-binding surfaces in opposite directions(3). Thus, PTBP1 might be able to reorganize the structure of an RNA target by bringing distant binding sites into proximity.

PTB binding sites were mapped on the IRES of encephalomyocarditis virus (EMCV) by hydroxyl radical probing(4). It was found that two molecules of PTBP1 bind to the IRES of EMCV in a unique orientation: One PTBP1-molecule binds the IRES-domains H-L and the second one to domains D-F. The footprinting data suggests that RRM1 binds the UCUUU-pentaloop of the IRES-domain F and RRM2 binds its regular stem. We investigated the binding of RRM12 to domain F by NMR titrations: In contrast, our NMR-data suggest that RRM12 binds the isolated domain F with RRM2 contacting the loop. We present a preliminary structure of RRM2 bound to domain F.

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367 B CPEB3-controlled translation regulates memory consolidation

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Long-term memory requires activity-dependent synthesis of plasticity-related proteins to strengthen synaptic efficacy and consequently consolidate memory. Cytoplasmic polyadenylation element binding protein(CPEB)3 is a sequence-specific RNA binding protein and represses translation via inhibiting the translation elongation factor 2 (eEF2)1. Activation of N-methyl-D-aspartate (NMDA) receptors in neurons triggers the cleavage of CPEB3 by calpain 2 and subsequently leads to activity-related translation of CPEB3-targeted RNAs2. Recent studies indicate that the Drosophila homolog of CPEB3, Orb2, of which prion-oligommeric property is required for memory persistence in the fly3 and the glutamine (Q)-rich prion domain in Orb2 can be substituted functionally with the Q-rich motif in CPEB34, raising the question whether mammalian CPEB3 employs prion-like mechanism to sustain long-term memory. To understand the cellular and molecular contributions of CPEB3-controlled translation to learning and memory, we have generated CPEB3 knockout (KO) mice. The null mice display enhanced hippocampus-dependent memory and abnormal synaptic transmission in the Schaffer collateral pathway. Molecular and cellular characterizations reveal that the elevated PSD95 expression results in altered dynamics of NMDA-induced biochemical and morphological changes in the KO neurons. Together, CPEB3 functions as a negative regulator to confine the strength of consolidated memory. Our results overrule the possibility that CPEB3 can function like Orb2 as a prion to promote and persist memory formation.

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368 C DDX3 Regulates Rac1 Translation, Modulates Wnt Signaling and Is Required for Cancer Cell Metastasis

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DDX3 is a DEAD-box RNA helicase involved in multiple steps of gene expression. DDX3 is upregulated in hepatocellular carcinoma tissues and in aggressive breast epithelial cancer cell lines, and is considered as a biomarker of metastasis in squamous cell/ adenosquamous carcinomas. The genetic and functional interactions between DDX3 and β-catenin have been observed in the Wnt type of medulloblastoma. Therefore, DDX3 is a potential oncogene. To reveal oncogenic roles of DDX3 in metastasis, we analyzed phenotypes caused by DDX3 depletion in HEK293, cervical cancer HeLa and neuroblastoma N2A cell lines. In these cell lines, DDX3 depletion increases cell-cell adhesion and decreases cell-extracellular matrix adhesion, cell migration and invasion. Metastasis assays further demonstrate that DDX3 is required for efficient metastasis of cancer cells. These results suggest that DDX3 may play an oncogenic role in controlling cancer cell adhesion, migration and invasion/metastasis via modulating cytoskeleton organization. To uncover potential targets of DDX3 involved in cytoskeleton remodeling, we performed pathway analysis of DDX3 targets that are regulated at the level of mRNA translation, and identified multiple pathways involved Rac1 functions. Our following experiments demonstrate that DDX3 may activate Rac1 translation perhaps by resolving its 5'UTR secondary structure. Moreover, we performed qRT-PCR array analysis of cell motility genes and found that transactivation of the Wnt/β-catenin target genes was down-regulated in DDX3 knockdown cells. Our results further showed that DDX3 depletion decreases β-catenin stability and attenuates Wnt/β-catenin signaling, and that Rac1 can rescue β-catenin expression and cell adhesion in DDX3 knockdown cells. Rac1 is a major factor of the Wnt PCP pathway that controls cell movement and also regulates the stability of β-catenin in the canonical Wnt pathway. Thus, our study unveils a molecular mechanism by which DDX3 controls cancer metastasis via regulating Rac1 and β-catenin signaling of cancer cells and supports DDX3 as a culprit in Wnt type tumors.

369 A Inactivation of the mTORC1-eIF4E Pathway alters Stress Granules Formation

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Stress granules (SG) are cytoplasmic multimeric RNA bodies that form under stress conditions known to inhibit cap-dependent translation. SG contain translation initiation factors, RNA binding proteins like the Fragile Mental Retardation Protein (FMRP) and signaling molecules. SG are known to inhibit apoptotic pathways, thus contributing to chemo- and radio-resistance in tumor cells. However, whether stress granules formation involves oncogenic signaling pathways is currently unknown. Herein, we report a novel role of the mTORC1-eIF4E pathway, a key regulator of cap-dependent translation initiation of oncogenic factors, in SG formation. mTORC1 specifically drives the eIF4E-mediated formation of SG through the phosphorylation of 4E-BP1, a key factor known to inhibit formation of the mTORC1-dependent eIF4E-eIF4GI interactions. Disrupting formation of SG by inactivation of mTOR with its specific inhibitor pp242 or by depletion of eIF4E or eIF4GI blocks the SG-associated antiapoptotic p21 pathway. Finally, pp242 sensitizes cancer cells to death *in vitro* and inhibits the growth of chemoresistant tumors *in vivo*. This work therefore highlights a novel role of the oncogenic mTORC1-eIF4E pathway, namely the promotion of formation of antiapoptotic SG.

370 B Identification of DDX6 as a cellular modulator of VEGF expression under hypoxia

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Vascular endothelial growth factor A (VEGF) is a crucial pro-angiogenic factor, which regulates blood vessel supply under physiologic and pathologic conditions. The VEGF mRNA 5' untranslated region (5'UTR) bears internal ribosome entry sites (IRES), which confer sustained VEGF mRNA translation under hypoxia when 5'cap-dependent mRNA translation is inhibited. VEGF IRES-mediated initiation of translation requires the modulated interaction of *trans*-acting factors. To identify *trans*-acting factors that control VEGF mRNA translation under hypoxic conditions we established an *in vitro* translation system based on human adenocarcinoma cells (MCF-7). Cytoplasmic extracts of MCF-7 cells grown under hypoxia (1% oxygen) recapitulate VEGF IRES-mediated reporter mRNA translation. Employing the VEGF mRNA 5'UTR and 3'UTR in an tobramycin-aptamer RNA affinity purification approach we isolated interacting proteins from translational active MCF-7 extract prepared from cells grown either under normoxia or hypoxia. Interestingly, mass spectrometry analysis identified the DEAD-box RNA helicase 6 (DDX6) that interacts with the VEGF mRNA 5'UTR. Recombinant DDX6 inhibits VEGF IRES mediated translation initiation in normoxic MCF-7 extract *in vitro*. Under hypoxia the level of DDX6 declines and its interaction with VEGF mRNA is diminished *in vivo*. Depletion of DDX6 by RNAi further promotes VEGF expression in MCF-7 cells. Increased secretion of VEGF from DDX6 knock down cells positively affects vascular tube formation of human umbilical vein endothelial cells (HUVEC) *in vitro*. Our results indicate that the decrease of DDX6 under hypoxia contributes to the activation of VEGF expression and promotes its pro-angiogenic function.

371 C Molecular characterization of SMG1-UPFs complexes

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Non-sense Mediated mRNA Decay (NMD) allows the recognition of Premature Termination Codon (PTC)-containing mRNAs1. This quality control mechanism is crucial to avoid putatively toxic truncated proteins in the cell2. PTCs are recognised by the SURF complex consisting of eRF1/eRF3/Upf1/SMG1-8-9 3, 4. Subsequent interaction of the SURF complex with the Upf2-3-Exon Junction Complex (EJC) triggers SMG1-mediated Upf1 phosphorylation3,5.This phosphorylation is the key event initiating the mRNA decay cascade 1,5.

In order to dissect the molecular details of activation of the NMD pathway *in vitro*, we generated SMG1 PIK-like kinase alone, SMG1-9 and SMG1-8-9 complexes. The SMG1 kinase alone or in complex with SMG9 or SMG8-9 is able to phosphorylate UPF1 expressed in insect cells as a non-phosphorylated form. We determined structures of SMG1-8-9 and a SMG1-8-9-UPF complex at low resolution by single particle electron microscopy. We solved the structures of all three UPF2 MIF4G domains and show that the MIF4G-3 domain not only binds UPF3 but also SMG1 in a non-competitive manner 6. In fact, this could be the key interaction between the SURF and the UPF2-3-EJC complexes triggering UPF1 phosphorylation, translation termination at the PTC and faulty mRNA degradation.

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372 A Changes in polysomal profiles in cells with aberrant RNA degradation

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Non-protein-coding RNAs (ncRNAs) are important in regulation of gene expression in many different ways. Very often they are connected with development, stress response and pathogenesis. One of the less known functions of ncRNA (excluding rRNAs, tRNAs and miRNAs) is the modulation of translation. Long non-coding RNAs for instance have been linked to both repression and stimulation of translation. We are interested in novel tRNA-derived small RNAs (tRFs) that are produced by endonucleases such as DICER, RNaseZ and angiogenin (1, 2). Angiogenin produced tRFs have a role in translational silencing and viral infection (3, 4).

Here we show, that 3' to 5' exoribonuclease DIS3L2 is involved in tRFs processing. DIS3L2 is a sequence homolog of the main exosomal ribonucleases DIS3 and DIS3L, that has been linked to Perlman syndrome (5). DIS3L2 is localized to cytoplasm where it acts on tRFs in exosome-independent manner. Our results indicate that DIS3L2 together with tRFs associates with ribosomes. Overexpression of wild type DIS3L2 in HEK293T cells causes changes in polysome to monosome ratios. We are currently investigating whether DIS3L2 is directly involved in translational regulation.

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373 B Homeostatic regulation of AMPA receptors in synaptic plasticity: a posttranscriptional interplay between Caspr1 and the RNA-binding protein ZBP1.

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Phenomena such as learning and memory rely on mechanisms of synaptic plasticity, which are highly dependent on the modulation of postsynaptic glutamate receptors of the AMPA-type (AMPARs). Moreover, a growing body of evidence suggests that posttranscriptional control of AMPAR subunits may also be fundamental for the expression of homeostatic synaptic plasticity, a mechanism known to adjust and stabilize synaptic strength upon chronic changes in neuronal activity. Despite evidence showing mRNA molecules for the GluA1 subunit of AMPARs strategically positioned for local protein synthesis in dendrites, little is yet known about the mechanisms regulating the availability of synaptic AMPAR transcripts and on how they underlie the expression of several forms of plasticity.

Our data identify the transmembrane protein Contactin associated protein 1 (Caspr1) as a novel potential posttranscriptional regulator of the GluA1 subunit of AMPARs. We show that Caspr1 regulates GluA1 protein by increasing GluA1 mRNA levels, an effect dependent on the presence of the 3'untranslated region of the transcript. Moreover, we propose that Caspr1 exerts its effects on GluA1 mRNA levels by activating a signaling cascade downstream of the tyrosine kinase Src, ultimately regulating the phosphorylation status of the Zipcode-binding protein 1 (ZBP1), a well described RNA-binding protein known to regulate the translation of several mRNAs. Furthermore, our results indicate that this mechanism may be regulated by neuronal activity. We demonstrate that a chronic blockade of activity significantly upregulates not only total and surface synaptic levels of GluA1, but also mRNA levels of this subunit. Moreover, protein and mRNA levels of endogenous Caspr1 are also increased upon homeostatic stimuli, as well as levels of phosphorylated ZBP1. Interestingly, it has been described that phosphorylation of ZBP1 disrupts its binding to target mRNAs, allowing the translation of those transcripts. Accordingly, through a ribonucleoprotein immunoprecipitation protocol, we show that although in basal conditions ZBP1 is able to bind to GluA1 mRNA, upon chronic changes in activity levels of ZBP1-bound GluA1 mRNA are significantly decreased.

Taken together, our results start to uncover a novel posttranscriptional mechanism that may be fundamental to regulate synaptic availability of GluA1 transcripts and contribute to the expression of homeostatic plasticity phenomena.

374 C When small non-coding RNAs meet the ribosome: Tuning the translational machinery

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The functions of ribosomes in translation are complex and involve different types of activities critical for decoding the genetic code, linkage of amino acids via amide bonds to form polypeptide chains, as well as the release and proper targeting of the synthesized protein. Non-protein-coding RNAs (ncRNAs) have been recognized to be crucial in establishing regulatory networks.[1] However all of the recently discovered ncRNAs involved in translation regulation target the mRNA rather than the ribosome. The main goal of this project is to identify potential novel ncRNAs that directly bind and possibly regulate the ribosome during protein biosynthesis. To address this question we applied various stress conditions to the archaeal model organism *Haloferax volcanii* and deep-sequenced the ribosome-associated small ncRNA interactome. In total we identified 6.250 ncRNA candidates. Significantly, we observed the emersed presence of tRNA-derived fragments (tRFs). These tRFs have been identified in all domains of life and represent a growing, yet functionally poorly understood, class of ncRNAs. Here we present evidence that tRFs from *H. volcanii* directly bind to ribosomes. In the presented genomic screen of the ribosome-associated RNome a 26 residue long fragment originating from the 5' part of valine tRNA was by far the most abundant tRF. The Val-tRF is processed in a stress- dependent manner and was found to primarily target the small ribosomal subunit *in vitro* and *in vivo*. As a consequence of ribosome binding, Val-tRF reduces protein synthesis by interfering with peptidyl transferase activity. Therefore this tRF functions as ribosome-bound small ncRNA capable of regulating gene expression in *H. volcanii* under environmental stress conditions probably by fine-tuning the rate of protein production.[2] Currently we are investigating the binding site of this tRF on the 30S subunit in more detail.

375 A Observation of mRNA Surveillance in Living Yeast by Ribosome Profiling

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The synthesis of proteins is a major step in gene expression and therefore represents a regulatory point for modulating cellular output. During elongation, ribosomes are thought to pause along the mRNA transcript upon colliding with various obstacles or translating specific sequence motifs. In addition, ribosomes halt if they arrive at the 3' end of a transcript after failing to properly terminate or if the message is endonucleolytically cleaved. Genetic studies have suggested that the protein Dom34 and a GTPase binding partner, Hbs1, target mRNAs associated with paused ribosomes for degradation (Doma and Parker 2006, *Nature* 440:461-4). While biochemical data support this idea by showing that Dom34 directly binds and dissociates ribosomes (Shoemaker *et al*. 2010, *Science* 330:369-72), the natural cellular targets of Dom34 remain unknown. To identify functions of Dom34 *in vivo*, knockout and wild type strains of *S. cerevisiae* were subjected to a high-throughput footprinting analysis (ribosome profiling) that reveals the places where ribosomes are bound throughout the transcriptome. Sites where ribosomes are selectively enriched in the knockout strain relative to those in the wild type strain likely represent places where Dom34 dissociates paused ribosomes. These data reveal that ribosome occupancy on known pauses is not generally enhanced in the strain lacking Dom34. Instead, the targets of Dom34 include a small number of specialized targets in open reading frames. In addition, ribosomes are enriched at the 3' end of many transcripts in the Dom34 deletion strain and generally covere <10 bp of polyadenine at the 3' end of the protected fragment. These data imply that Dom34 dissociates ribosomes that bypass the stop codon and thus serves as a quality control factor required for recycling ribosomes that fail to terminate by the conventional pathway (mediated by eRF1, eRF3, and Rli1). These results support a general role for Dom34 in ribosome recycling in addition to a more specialized role on particular gene products.

376 B The connection between mRNA binding by the DEAD-box helicase Ded1p and the kinetics of translation initiation.

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DEAD-box helicases are involved in virtually all steps of the RNA metabolism, but it remains challenging to define the molecular basis of their functions. Here we address this problem for the highly conserved DEAD-box helicase Ded1p from *Saccharomyces cerevisae*, which functions in translation initiation. Using a modified CLIP assay, we find that Ded1p binds to a large cross-section of cellular mRNAs. Binding sites are found throughout ORFs and on UTRs. Cognate sites do not display apparent sequence signatures. The most pronounced binding sites on the majority of mRNAs are located 30 to 80 nucleotides downstream of the initiation codon, suggesting position-dependent binding pattern of Ded1p on mRNAs. To examine the impact of mRNA binding by Ded1p on protein synthesis rates, we used a temperature-sensitive yeast strain with a genomically encoded Ded1 mutation which only impairs RNA binding, but causes a defect in translation initiation. We quantitatively measured the response of translation initiation to a sudden shift to non-permissive temperature in short time intervals over several minutes and found significant changes in the kinetic profile in the Ded1p mutant, compared to wt Ded1p. This kinetic approach provides a quantitative measure of the effect of Ded1p on translation initiation. We are currently analyzing the kinetics of this process on a transcriptome-wide level, to determine the impact of Ded1p on rate constants of translation initiation for each individual mRNA.

377 C Splicing factor SRSF3 represses the translation of Programmed Cell Death 4 mRNA by associating with the 5'UTR

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SRSF3, an SR family of RNA binding proteins, is known to regulate alternative splicing of pre-mRNA in the nucleus and facilitate export of spliced mRNA to the cytoplasm. Despite its well established roles in mRNA processing in the nucleus, the mechanism by which it regulates the fate of exported mRNA in the cytoplasm remains poorly understood. Here, we provide evidence that SRSF3 not only regulates alternative splicing of the programmed cell death 4 (PDCD4) gene in the nucleus, but also modulates the translation of the PDCD4 mRNA in the cytoplasm. We showed that SRSF3 knockdown specifically enhanced PDCD4 translation as indicated by increased mRNA in polysome fractions whereas SRSF3 overexpression showed the opposite effect, suggesting that SRSF3 is involved in repressing PDCD4 translation. Moreover, we found that SRSF3 and PDCD4 mRNA colocalized in P-bodies (PBs) where translationally silenced mRNAs are deposited and the localization of PDCD4 mRNA in PBs was abrogated in SRSF3 knockdown cells. Ribo-IP experiments established that SRSF3 binds to PDCD4 mRNA, preferentially via its 5'UTR region, to mediate translational repression. Together, these data suggest that SRSF3 may function as a oncoprotein in mammalian cells, at least in part, through repressing the translation of a critical cell death regulator.

378 A Activation of HRI Kinase and Translation Control by Oxidative Stress

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Oxidative stress causes rapid inhibition of cellular protein synthesis through phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α) by the heme-regulated inhibitor (HRI) kinase. Arsenite is a potent inducer of oxidative stress, and arsenite-induced translation inhibition leads to the assembly of stalled mRNAs in cytoplasmic stress granules (SGs). In line with the central role of HRI as a mediator of translation suppression, we observe that cells lacking HRI do not form SGs after arsenite treatment. The mechanism by which HRI is activated under conditions of oxidative stress, however, is not understood.

To address the mechanism of HRI activation, we asked whether HRI might undergo thiol conjugation under oxidative conditions. To this end, HEK293 cells were transfected with YFP-HRI and subjected to control conditions or oxidative stress. Prior to lysis, cells were treated with N-ethylmaleimide (NEM), which binds free thiols and thus prevents disulfide bond formation post lysis. YFP-HRI was immunoprecipitated and analyzed by western blot under non-reducing conditions, whereby disulfide bonds formed inside cells are retained. Our results show that HRI forms a covalent, high molecular weight complex, most likely a dimer, in cells exposed to arsenite- and hydrogen peroxide-induced oxidative stress. Further evidence for HRI dimerization was obtained by co-immunoprecipitation of HA-tagged HRI with YFP-HRI. Mass spectrometry analysis showed that HRI kinase is the only major component of the high molecular weight complex, compatible with HRI dimerization. Interestingly, we found that HRI forms a non-covalent dimer under normal conditions, whereas the covalent complex occurs only during oxidative stress, most likely by disulfide bond formation. Our results suggests that dimerization of HRI may be important for kinase activation. We are currently in the process of identifying cysteine residues involved in covalent complex formation, which will help us to fully understand the mechanism by which HRI is activated under conditions of oxidative stress.

379 B Structural basis for translation termination by archaeal RF1 and GTP-bound EF1A complex

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Translation termination occurs when one of the three stop codons, UAA, UAG, or UGA, appears at the ribosomal A site. In this process, the stop codons are recognized by the class-I release factors (RFs). The single eukaryotic class-I RF, eRF1, recognizes all three stop codons and catalyzes polypeptide-chain release. The overall shape of eRF1 resembles that of a tRNA molecule. In addition, eRF1 forms a heterodimeric complex with eRF3, an elongation factor 1 alpha (EF1A)-related essential factor, to complete the overall translation termination process in a GTP-dependent manner. eRF3 is considered to deliver eRF1 to the ribosomal A site in the translation termination process. However, the structural details for the GTPase-mediated decoding of stop codons by eRFs and the detailed molecular mimicry of tRNA remain to be clarified. eRF1 homologues are widely conserved in archaea, suggesting the conservation of a similar translation termination mechanism in archaea. In archaea, archaeal EF1A (aEF1A) functionally interacts not only with tRNA but also archaeal RF1 (aRF1) and aPelota, which facilitates mRNA surveillance, in a GTP dependent manner, and considered to perform three different functions: translation elongation and termination as well as mRNA surveillance. Here, we will report the structure of aRF1 and GTP-bound aEF1A complex determined at 2.3 Å resolution. In the structure, aRF1 interacts with the tRNA recognition site of aEF1A by mimicking the shape of tRNA, and, as a result, the overall structure of the aRF1·aEF1A complex resembles that of the tRNA·EF-Tu (elongation factor from bacteria) complex. This strongly supports our proposal that aEF1A delivers aRF1 to the ribosomal A site in the translation termination as well as tRNA during the translation elongation. Furthermore, we will describe structural basis for the translation termination process in archaea.

380 C Translation of HTT mRNA with expanded CAGrepeats is regulated by the MID1–PP2A proteincomplex

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Expansion of CAG repeats is a common feature of various neurodegenerative disorders, including Huntington's disease. Here we show that expanded CAG repeats bind to a translation regulatory protein complex containing MID1, protein phosphatase 2A and 40S ribosomal S6 kinase. Binding of the MID1–protein phosphatase 2A protein complex increases with CAG repeat size and stimulates translation of the CAG repeat expansion containing messenger RNA in a MID1-, protein phosphatase 2A- and mammalian target of rapamycindependent manner. Our data indicate that pathological CAG repeat expansions upregulate protein translation leading to an overproduction of aberrant protein and suggest that the MID1-complex may serve as a therapeutic target for the treatment of CAG repeat expansion disorders.

381 A Posttranscriptional control of the DNA damage response through TIAR

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The RNA-binding proteins TIA1 and TIAR regulate splicing of pre-mRNAs in the nucleus and repress translation of mRNAs in the cytoplasm. In response of UVB treatment, cells reduce the global rate of protein synthesis, and TIAR was shown to mediate inhibition of translation after UVB. While UV induces single-strand DNA breaks that are repaired through nucleotide excision repair, g-radiation causes more severe double-strand breaks, which require homologous recombination or error-prone non-homologous end-joining for repair.

To date, regulation of translation in response to γ-radiation has not been investigated. Using human HCT116 colon carcinoma cells, we found that the global rate of translation is attenuated in response to low dose γ-radiation in a TIAR-dependent manner. Importantly, knock down of TIAR reduces activation of the checkpoint kinase Chk2 and specifically impairs maintenance of the G2/M checkpoint in response to γ-radiation. In the absence of TIAR, cells exposed to γ-radiation enter M-phase prematurely and show enhanced proliferation rates. This goes along with strongly increased numbers of micronuclei, which result from the failure to properly segregate damaged chromosomes. When we combined TIAR depletion with p53 deletion, we observed an additive effect on checkpoint de-regulation, indicating that TIAR is an important component of a p53-independent checkpoint mechanism. We have identified putative targets of TIAR involved in G2/M checkpoint maintenance by deep sequencing of TIAR-associated mRNAs. This approach is combined with the analysis of mRNAs whose translation is specifically altered in the absence of TIAR. Taken together, out data provide evidence for the important role of a posttranscriptional, TIAR-dependent mechanism in the maintenance of genome integrity.

382 B Human DDX3 interacts with the HIV-1 Tat protein to facilitate viral mRNA translation

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Nuclear export and translation of intron-containing viral mRNAs are required for HIV-1 gene expression and replication. In this report, we provide evidence to show that DDX3 regulates the translation of HIV-1 mRNAs. We found that knockdown of DDX3 expression effectively inhibited HIV-1 production. Translation of HIV-1 early regulatory proteins, Tat and Rev, was impaired in DDX3-depleted cells. All HIV-1 transcripts share a highly structured 5' untranslated region (UTR) with inhibitory elements on translation of viral mRNAs, yet DDX3 promoted translation of reporter mRNAs containing the HIV-1 5' UTR, especially with the transactivation response (TAR) hairpin. Interestingly, DDX3 directly interacts with HIV-1 Tat, a well-characterized transcriptional activator bound to the TAR hairpin. HIV-1 Tat is partially targeted to cytoplasmic stress granules upon DDX3 overexpression or cell stress conditions, suggesting a potential role of Tat/DDX3 complex in translation. We further demonstrated that HIV-1 Tat remains associated with translating mRNAs and facilitates translation of mRNAs containing the HIV-1 5' UTR. Taken together, these findings indicate that DDX3 is recruited to the TAR hairpin by interaction with viral Tat to facilitate HIV-1 mRNA translation.

383 C Exploring the role of the GW182 protein, Gawky (Gw) during early Drosophila melanogaster embryogenesis

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The GW182 protein family is composed of multiple members, each having a high percentage of glycine-tryptophan (GW) and troptophan-glycine (WG) repetitive amino acid sequences and an RNA recognition motif on the C-terminal end. The *Drosophila* GW182 single homologue, *Gawky* (*Gw*), has been shown to participate in the microRNA (miRNA) repression pathway. A mutation in the *gw* gene results in an abnormal number of centrosome, chromosome mis-segregation and disrupted microtubule network in the nuclear mitosis during early embryogenesis (0-2hrs after egg deposition). Our work shows that Gw interacts with centrosomal structural protein centrosomin (CNN) and the interaction is mediated by RNA. Centrosomes have been seen adjacent to endogenous Gw bodies and the adjacent pattern is mitosis-phase specific in the early embryo. A subset of mRNA related to centrosomal organization function were found to associate with Gw. Notably, conserved miRNA-277-binding sites were predicted to be in the 3' untranslated regions of 4 transcripts responsible for centrosomal organization that also interact with Gw. These results suggested that Gw potentially plays an vital role of organizing centrosome duplication.

384 A Global translational control during norovirus infection

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Human norovirus (HuNV) is a member of the calicivirus family and is responsible for more than half of the viral gastroenteritis outbreaks, infecting over one million people last winter in Great Britain. Due to the lack of a suitable cell culture system, its replication mechanisms are poorly understood but two animal caliciviruses, the feline calicivirus (FCV) and mouse norovirus (MNV) provide models to increase our understanding of norovirus translation and replication.

Caliciviruses possess a positive-sense, single-stranded RNA genome of 7-8 kb, which functions as mRNA. Unlike most cellular mRNAs, the calicivirus RNA genome does not possess a 5' cap structure but instead has a 13–15 kDa viral protein, genome linked (VPg) acting as a novel proteinaceous "cap substitute" to direct translation and hijack the host protein synthesis machinery. In addition, viruses can silence the expression of genes involved in the anti-viral responses and the viral life cycle completion requires separated events occurring at different times since viral transcripts are used as template for both translation (mRNA) or replication (genomic RNA). Recent evidence suggests that the dynamic nature of both host and viral mRNA expression is a key coordinator of viral pathogenesis, with different host genes expressed at different times during infection, regulated through their storage and/or decay in subcellular compartments such as stress granules, to stall their translation, or processing bodies (P-bodies), for their further degradation. Viral proteins can also be found in these compartments, suggesting an important interplay between RNA turnover and viral life cycle.

Therefore to investigate global translational control during norovirus infection, and dissect host-pathogen interactions, we adopted a three-pronged strategy:

1. We analysed the regulation of translational factors during MNV and FCV infection and observed changes in the phosphorylation levels of eIF4E, eIF2alpha and 4E-BP1, and identified how MAPK and mTOR signalling pathways are controlling these events.

2. We investigated how infection affects the formation of P-bodies and stress granules and have evidence that MNV and FCV infection modulates the formation of stress granules.

3. We set out to dissect the global regulation of translational control by performing polysome profiling analysis to identify how host mRNAs expression is affected during MNV infection and will provide preliminary results.

The dissection of host-virus interactions during norovirus infection should ultimately contribute to the development and design of new antiviral therapy for this important human pathogen.

385 B Cap-independent translational regulation of mammalian target of rapamycin (mTOR)

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Regulation of translation is a key mechanism by which cells and organisms can rapidly change their gene expression patterns in response to extra- and intracellular stimuli. Translational control can occur on a global basis by modifications of the basic translation machinery, or selectively target defined subsets of mRNAs to maintain the synthesis of certain proteins required either for the stress response or to aid recovery from the stress. These pathways are evolutionary conserved and have been shown to significantly impact translation in organisms as diverse as yeast and humans. In many cases, features in the 5' untranslated regions (5'UTRs) of the corresponding mRNAs, such as regulatory upstream open reading frames (uORFs) and internal ribosome entry sites (IRESs) are important for them to evade global repression of translation. IRES-mediated translation is an alternative to the cap-dependent mechanism of translation initiation that involves the direct recruitment of the ribosome to the vicinity of the initiation codon and may require several trans-acting proteins known as IRES trans-acting factors (ITAFs).

Mammalian target of rapamycin (mTOR) is a highly conserved kinase that is responsive to several cellular stimuli. Deregulation of mTOR signalling is implicated in major diseases, such as cancer, mainly due to its role in regulating protein synthesis. The main mTOR targets are proteins responsible for ribosome recruitment to the mRNA, thus, a specific inhibitor of mTOR, for example rapamycin, leads to global inhibition of translation. Knowing that in stress conditions such as hypoxia, overall protein synthesis is reduced, but synthesis of mTOR protein is not inhibited, we hypothesized that mTOR 5'UTR harbours an IRES allowing cap-independent synthesis of mTOR protein in stress conditions. By using dicistronic reporter plasmids we have tested and confirmed this hypothesis. Our findings provide a framework for understanding how translational reprogramming of mTOR mRNA is regulated in response to cellular stress conditions.

386 C NALM-6 acute lymphoblastic leukaemia cell line has elevated level of the subunit b of translation initiation factor 3

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About 75 cases of acute lymphoblastic leukemia (ALL) are diagnosed yearly in the Czech Republic and many more throughout the world. The majority of these cases are in children and young adults, making ALL the most common form of malignancy in these age groups. The treatment protocols of ALL are complex and use 6–12 drugs. Many of those drugs directly or indirectly target translation, for example mTOR pathway. We studied possible role of translation initiation factor 3 (eIF3) in ALL pathogenesis. eIF3 is a multi-subunit complex participating in all steps of translation initiation. Some of its 13 subunits are also connected to fundamental cell processes such as protein degradation, cell cycle control, differentiation and apoptosis. Down-regulated or up-regulated expression of some of the eIF3 subunits was found to be typical for a variety of human cancers.

We quantified expression of individual eIF3 subunits (a, b, d, e, f, g, h, i, and j) by Real-time RT-PCR in lymphoblastic cell lines NALM-6, RS4;11, REH, TOM-1 carrying chromosomal aberrations characteristic for main types of ALL. As a non-leukemic control, we employed NC-NC cell line that represents B-cell precursor immortalized by EBV. Statistical evaluation of results revealed that the expression of eIF3b is significantly higher in NALM-6 than in REH, RS4;11, and TOM-1 cell lines. The level of eIF3b mRNA is comparable between NC-NC and NALM-6. The up-regulation of eIF3b in NALM-6 was also confirmed by quantitative Western blot analyses. Next, we tested an effect of eIF3b on growth speed of the ALL cell lines and found strong correlation between eIF3b expression level and their doubling time. NALM-6 and NC-NC grew faster and better than the other cell lines. On the contrary, TOM-1 grew the most slowly even in the presence of 20% FBS.

However, eIF3b subunit has not been considered to be the subunit of eIF3 which affects general translation primarily; we assume that eIF3b level could be somehow connected to the rate of translation. Our preliminary experiments also suggest that eIF3b expression level reflects rather a particular stage of B-cell development, which the particular ALL cell line is derived from, than a direct or indirect influence of specific chromosomal gene fusion.

Regarding NALM-6, the corresponding leukaemia is very aggressive, refractory to treatment, and patients have very poor chances of recovery. We presume that deregulation of translation can contribute to poor prognosis of those patients.

387 A Caspase-3 cleaves hnRNP K in erythroid differentiation

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Post-transcriptional control of gene expression is crucial for the control of cellular differentiation. Erythroid precursor cells loose their organelles in a timely controlled manner during terminal maturation to functional erythrocytes. Extrusion of the nucleus precedes the release of young reticulocytes into the blood stream. The degradation of mitochondria is initiated by reticulocyte 15-lipoxygenase (r15-LOX) in mature reticulocytes. At that terminal stage the release of r15-LOX mRNA from its translational silenced state induces the synthesis of r15-LOX. Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a key regulator of r15-LOX mRNA translation. HnRNP K that binds to the differentiation control element (DICE) in the 3' untranslated region (UTR) inhibits r15-LOX mRNA translation initiation. During erythroid cell maturation, activation of r15-LOX mRNA translation is mediated by post-translational modifications of hnRNP K and a decrease of the hnRNP K level. To further elucidate its function in the post-transcriptional control of gene expression we investigated hnRNP K degradation employing an inducible erythroid cell system that recapitulates both nuclear extrusion and the timely controlled degradation of mitochondria mediated by activation of r15-LOX synthesis. Interestingly, we detected a specific N-terminal cleavage intermediate of hnRNP K lacking DICE binding activity that appeared during erythroid differentiation and puromycin induced apoptosis. Employing mass spectrometry and enzymatic analyses we identified Caspase-3 as the enzyme that cleaves hnRNP K specifically. In vitro studies revealed that cleavage by Caspase-3 at amino acids (aa) D334-G335 removes the C-terminal hnRNP K homology (KH) domain 3 that confers binding of hnRNP K to the DICE. Our data suggest that the processing of hnRNP K by Caspase-3 provides a save-lock mechanism for its timely release from the r15-LOX mRNA silencing complex and activation of r15-LOX mRNA synthesis in erythroid cell differentiation.

388 B Translational regulation of human hemojuvelin expression via upstream open reading frames

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Hemojuvelin (HJV) is a glycosylphosphatidylinositol (GPI)-linked membrane protein shown to be a co-receptor for class of ligands called bone morphogenetic proteins (BMPs). The HJV is involved on iron homeostasis through regulation of hepcidin transcription levels. Mutations on the hepcidin gene or in the HJV gene cause an early-onset inherited disorder associated to iron overload named juvenile hemochromatosis.

A better knowledge of the mechanisms implicated in HJV gene expression is crucial to understand its role in the iron homeostasis. The 5' leader sequence of the human HJV mRNA has two upstream open reading frames (uORF) with 28 and 19 codons created by two upstream AUGs (uAUGs) that share the same stop codon. Reporter constructs containing several HJV 5' leader sequences fused to the Firefly luciferase cistron were tested in HeLa and HepG2 cells to evaluate the effect of these uORFs in the translational regulation of HJV mRNA. Luciferase activity was measured by luminometry and the corresponding mRNA levels, quantified by real-time RT-PCR.

The results revealed that the HJV uORFs decrease the translational efficiency of the main ORF in about 6-fold. Furthermore, we have observed that the production of HJV protein is mainly due to translation reinitiation. Thus, HJV mRNA has a low leaky scanning ability that contributes to the translational repression of the main ORF. We also observed that the amino acid sequence of the uORF2-encoded peptide seems to cause ribosomal stalling, which also impedes translation of the downstream main ORF. In addition, our preliminary results show that in HepG2 cells submitted to eIF2α phosphorylation or iron overload, the HJV uORFs-mediated translational repression is released. These results suggest that these uORFs play a role in regulating HJV expression levels in response to iron overload.

389 C Gene silencing using artificial small RNAs derived from a natural RNA scaffold in Escherichia coli

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Small noncoding RNAs (sRNAs) function as central regulators in *E. coli* in response to diverse environmental growth conditions. While gene silencing by sRNAs is known to occur through RNA-RNA interactions, the detailed mechanisms underlying base-pairing between sRNA and target mRNAs in the cell remain unclear at present. In the current study, the mechanism of base pairing *in vivo* was explored using artificial sRNA (afsRNA) loaded with various target recognition sequences. An artificial small RNA (afsRNA) scaffold was designed from a natural sRNA, SibC. Using the *lacZ* reporter system, the gene silencing effects of afsRNAs were examined in *E. coli*. Substitution of the original target recognition sequence with a new sequence recognizing *lacZ* mRNA led to effective reduction of *lacZ* gene expression. The target recognition sequence was shortened to 10 nt without significant loss of gene silencing, although this minimal length was limited to specific target mRNA sequences. The presence of mismatched or unmatched regions in the middle of the target recognition sequences of afsRNAs hindered gene silencing, but internal loop-forming afsRNAs were more effective in gene silencing than bulge-forming afsRNAs. Notably, gene silencing by afsRNA was not decreased, but increased upon *hfq* disruption in *E. coli*, particularly when interactions between afsRNA and mRNA were weak, suggesting that Hfq is possibly involved in destabilization of the RNA-RNA duplex. This Hfq function, opposite to its known role in enhancing gene silencing, may contribute to reducing the off-target effects of sRNA caused by unwanted target recognition through short base-parings, thereby increasing target specificity.

390 A Translation of human LAT2 mRNA is controlled by a short upstream open reading frame

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Ribosome profiling has shown that ~25% of mammalian mRNA transcripts contain ribosome densities on short upstream open reading frames (uORFs) in the 5' UTR. The functions and mechanisms of these uORFs are still poorly defined. Human LAT2 hydrophobic amino acid transporter mRNA is an example of a uORF containing transcript. A short polypeptide composed of ~40% phenylalanine and leucine is encoded by the LAT2 uORF.

We are investigating how this uORF regulates the translation of the LAT2 gene. Mutation of the uORF AUG start codon to AUC or AAG significantly decreases translation of the LAT2 protein coding open reading frame *in vivo*. Toeprinting experiments demonstrate uORF translation *in vitro*. These results are in contrast to known models of uORF function wherein uORF translation is generally inhibitory to translation and elimination of uORF would lead to increased levels of translation. To elucidate the mechanism of uORF dependent translational regulation of LAT2, we performed additional toeprinting and photo-crosslinking experiments which indicate that the LAT2 5' UTR can recruit ribosome independently of a 5' cap. An additional ribosome binding site seems to be present between the uORF and the start of the LAT2 coding sequence. We propose a model where an inactive ribosome is initially bound within the LAT2 5'UTR. 5' Cap dependent translation of the uORF by a second ribosome leads to RNA structural switch and thereby activates the first ribosome. We are working on the validations of this model *in vitro* and *in vivo*.

391 B Regulation of mRNA translation in late-phase activated macrophages

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Macrophages strongly respond to different inflammatory stimuli such as bacterial lipopolysaccharide (LPS) with a coordinated release of cytokines, the production of oxygen radicals and by stimulating phagocytosis. In the early phase of activation, macrophages primarily secrete pro-inflammatory cytokines that trigger the immune response. Late after activation, macrophages produce anti-inflammatory cytokines to dampen the immune reaction and resolve inflammation. Moreover, macrophages in the late phase enter a state of unresponsiveness (endotoxin tolerance), during which they cannot be re-activated. This sequence of events requires tightly coordinated changes in the gene expression program of macrophages. While the regulation of transcription and mRNA stability during macrophage activation has been studied extensively, much less is known about the regulation of translation. Here we present a systematic analysis of changes in mRNA translation that occur in late-phase activated macrophages.

By recording polysome profiles of mouse RAW264.7 macrophages, we observed a global suppression of mRNA translation in late-phase activated macrophages. The percentage of polysomal ribosomes was reduced by 50% after 16 hours of LPS treatment. To identify mRNAs whose translation rate is regulated specifically during this response, we performed microarray analysis of mRNAs associated with light and heavy polysomes. Thereby, we discovered 61 messenger RNAs that escape global translation suppression, indicating that their protein products are important for the late phase of macrophage activation. These mRNAs encode inhibitors of the NFkB transcription factor complex, proteins involved in producing reactive oxygen and nitrogen species, and a family of small GTPases known to participate in microbial defense. In addition, our analysis identified 52 mRNAs whose translation is actively suppressed in late-phase activated macrophages, including mRNAs encoding transcription factors and activators of inflammation. Our results strongly suggest that translation control contributes to the unresponsive state of late-phase activated macrophages. Currently, we are in the process of identifying regulatory elements within a selected number of the identified mRNAs. This will serve as a basis for further investigating the mechanisms by which specific control of translation is achieved.

392 C Two Retinoblastoma associated SNVs in RB1 form a RiboSNitch.

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Retinoblastoma (RB1) is a negative regulator of the cell cycle and also involved in tumor suppression. Recent computational analysis of known, disease-associated Single Nucleotide Variants (SNVs) in the human genome suggest that two disease-associated SNVs found in the 5' UTR of the RB1 gene alter the mRNA transcript structure. These two mutations, G17C and G18U in RB1's 5' UTR, are also predicted to affect the structure of a putative Internal Ribosome Entry Site (IRES). Using Selective 2'-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) we have analyzed both the wild type (WT) RB1 and the RB1 mutants G17C and G18U. The data reveal significant changes in SHAPE reactivity in both mutants compared to the WT along with structural changes to the IRES site, consistent with the computational prediction. The RB1 5' UTR is thus a RiboSNitch, and our data suggest that SNV induced conformational change in mRNA is likely a drive of oncogenesis.

393 A Regulation of translation of the most abudant protein in human body, type I collagen.

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Type I collagen is the most abundant protein in human body and is composed of two α1(I) and one α2(I) polypeptides. During wound healing or in fibrosis the synthesis of type I collagen is increased several hundred fold, predominantly due to increased half life and more efficient translation of collagen mRNAs. A unique stem-loop structure in the 5'UTR of collagen mRNAs (5'SL) regulates translation by binding LARP6. LARP6 recruits positive and negative regulators of translation to collagen mRNAs to balance the synthesis of α1(I) and α2(I) chains. RNA helicase A (RHA) is recruited to facilitate translation initiation, without RHA collagen mRNAs can not be loaded onto polysomes. LARP6 also associates collagen mRNAs with vimentin and nonmuscle myosin filaments. Binding to vimentin stabilizes collagen mRNAs, while nonmuscle myosin is required for coordinated translation of α1(I) and α2(I) mRNAs. When nonmuscle myosin filaments are disrupted, collagen mRNAs fail to co-localize to discrete sites of their translation and the cells can secrete only homotrimers of α1(I) polypeptides. This suggests that for the proper assembly of type I collagen, collagen mRNAs must be co-localized and translated in coordination. On the other hand, Serine-Threonine kinase Receptor Associated Protein (STRAP) is recruited to restrict random translation of collagen mRNAs. Like RHA, STRAP is tethered to collagen mRNAs by interaction with LARP6. STRAP prevents unrestricted translation of collagen mRNAs by interacting with eIF4A. In the absence of STRAP eIF4A freely associates with collagen mRNAs and promotes their loading onto the polysomes. This results in overproduction of collagen polypeptides, in imbalance of synthesis of α1(I) and α2(I) polypeptides and in their inefficient assembly into collagen heterotrimer. These defects can be partially restored by supplementing STRAP. The mechanism of coordinated translation of collagen mRNAs, mediated by binding of LARP6 to 5'SL of collagen mRNAs, will be presented.

394 B Regulation of translation of collagen mRNAs by STRAP

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Type I collagen is the most abundant protein in human body and is composed of two α1(I) and one α2(I) polypeptides, which are encoded by two different mRNAs. However, both collagen mRNAs contain in their 5'UTR a conserved structure, termed the 5' stem-loop (5'SL). We have cloned LARP6 as the protein which binds 5'SL with high affinity and specificity. This binding regulates translation of collagen mRNAs. Among other interactions, LARP6 also interacts with protein STRAP (also called unrip). STRAP is involved in translation of IRES containing mRNAs by interacting with protein unr and in formation of snRNPs by interacting with Gemin 7. The last 20 amino-acids at the C-terminus of LARP6 are required for its interaction with STRAP, these amino-acids are conserved in unr and Gemin 7, representing the STRAP interacting epitope. Overexpression of STRAP restricts translation of collagen mRNAs, as evidenced by lower protein level and shift of collagen mRNAs from heavy polysomes. Knock out of STRAP results in overexpression of collagen α1(I) and α2(I) polypeptides and high loading of collagen mRNAs onto polysomes. However, collagen polypeptides in the STRAP knock out cells are hyperglycosylated and fail to assemble into collagen triple helix. The hyperglycosylation takes place if the synthesis of α1(I) and α2(I) polypeptides is not coordinated, the randomly made polypeptides fail to productively fold into triple helix and are exposed to the modifying enzymes for prolonged period of time. These results suggest that STRAP restricts random translation of collagen mRNAs and that this inhibition is necessary to coordinate translation of collagen α1(I) mRNA to that of α2(I) mRNA. A working model of regulation of collagen translation by STRAP will be presented.

395 C Dissecting the regulation of vFLIP expression, a Kaposi's Sarcoma-associated Herpesvirus tumorigenesis factor

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Kaposi's Sarcoma-associated Herpesvirus (KSHV) is an oncogenic virus, the etiological agent of Kaposi's Sarcoma (KS); it is also associated with multicentric Castleman's disease (MCD) and primary effusion lymphoma (PEL). The infection is mainly latent in KSHV-induced tumour cells with only a few viral genes expressed that contribute to tumorigenesis. Among these, vFLIP interacts with the NFkB pathway to trigger the expression of anti-apoptotic and pro-inflammatory genes.

The expression of vFLIP is mediated by an unusual internal ribosomal entry site (IRES) element. Using an in vitro system to dissect vFLIP IRES function we identified that a minimal IRES domain is located within a coding region upstream of the vFLIP gene. Furthermore, using specific inhibitors and proteomic analysis, we found that eIF4A and eIF4G, but also eIF4E, are required for initiation of translation on the vFLIP IRES. Novel IRES-interacting proteins, such as Y box-binding protein 1, were also identified. Currently, we are investigating the requirement of the IRES for these factors and analysing the RNA structure of the vFLIP IRES. By dissecting the structure and function of the vFLIP IRES we will determine how vFLIP expression is regulated during latent KSHV infection. This will deepen our understanding of the viral trigger responsible for NFkB activation and the oncogenic properties of KSHV. The ultimate aim is to define new mechanisms to inhibit KSHV-induced tumour formation.

396 A The role of human RLI in cell proliferation and translational regulation

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RNase L inhibitor (RLI) belongs to the family of ABC (ATP-binding cassette) proteins, which carry out a broad range of functions. Human RLI was first characterized as an inhibitor of the antiviral 2-5A/RNase L system. The RNase L pathway, however, is only found in vertebrates, whereas RLI proteins are highly conserved from archaea to eukaryotes. Moreover, RLI is essential for the viability and development of several organisms. The central role of RLI remains therefore unexplained.

Recent studies link the role of RLI to several stages of eukaryotic translation, including initiation, termination and ribosome recycling. RLI has also been found to interact with a number of translational factors.

In this study, we show that human RLI downregulation has a strong effect on cultured cell proliferation, but does not significantly affect the total protein synthesis. We demonstrate the interaction of human RLI with some translation initiation factors, which are also essential for cell cycle regulation. We finally suggest that this interaction might be essential for the translational regulation of specific proteins involved in the cell cycle rather than for general translation.

397 B Dom34-mediated dissociation of non-translating ribosomes allows efficient restart of translation after stress**

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Dissociation of terminating ribosomes ensures the availability of the resulting subunits for new rounds of translation. Upon glucose depletion in yeast, translation rapidly ceases and free ribosomal subunits tightly associate, tethered by the Stm1 factor, forming a large pool of non-translating ribosomes. A mechanism dissociating Stm1-bound ribosome and allowing the reentry of these subunits in translation is thus required upon glucose repletion. Similar mechanisms must also exist in all cells as non-translating ribosomes temporarily accumulate under a variety of stress conditions and need to be released when translation resume.

The Dom34 and Hbs1 factors form a complex structurally similar to the one resulting from association of translation termination factors eRF1 and eRF3. Dom34 and Hbs1were initially characterized as implicated in the NGD RNA Quality Control Pathway. Consistently, recent biochemical analyses demonstrated that Dom34 and Hbs1, together with the factor Rli1, dissociated ribosomes stalled on a messenger RNA. These factors were also shown to dissociate associated ribosomal subunits in vitro. Dom34 and Hbs1are not essential for viability in yeast and the extent of their physiological function(s) remains unclear. Using a variety of assays, we found that Dom34-Hbs1 mediates the dissociation of non-translating ribosomes present in glucose-starved yeast, facilitating thereby an efficient restart of translation when glucose is supplied again. Our data show for the first time that Dom34 and Hbs1 dissociate non-stalled, mature ribosomes in vivo. This suggests that these factors might have a general physiological role in affecting ribosomal subunit availability, thus impacting on translation initiation, possibly even in non-stress conditions.

**Abstract presented as an oral in Plenary Session 1 - Ribosome biogenesis and translation

398 C Unravelling the role of dimerization for the STAR-domain RBP, GLD-1

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RNA-binding proteins (RBPs) are critical regulators of gene expression. Indeed, whether a particular mRNA is translated, repressed, or degraded, depends on its RBP and regulatory RNA interactions. The STAR family of RBPs includes developmental regulators such as C. elegans GLD-1, which is a key regulator of germ cell development with loss of GLD-1 resulting in a germline tumour. GLD-1 binds approximately 10% of all germline transcripts via GLD-1 binding motifs (GBMs) in UTRs[1] resulting in translational repression and/or stabilisation of its target mRNAs[2]. Dimerization of RBPs is generally thought to be required for their functions in RNA-recognition, mRNP complex formation, RNA oligomerization and controlling the balance between an RBP and its targets. GLD-1 and its closest STAR protein family homologs, e.g. Quaking (QKI), can homodimerize[3,4]. Inhibiting QKI dimerization causes embryonic lethality although the molecular basis for this is unknown[5]. We find that dimerization of GLD-1 is generally not required for its function as it does not phenocopy the null mutant and the global RNA binding ability of dimerization mutant GLD-1 is largely unchanged. However, inhibiting GLD-1 dimerization does leads to a high percentage of sterile worms. We hypothesize that GLD-1 dimerization is involved in regulating specific mRNAs, possibly affecting different aspects of mRNA regulation for different targets.

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399 A Tdrd7, a RNA binding protein, acts to restrict Epha2 protein synthesis in space and time during lens development

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The formation of tightly controlled cell-cell adhesions and adherens junctions with each other enables lens fiber cells to adopt a highly ordered structure that allows for the optical transparency of the mature ocular lens. The precise spatial and temporal regulation of mRNA and local protein synthesis is essential for establishing functional compartments within eukaryotic cells, but whether such a process occurs during lens development is unclear. We report here that Tdrd7, a Tudor domain-containing RNA binding protein, is required for maintaining lens fiber cell morphology and cell-cell interactions. Lens fiber cells in cross section adopt a regular hexagonal pattern. In contrast, lens fiber cells in *Tdrd7* mutant mice appear irregular in cross section with blebbing of the cell membrane. These phenotypes resemble those observed in the lenses of mice lacking Epha2, a receptor tyrosine kinase (RTK) in the Eph-Ephrin signaling pathway that can mediate diverse processes in development. Tdrd7 expression is spatially and temporal regulated during lens development. Tdrd7 is initially expressed in distinct cytoplasmic granules in lens fiber cells at the onset of lens vesicle formation, and then shifts to nuclei when lens fiber cells differentiation commences. Previous RNA-immunoprecipitation (RIP) experiments by us have shown that Tdrd7 can bind Epha2 mRNA. Examination of Tdrd7 null lenses shows decreased Epha2 protein but not mRNA. Moreover, Epha2, which is localized primarily to the short ends of fiber cells, is mislocalized in the Tdrd7-null lens, suggesting that Tdrd7 acts to help restrict Epha2 protein synthesis in time and space.

400 B Alternative polyadenylation in CD2 expression

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Alternative polyadenylation (APA) in the 3'UTR has a fundamental role in gene expression in a variety of cellular programs: cellular differentiation, leukocyte activation and cancer. The physiological relevance of APA was accentuated by the observation that human T lymphocytes stimulated through T cell receptors express preferentially mRNA isoforms with shorter 3'UTRs. CD2 is a T cell surface glycoprotein involved in signaling transduction and T cell activation. Transcription of CD2 results in the formation of multiple mRNA species whose physiological function has remained undisclosed. CD2 contains a conserved 3'UTR across species, from rodents to humans, which may indicate the presence of regulatory elements in this region. CD2 3'UTR contains a proximal and a distal poly(A) signals that are used in human T lymphocytes to generate two mRNA species with different 3'UTR. In mouse, however, only the shorter mRNA produced by usage of the first poly (A) site has been reported in T cells. By immunohistochemistry, Northern blotting and RT-PCR using perfused rat brains, we now present evidence that CD2 is also expressed in different regions of the brain, specifically in the cortex, cerebellum, hippocampus and thalamus, which represent a significant part of the encephalon. CD2 appears to have a very low expression in the hippocampus, contrary to what happens in the cortex where relatively high levels of expression were detected. Additionally, we have mapped the 3' ends of the CD2 mRNA isoforms by 3'RACE/sequencing to show that the two poly(A) signals are used generating two mRNAs with different 3'UTR lengths, both in the Jurkat T cell line as well as in the rat brain. Although some reports have described the expression of immune molecules in neurons the role of these molecules in the nervous system is still unknown. As the nervous system is similar to the immune system with respect to many phenomenological, functional and molecular properties, a potential role for CD2 in the brain will be discussed.

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401 C Post-transcriptional regulation of COX-2

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The oxidative conversion of arachidonic acid to prostaglandin H_2 is carried out by a set of two enzymes termed cycloxygenases, abbreviated as COX. COX-1 is constitutively expressed in normal tissues, while COX-2, which is not expressed in normal tissues, is transiently induced from external stimuli, such as pro-inflammatory cytokines. COX-2 is also overexpressed in numerous cancers. We show that COX-2 protein expression is constitutive in a lung cancer cell line, A549, but not expressed in a normal bronchial cell line, Beas2B. Previous work from our lab has shown that COX-2 has two polyadenylation signals present in its 3'UTR that can potentially be utilized. Alternative polyadenylation is a post-transcriptional mechanism by which mRNAs can produce variable 3' untranslated region (UTR) lengths is through usage of alternative poly(A) sites. Our RNAse H-coupled RT-PCR data indicates that both COX-2 mRNA isoforms, resulting from usage of two different poly(A) sites, are transcribed in A549 lung cancer cells. Another means of post-transcriptional regulation is mediated through microRNA repression. We have Real-Time qPCR data and microarray data that show decreased expression of specific miRs in lung cancer cells as compared to normal lung cells. The biological function of COX-2 is to produce prostaglandins; we have also demonstrated that COX-2 specific miRs can modulate resulting prostaglandins produced. We speculate that many post-transcriptional mechanisms work in concert to regulate COX-2 expression, which may explain the employment of an alternative poly(A) signal.

402 A Assessing the "geometry" of the TRAMP and exosome complexes.

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The exosome complex plays major roles in RNA 3'->5' processing and surveillance activities. The TRAMP polyadenylation complexes are major cofactors for the nuclear exosome and play key roles in nuclear RNA quality control. The TRAMP complexes include the RNA helicase Mtr4 together with a Zn-knuckle RNA binding protein (Air1 or Air 2) and a poly(A) polymerase (Trf4 or Tr5). However, Mtr4 is an essential exosome cofactor of all its characterized nuclear RNA degradation and processing activities, whereas only surveillance activities are known to require the other TRAMP components. Recent structural analyses of Mtr4 have identified an "Arch" domain, which acts independently of the helicase activity and is required to stimulate exosome function *in vivo* and *in vitro*. An *in vivo* crosslinking technique (CRAC) was used to compare RNA targets of intact Mtr4 and an Mtr4-Archless construct, lacking the Arch domain. Substantial differences between RNA species associated with the two constructs were revealed. In particular, Mtr4-Arch shows reduced targeting of pre-rRNA species, consistent with observed defects in pre-rRNA processing and growth. In addition, the fraction of Mtr4-associated RNA sequences that carry TRAMP-generated, non-encoded oligo(A) tails was lower in strains lacking Arch domain. This is consistent with the reported role for Mtr4 in the regulation of oligoadenylation of target RNAs by the TRAMP complex.

In vitro functional and structural analyses show that RNA is threaded through the central channel of the exosome. However, it is also feasible that substrates could be "docked" directly to the ribonucleases Rrp44 and Rrp6. We have proposed that highly structured RNAs may be docked, rather than threaded through the lumen of the exosome core structure, and that this might help distinguish RNAs targeted for maturation or decay. To assess how Mtr4 affects the interaction of the core exosome with these different substrate classes, we performed CRAC on the exonuclease Rrp44 (WT and catalytic mutant) in strains expressing Mtr4-Archless and in strains with mutation that occlude the RNA channel within the exosome. High-throughput sequencing is currently being carried out. These analyses should clarify the role of Mtr4 and the Arch domain in substrate recruitment, exosome activation, and mode of degradation.

403 B Role of mRNA 3′ processing in the progression of the DNA damage response (DDR)

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The cellular response to DNA damage could be either in the survival mode, where DNA repair and cell cycle arrest occur, or in the death mode, where apoptosis is induced. The mRNA 3'-end processing machinery is involved in the response to DNA damage. As the poly(A) tail in the 3' end of mRNAs is important to control mRNA stability, mRNA export and translation; the regulation of 3'-end processing during DDR represents an important mechanism to control gene expression. Recently, we have described a novel feedback loop between p53 and polyA specific ribonuclease (PARN), where PARN deadenylase keeps p53 levels low in non-stress conditions by destabilizing p53 mRNA through the AU-rich elements (AREs) present in the 3'UTR. After UV, the p53 expression levels increase resulting in the p53-mediated activation of PARN deadenylase, which represents a mechanism of p53-mediated regulation of gene expression in a transactivation-independent manner.

As the levels of p53 expression increase after DNA damage treatment, the PARN-mediated down-regulation of p53 mRNA should be reverted during the progression of DDR. It has been shown that HuR, an ubiquitously expressed ARE-binding protein, binds the AREs in p53 3'UTR resulting in the regulation of p53 mRNA translation and expression upon stress. Our results also indicate that under normal conditions Ago2 and PARN bind to a region in the p53 3'UTR where a miRNA-binding site and ARE sequence overlap, and both of them dissociate after UV treatment. Interestingly, our results indicate that under DNA damaging conditions HuR can compete for binding to the p53 3'UTR with both PARN and Ago2 in *in vitro* and *in vivo* assays, resulting in the release of PARN and Ago2 and in the increase of p53 expression levels. Based on these results, our working model is that the dynamic binding of PARN, Ago2 and HuR to the p53 3'UTR plays a role in the progression of DDR.

Together our studies provide new insights into p53 function and the mechanisms behind the regulation of mRNA 3' end processing in different cellular conditions, providing new approaches in the design of new cancer therapies.

404 C Transcriptional and translational profiles in stimulated T lymphocytes

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The many processing steps to which RNA polymerase II transcripts are subjected include cleavage and polyadenylation within the 3' untranslated region. Many genes have multiple polyadenylation sites, whose differential use results in different mRNA isoforms. Although the processing efficiency for pre-mRNAs is largely determined by the strength of their polyadenylation signals, recent studies showed that polyadenylation site choice can be influenced by several protein factors that have long been known to be involved in pre-mRNA processing. One of the model systems in which systematic changes in the use of polyadenylation sites are studied is T cell activation, but similar changes have also been observed in development and cell differentiation.

In this study we undertook a systematic investigation of the pattern of poly(A) site selection and its influence on the level of protein synthesis during activation of T cells. By deep sequencing of mRNA 3' ends we mapped polyadenylation sites in resting and activated murine and human lymphocytes. At the same time we measured global transcript and protein levels. Through computational analysis we inferred genes that undergo a significant shift in polyadenylation site use between the two states and we characterized the properties of the respective sites. Because shorter transcripts isoforms lack sequence elements with negative impact on mRNA stability and translation -such as miRNA binding sites- it has been hypothesized that the shorter transcript isoforms are associated with an increased protein production as found in highly proliferating cancer cells. Here, we investigated this hypothesis by measurements of mRNA, 3' end processing and protein levels in primary cells under noncancerous conditions.

405 A Shifting targets: microRNA variants and alternative polyadenylation in cardiac hypertrophy

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Emerging findings indicate that cells can produce both miRNAs and their mRNA targets in multiple processing variants as a means to increase the complexity of miRNA-mediated control in a tissue and developmental stage-specific manner. MiRNAs play critical roles in the heart, and we hypothesise that during the cardiac hypertrophic response there are changes to both miRNA processing and mRNA 3' polyadenylation site selection, which will alter miRNA/mRNA interactions. Transverse Aortic Constriction (TAC), a model of left ventricular hypertrophy, was used to obtain pre-hypertrophic and hypertrophic cardiomyocytes. TAC led to pressure overload and left ventricular hypertrophy, characterised by an increase in left ventricular weight and induction of hypertrophic markers.

RNA was then extracted from purified cardiomyocytes for next-generation sequencing of small RNAs and mRNA 3' ends. Several miRNAs were deregulated prior to the development of hypertrophy and in the hypertrophic hearts. Processing variants, such as isomiRs and unexpected arm bias, of key cardiac miRNAs were also identified that could potentially alter their targeting specificity. Furthermore, numerous mRNAs encoding important cardiac functions are subjected to alternative polyadenylation that alters the length of the 3'UTR. The 3'UTR length changes may alter the extent to which miRNAs can regulate these mRNAs. The sequencing has produced global information on expression changes to both miRNA sequence and mRNA 3'UTR lengths, allowing us to form a systems level understanding of miRNA-regulation during cardiac hypertrophy.

The realisation that cardiac miRNAs and their targets exist as currently under-appreciated variants with potentially complex effects on target specificities has important implications for the role of miRNAs in cardiac disease.

406 B Towards the understanding of the CTD-code

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RNA polymerase II (RNAPII) is not only the fundamental enzyme involved in the gene expression but also the central coordinator of co-transcriptional processing. RNAPII associates with a large number of enzymes and protein/RNA-binding factors through its C-terminal domain (CTD). The CTD consists of tandem repeats of the heptapeptide consensus Y1S2P3T4S5P6S7. The CTD is dynamically post-translationally modified, e.g. phosphorylated at various positions. These phosphorylations creates specific patterns of the CTD, which predominate at different stage of the transcription cycle, control the recruitment, activation, and displacement of various factors involved in transcription and RNA processing.

The CTD is flexible but it can acquire diverse structures upon binding with corresponding protein factors. Our study that combines various approaches ranging from nuclear magnetic resonance (NMR), through fluorescent anisotropy (FA) to small angle x-ray scattering (SAXS) aims to understand how the full-length CTD is modulated upon binding with multiple copies of protein factors containing CTD-interacting domains. By combining the recombinant protein technology, with the afore mentioned techniques we are trying to uncover how proteins are organized along the whole CTD length at atomic level. Revealing the structural behavior of the CTD will help to understand the role of CTD and its post-translational modifications in the regulation of the transcription cycle.

407 C Enhancer of RNA Interference -1-LIKE-1: One More Player in the RNA Processing Game of the Chloroplast

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Ribonucleases are a group of enzymes widely distributed in nature. In the chloroplast, a network of post-transcriptional modifications of RNA molecules is mediated by ribonucleases. Our lab found that ENHANCER OF RNA INTEREFERENCE-1-LIKE-1 (ERL1, named following plant nomenclature conventions) in *Arabidopsis thaliana*, is such an enzyme. ERL1 belongs to a family of exoribonucleases which share a common 3'-5'exonuclease domain (EXOIII domain) containing a highly conserved DEDD motif. Homologues of ERL1 fullfil various functions in RNA metabolism by participating in tRNA and rRNA processing in bacteria and in the regulation of RNAi and rRNA maturation pathways in eukaryotes. By confocal microscopy we showed that ERL1 is targeted to the chloroplasts. We have generated *Arabidopsis thaliana* and *Nicotiana benthamiana* plants that misexpress ERL1. The misexpression of ERL1 leads to phenotypes indicative of defects in chloroplasts development. Since ERL1 acts in the chloroplast, we used these transgenic lines to study the impact of ERL1 on chloroplastic related genes and measured the chlorophyll content. In addition we showed that *in vitro* purified ERL1 protein is capable of processing different RNA substrates. Altogether our results suggest that ERL1 is another piece in the puzzle of the complex posttranscriptional regulatory machinery of the chloroplasts.

408 A FUS protein interacts with U7 snRNP and plays a role in replication-dependant histone genes expression

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The U7 small nuclear ribonucleoprotein (U7 snRNP) is an essential factor mediating the unique 3'end processing of non-polyadenylated, replication-dependent histone mRNAs in metazoans. These histone genes expression and processing of their transcripts are cell cycle-regulated mechanisms that recruit a number of specific proteins as well as common factors required for expression and maturation of polyadenylated mRNAs. However, despite all the knowledge we have so far, there are still gaps in understanding of core histone RNA 3' end processing, its coupling to transcription and regulation during cell cycle. To further elucidate this phenomena we used affinity chromatography based on tagged version of U7 snRNA molecule to identify proteins associated with U7 snRNP/U7 snRNA that could be potentially involved in core histone genes expression in human cells.

Mass spectrometric analysis of affinity-purified fraction revealed, among others, multifunctional RNA/DNA-binding protein FUS/TLS (fused in sarcoma/translocated in liposarcoma) as a new factor interacting with U7 snRNA/RNP. Co-immunoprecipitation and RIP experiments confirmed the binding between FUS and the U7 RNA/snRNP. Interestingly, FUS:U7 snRNA interaction seems to be activated in S phase where the core histone genes are expressed. Moreover, FUS co-fractionates in 10-50% continuous glycerol gradient with other factors involved in histone pre-mRNAs 3'end processing. However, this unique 3'end maturation was not disturbed upon FUS knockdown. Instead, we found that FUS depletion leads to a de-regulation of expression from selected histone promoters, suggesting that FUS is rather involved in regulation of core histone genes transcription. Thus, FUS bound to U7 snRNP can play a role in coupling between transcription and 3'end processing of replication dependant histone mRNAs.

409 B Mammalian mRNA 3'end formation: is this the end? New insight on CstF64

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Constitutive polyadenylation (CPA) and alternative polyA site selection (APS) participate in the control of cell life. Together with splicing, they generate alternative mRNA/protein isoforms, and/or mRNAs containing different regulatory elements in their 3' untranslated regions. Alterations of these reactions often occurs in cancer and in neurological disorders. In this context CstF plays a critical role. This complex consists of three subunits of 77, 64 and 50 kDa and seems to act as a hexamer at least in some steps of CPA. CstF64 binds preferentially to a G/U rich downstream sequence element and thereby helps to define the polyadenylation site. Several reports indicate that CstF64 is crucial in cell growth and development. Importantly, several tissue-specific splicing isoforms of CstF64 and a paralogue on chromosome 19 (CstF64Tau) have been described. This leads to our main hypothesis that changes in CstF64/CstF64Tau ratio or in the abundance of various CstF64 splicing isoforms may be important in regulating APS in cell growth and development. Interestingly, CstF77 and 64, but not 50, are also involved in replication-dependent histone RNA 3' end processing even if there is no direct evidence of any RNA binding activity. This obviously raises questions about the function of CstF in histone mRNA processing.

Firstly, we analysed the CstF64/CstF64Tau ratio in HeLa cells throughout the cell cycle. This revealed a small but interesting differential expression of the two paralogues in diverse phases. Moreover, by using an inducible knock-down system, we were able to show a defect of CstF64 (and for less extends CstF64T) lacking cells in G1 to S phase transition. Our current research aims to shed light on the role of CstF64 and CstF64Tau in cell cycle progression and/or histone mRNA 3'end processing. We are currently performing IPs, in vivo processing assay for histone mRNAs and dissecting the molecular basis of this deregulation by studying recombinant protein interactions.

Secondly, we investigated the CstF64 splicing pattern during retinoic acid-induced differentiation of the human neuroblastoma cell line SH-SY5Y. In this system we detected a concrete and substantial change in the ratio of specific splicing isoforms. Interestingly, the protein variants differ in their predicted structures and possibly protein:protein interaction properties. We are characterizing the biological implication of these changes by knocking down specifically all the endogenous CstF64 isoforms and expressing RNAi-resistant versions of individual variants. In such context, we plan to study global polyadenylation site preferences by polyA-seq experiments and proteins associated with the expressed CstF64 variants by immunoprecipitation. Furthermore, we will analyse the functional impact of these CstF64 isoform manipulations on cell differentiation and growth.

410 C The U7 snRNP revisited: a complex of the core U7 snRNP, FLASH and multiple polyadenylation factors controls 3' end processing of histone pre-mRNAs in vertebrates and invertebrates

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Animal replication-dependent histone pre-mRNAs are processed at the 3' end by endonucleolytic cleavage that is not followed by polyadenylation. The cleavage reaction is catalyzed by CPSF73 and depends on binding of the U7 snRNP to a sequence in histone pre-mRNA downstream of the cleavage site. In the past 10 years the U7 snRNP has been portrayed as a simple complex of an approximately 60-nucleotide U7 snRNA and a unique Sm ring in which the spliceosomal-specific proteins SmD1 and SmD2 are replaced by the related Lsm10 and Lsm11. We recently showed that in both vertebrates and invertebrates, Lsm11 interacts with a protein called FLASH and that this interaction is essential for processing. We now demonstrate that the U7 snRNP isolated from mammalian and *Drosophila* nuclear extracts is stably associated with FLASH and a number of polyadenylation factors that we refer to as the Histone pre-mRNA Cleavage Complex (HCC). The association of the HCC with the core U7 snRNP critically depends on the interaction between Lsm11 and FLASH. The mammalian HCC consists of symplekin, CstF64 and all six CPSF subunits, including the CPSF73 endonuclease. FLASH is severely limiting in mammalian nuclear extracts, likely explaining the inability of previous studies to identify the composite structure of the U7 snRNP and its association with the polyadenylation factors. In *Drosophila* nuclear extracts, FLASH is abundant and quantitatively associated with the U7 snRNP. The*Drosophila* HCC has a similar composition to the mammalian HCC but lacks two CPSF subunits, Fip1 and CPSF30. In both mammalian and *Drosophila* nuclear extracts, the composite U7 snRNP bearing FLASH and multiple polyadenylation factors is subsequently recruited to histone pre-mRNA for 3' end processing. However, of these polyadenylation factors only three are essential for the cleavage reaction *in vivo*: CPSF73, CPSF100 and symplekin. The other subunits are likely passive spectators or play nonessential regulatory roles. Collectively, our studies revealed an unexpected complexity of the U7 snRNP and suggest that this factor functions as an RNA-guided multi-subunit nuclease where the U7 snRNA recognizes the substrate and together with three proteins of the Sm ring, SmD3, SmB and Lsm10, defines the site of cleavage that is carried out by the catalytic component, CPSF73.

411 A A search for new factors involved in 3′ end processing of histone pre-mRNAs: proteins interacting with a complex of the conserved stem-loop and the Stem-Loop Binding Protein (SLBP) in Drosophila.

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3' end processing of animal replication-dependent histone pre-mRNAs occurs through a single-step endonucleolytic cleavage that is not followed by polyadenylation. Cleavage depends on two cis-elements in histone pre-mRNA: a highly conserved stem-loop structure (SL) and a variable purine-sequence, the Histone Downstream Element (HDE). The SL interacts with the Stem-Loop Binding Protein, whereas the HDE is a binding site for U7 snRNP. We recently showed that U7 snRNP in addition to the U7-specific Sm ring and U7 snRNA contains FLASH and a number of polyadenylation factors, including the CPSF73 endonuclease. Mammalian histone pre-mRNAs are cleaved by this enzyme at a fixed distance from the HDE. SLBP and the SL structure are dispensable for processing, although they have a stimulatory effect by stabilizing U7 snRNP on histone pre-mRNA. This indicates that the mammalian U7 snRNP alone can recruit the entire machinery required for processing. In contrast, cleavage of *Drosophila* histone pre-mRNAs depends on both the SLBP and the U7 snRNP and invariably occurs 4 nucleotides after the stem. In *Drosophila* nuclear extracts, the U7 snRNP containing CPSF73 and other polyadenylation factors is efficiently recruited to the HDE even in the absence of SLBP. Thus, *Drosophila* SLBP must function in processing by recruiting an essential factor that in turn may interact with the machinery contributed by U7 snRNP. We used *Drosophila* histone pre-mRNA prebound to recombinant SLBP to purify proteins from *Drosophila* nuclear extracts that interact with the complex but not with the histone pre-mRNA lacking SLBP. Mass spectrometry identified three proteins that fit these criteria: NOT1, a DHX-type helicase and the methyltransferase responsible for modifying the 2' hydroxyl of the cap. Importantly, these three proteins do not interact with the complex of SLBP and the SL followed by 4 only nucleotides, suggesting that they are involved in 3' end processing of histone pre-mRNAs rather than in processes operating on mature histone mRNA. We identified a different complex that interacts with the 3' end of mature histone mRNA. This complex consists of multiple aminoacyl-tRNA synthetases and binds to the terminal portion of the SL in a manner independent of binding of SLBP. Potential roles of proteins that interact with the SL in histone pre-mRNA and mRNA in 3' end processing and post-processing events and their precise mode of binding in the presence and absence of SLBP are being currently investigated.

412 B Enhancer of RNA INTERFERENCE -1-LIKE-1: The ERI1 Plant Homologue Involved in the RNA Processing Game of the Chloroplast

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Ribonucleases are a group of enzymes widely distributed in nature. In the chloroplasts, a network of post-transcriptional modifications of RNA molecules, is mediated by ribonucleases. Our lab found that ENHANCER OF RNA INTEREFERENCE-1-LIKE-1 (ERL1, named following plant nomenclature conventions) in Arabidopsis thaliana, is such an enzyme. ERL1 belongs to a family of exoribonucleases which share a common 3'-5'exonuclease domain (EXOIII domain) containing a highly conserved DEDD motif. Homologues of ERL1 fulfill various functions in RNA metabolism by participating in tRNA and rRNA processing in bacteria and in the regulation of RNAi and rRNA maturation pathways in eukaryotes. By confocal microscopy we showed that Arabidopsis ERL1 is targeted to the chloroplasts. We have generated *Arabidopsis thaliana* and *Nicotiana benthamiana* plants that misexpress ERL1. The misexpression of ERL1 leads to phenotypes indicative of defects in chloroplasts development in both plant species. Since ERL1 acts in the chloroplast, we used these transgenic lines to study the impact of ERL1 on chloroplastic related genes and measured the chlorophyll content. In addition we showed that in vitro purified ERL1 protein is capable of processing different RNA substrates (5S rRNA precursor and siRNA-like oligos).Recently, an RNA sequencing analysis complemented by Northern blot analysis has showed alterations at the expression and processing levels of certain operons in the absence of plant ERIL1. Altogether our results suggest that ERL1 is another piece in the puzzle of the complex posttranscriptional regulatory machinery of the chloroplasts.

413 C A triple helix structure is able to functionally replace a poly(A) tail

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The MALAT1 locus is commonly misregulated in many human cancers and produces an abundant long nuclear-retained noncoding RNA. Despite being transcribed by RNA polymerase II, the 3' end of MALAT1 is produced not by canonical cleavage/polyadenylation but instead by recognition and cleavage of a tRNA-like structure by RNase P. Mature MALAT1 thus lacks a poly(A) tail yet is expressed at a level higher than many protein-coding genes in vivo. We find that the 3' ends of MALAT1 and the MEN beta long noncoding RNAs are protected from 3'-5' exonucleases by highly conserved triple helical structures. Surprisingly, when these structures are placed downstream from an ORF, the transcript is efficiently translated in mammalian cells despite the lack of a poly(A) tail. The triple helix therefore also functions as a translational enhancer, and mutations in this region separate this translation activity from simple effects on RNA stability or transport. This unusual form of translational control appears to be highly evolutionarily conserved as we now find that reporter mRNAs ending in a triple helix are efficiently translated in yeast. We are additionally investigating if endogenous MALAT1 may actually produce short peptides. These results provide new insights into how transcripts that lack poly(A) tails are stabilized and regulated and suggest that RNA triple helical structures likely have key regulatory functions in vivo.

414 A FPA, a regulator of alternative polyadenylation, is closely associated with cleavage and polyadenylation factors in vivo

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Alternative cleavage and polyadenylation influences the coding and regulatory potential of mRNA and where transcription termination occurs. Although widespread, few regulators of this process are known. First identified because of its role in flower development, the *Arabidopsis thaliana* SPEN family protein FPA is a rare example of a *trans*-acting regulator of poly(A) site choice (Hornyik et al., 2010). The characterization of FPA therefore provides an opportunity to reveal novel mechanisms by which poly(A) site selection can be controlled. Here we show that FPA is closely associated with core components of the cleavage and polyadenylation apparatus *in vivo*.

In order to understand how FPA might function, we developed a novel proteomics procedure based on the cross-linking of native protein interactions in living cells using formaldehyde. We then used statistical analysis of multiple biological replicates to compare proteins co-purified with FPA and the well-studied splicing factor U2B" as a control. We found that U2B" associates with known components of the U2snRNP and spliceosome as expected, but found little overlap between these proteins and those associated with FPA. Most proteins associated with FPA had domains connected to RNA processing and several had previously been identified as components of the same genetic pathway controlling flower development as FPA. Strikingly, almost all core components of the cleavage and polyadenylation machinery co-purified with FPA. We have substantiated these data with genetic analyses, revealing for example, that the function of FPA in promoting flower development requires an Arabidopsis protein related to the core cleavage, polyadenylation and termination factor Pcf11. Experiments designed to identify which of these co-purified proteins FPA interacts with directly are now in progress.

These findings establish a simple, generally useful procedure for label-free *in vivo* proteomic analysis of proteins involved in RNA processing. In addition, they suggest that the mechanism by which FPA regulates poly(A) site choice involves close, and possibly direct, association with the core cleavage and polyadenylation machinery.

415 B Regulation of stress granules formation during calicivirus infection

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Caliciviruses are single-stranded positive RNA viruses that are responsible for several important diseases in human and animal hosts. To date, the replication mechanisms of human caliciviruses are poorly understood because of a lack of a suitable cell culture system. Feline calicivirus (FCV) or mouse norovirus (MNV) share many properties with the human caliciviruses, and provide models to increase our understanding of calicivirus translation and replication.

Recent evidence suggests that the dynamic nature of mRNA expression is a key coordinator of viral pathogenesis, with different host genes expressed at different times during infection. The expression of mRNAs can be regulated through their storage and/or decay in subcellular compartments such as stress granules, to stall their translation, or processing bodies (P-bodies), for their further degradation. Moreover, proteins within P-bodies or stress granules can enhance or limit viral infection. Viral proteins can also be found in these compartments, suggesting an important interplay between RNA turnover and viral life cycle.

This is an exciting emerging field in virology and we have set out to investigate how calicivirus infection affects and regulates the formation of P-bodies and stress granules for efficient replication and will present evidence that calicivirus infection modulates the formation of stress granules.

416 C Exploring the role of mouse DEAH helicase, skiv2l2, in processing and degradation of non coding RNAs in neuronal N2A cells using high throughput pA-seq analysis

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Production and function of RNAs demands accurate transcription, processing and protein binding that is interogated by a number of mechansims. RNA surveillance and degradation insures that inaccurately transcribed or processed RNAs are efficiently removed by nuclease degradation. The RNA surveillance complex, TRAMP, has been characterized and studied quite effectively in the yeast model organism, Saccharomyces cerevisiae. Proteins orthologous to yeast TRAMP complex proteins were identified in mus musculus, however the study of RNA surveillance in mammals has only recently begun. In this work we have used small interfering RNAs to target orthologs of yeast proteins that function in RNA surveillance (Mtr4p and Rrp6p) in mouse N2A cells.

We used paired-end high-throughput sequencing of polyadenylated RNAs (pA-seq) to quantify the effects of *mMtr4* and *mRrp6* knockdowns on RNA surveillance and processing. We demonstrate that there is no difference in the accumulation or location of polyadenylation in protein coding mRNAs upon depletion of mMtr4 or mRrp6. Several targets of TRAMP mediated RNA surveillance from work in yeast do accumulate as polyadenylated RNAs in the *mMtr4*KD compared to control knockdowns. We also identified a novel target of *mMtr4* dependent RNA surveillance. The 5' leading portion of a pri-miRNA is liberated from the pre-miRNA by Drosha cleavage, and generally thought to be degraded. Our data demonstrate that *mMtr4* plays an important role in degrading these byproducts of miRNA processing. We continue to mine the current dataset for familiar, new or novel targets of mMtr4 and will report our findings.

417 A Molecular genetic exploration of the yeast DEAH helicase, Mtr4, arch domain

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Our interest in understanding how RNAs are recognized and tagged for degradation by the yeast TRAMP complex led us to explore the role of the Mtr4 arch domain in how it functions as an enzyme and a member of the TRAMP complex. Mutations in the Mtr4 arch domain were identified using a genetic screen to uncover mutations in the arch domain and c-terminus that inhibit the ability of Mtr4 to support degradation of hypomodified tRNAiMet. From this screen, 40 independent mutants were identified that had either one or more mutations in the arch domain. Six mutants within the arch domain and two mutants in the c-terminus, each with the strongest inhibitory effect on Mtr4 were chosen for further characterization.

Recombinant Mtr4 proteins and each mutant were expressed and purified from E. coli and tested for ATPase activity in the presence and absence of total tRNA and synthetic RNAs of 108 nt and 76 nt and predicted to have little to no structure were used in stimulating Mtr4 enzymatic activity. Enzymatic assays run in triplicate were used to determine the Km for ATP, Kapp for RNA and Vmax for RNAs. Our analysis demonstrated that total E coli tRNA and synthetic yeast tRNAiMet are poor at stimulating Mtr4 ATPase activity. The predicted unstructure RNAs stimulated Mtr4 ATPase activity much more robustly than did tRNA as we would have expected based on ours and other peoples work.

418 B mRNA degradation on the ribosome in Drosophila cells

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The translation and degradation of mRNAs are two key steps in gene expression. Consequently, both processes are highly regulated and targeted by many factors including miRNAs. Even though translation and mRNA degradation are tightly coupled, it was suggested that mRNAs are degraded after their dissociation from the ribosomes in cytoplasmic bodies, named P-bodies. Only recently the possibility of co-translational mRNA degradation in yeast was discussed. However, at this point it is unclear whether mRNA degradation on the ribosome would be limited to particular mRNA species and could also occur in multicellular eukaryotes.

We have investigated the possibility of co-translational mRNA degradation in *Drosophila* cells. The co-purification of mRNA degradation factors with heavy fractions of polysome profiles of cell lysates was an important first step. However, since P-bodies could co-migrate with heavy polysome fractions we further established the affinity purification of ribosomes from *Drosophila* S2 cell lysates. We could demonstrate the co-purification of various deadenylation and decapping factors with ribosomes. Interestingly, also the factors of the miRNA effector components, AGO1 and GW182, co-purify with ribosomes. Ongoing experiments investigate the general abundance of decapped mRNAs on ribosomes. In summary our findings strongly suggest the ribosome as an alternative site for mRNA degradation in *Drosophila*.

419 C The NOT2/NOT5 Module of the CCR4-NOT Complex is Required for Assembly of the Exosome

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The exosome is a conserved eukaryotic multi-subunit complex that plays a key role in processing and/or degradation of essentially all types of cellular RNAs. A different conserved multi-subunit complex known to specifically contribute to degradation of mRNAs is the Ccr4-Not complex. It carries Ccr4, the major cellular deadenylase in the yeast *S. cerevisiae,* several Ccr4-associated factors (Caf1, Caf40 and Caf130) and 5 Not proteins (Not1-5). In this study, we determine that one functional module of the Ccr4-Not complex composed of Not5 and Not2 contributes to the functional assembly of the exosome. Indeed, both Not2 and Not5 are important for exosome-dependent processing of 7S rRNA *in vivo*, and their deletion suppresses improper processing of the 5.8 S + 30 rRNA due to lack of the Rrp6 exonculease. The N-terminal domain of Rrp4 interacts with Rrp44 in the 10-subunit exosome associated with RNA and is improperly integrated into exosome complexes in the absence of Not2 or Not5. These results identify for the first time cellular components important for exosome assembly *in vivo*.

420 A Adipogenesis is efficiently regulated by SMG1 via staufen1-mediated mRNA decay

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Suppressor of morphogenesis in genitalia 1 (SMG1), a member of the phosphatidylinositol 3-kinase-related kinases family, is involved in nonsense-mediated mRNA decay (NMD). SMG1 phosphorylates Upf1, a key NMD factor. Subsequently, hyperphosphorylated Upf1 associates with SMG5-7 or proline-rich nuclear receptor coregulatory protein (PNRC2) to elicit rapid mRNA degradation. Upf1 is also known to be involved in staufen 1 (Stau1)-mediated mRNA decay (SMD), closely related to NMD. However, the biological and molecular roles of SMG1 in SMD remain unknown. Here, we provide evidence that SMG1 is involved in SMD. SMG1 is complexed with SMD factors and overexpression of a kinase-inactive mutant of SMG1 inhibits SMD efficiency. Accordingly, the cellular localization of Stau1 into processing bodies is dependent on the level of Upf1 phosphorylation. We also find that the level of SMG1 increases during adipogenesis and downregulation of SMG1 delays efficient adipogenesis, suggesting the functional involvement of SMG1 in adipogenesis via SMD.

421 B Control of mRNA metabolism by deadenylation

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The presence of a polyadenosine tail is an important determinant of mRNA translation and stability. The regulated removal of the tail, i.e. deadenylation, leads to either translational quiescence or mRNA degradation. Deadenylation, therefore, is a critical node in gene expression and is especially important in certain biological contexts such as in the early embryo and in neurons. All mRNAs deadenylate at different rates; some fast, some slow. In addition, deadenylation is enhanced by message-specific regulatory factors. For instance, many 3' UTR binding proteins and the miRNA machinery drive post-transcriptional regulation (in part) by facilitating deadenylation. Despite the importance of deadenylation, little is known about how differential rates of poly(A) tail shortening are achieved. One clue comes from studies that show that mRNA translation rates are intimately connected to deadenylation rate. Our recent work has begun to tease out the complex molecular details that intertwine translation and deadenylation. Moreover, we have concentrated on understanding the function of protein factors that facilitate removal of the poly(A) tail. Deadenylation is catalyzed by the CCR4-NOT protein complex. The function of distinct members of this complex as well as how these factors influence rates of deadenylation will be discussed.

422 C The structural and functional organization of the TRAMP complex

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The TRAMP complex is involved in the processing and surveillance of various non-coding RNAs produced by all three RNA polymerases in the cell nucleus. TRAMP adds an oligo-adenylated tail to the 3' end of selected RNAs and prompts their degradation by the exosome. The polyadenylation core of the TRAMP complex consists of the non-canonical poly(A)polymerase Trf4 and the zinc-knuckle protein Air2 (or their paralogues Trf5 and Air1). The delivery of polyadenylated RNAs to the exosome is likely mediated by the third subunit of the TRAMP complex, the RNA helicase Mtr4.

The RNA targets of TRAMP have been found to carry short oligoadenylated tails *in vivo*. It has been suggested that Mtr4 restricts the number of adenosines added by Trf4-Air2 by recognizing the 3' end of the RNA that is being polyadenylated. To visualize how the interaction between Mtr4 and Trf4-Air2 might underpin their regulation, we have studied the overall architecture of the *S. cerevisiae* TRAMP complex by combining SAXS reconstructions and crystal structures with interaction data obtained by crosslinking, mass spectrometry, biochemical and biophysical experiments. The results challenge a direct read-out mechanism of the poly-adenylated 3' end by Mtr4 and instead suggest a model where RNAs are first unwound by the Mtr4 helicase and then fed to the poly(A)polymerase.

423 A Control of mRNA decay by Puf proteins regulates ribosome biogenesis

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The Puf family of eukaryotic RNA-binding proteins plays important roles in stem cell maintenance, cell development, and differentiation by binding conserved elements within the 3' UTR of target mRNAs, typically resulting in mRNA degradation and/or translational repression. Puf proteins are characterized by their repeat domains composed of eight tandem repeats of ~36 amino acids, whereby each repeat contacts one base within an 8-10 nt target sequence containing a conserved UGU. Once bound to an mRNA target, Puf proteins elicit RNA repression by inhibition of cap-binding events or recruitment of mRNA decay factors. The yeast*Saccharomyces cerevisiae* expresses six Puf proteins, and hundreds of candidate mRNA targets of these Pufs have been predicted from physical association or bioinformatic screens. We have focused on understanding the mechanistic roles of Pufs in regulating such target mRNAs. One of our studies analyzed several of the predicted Puf4p targets that had the highest conservation of consensus binding sequence for Puf4p, and are all involved in various steps of ribosome biogenesis. Analysis of target mRNA steady state levels in wild-type versus *PUF4Δ* strains revealed only small differences between the two strains, which prompted us to examine these mRNA targets in other PUF deletion strains. Our data demonstrate that regulation of all the targets examined is controlled by the combination of Puf4p and Puf5p, suggesting a "partial redundancy" of these two Puf proteins. Over-expression of Puf4p causes delays in processing of the rRNA 35S precursor. Puf4p also appears to play a role in the export of pre-ribosomal subunits. Together, the data suggests that Puf proteins play a global role in ribosome biogenesis through the decay of their target mRNAs.

424 B Transcriptome analysis reveals thousands of targets of nonsense-mediated mRNA decay that offer clues to the mechanism in human, fish, and fly

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Many alternatively spliced isoforms contain a premature termination codon that targets them for degradation by the nonsense-mediated mRNA decay RNA surveillance system (NMD). Some such unproductive splicing events have a regulatory function, whereby alternative splicing and NMD act together to impact protein expression. Numerous RNA-binding proteins, including all the human SR splicing factors, are regulated by alternative splicing coupled to NMD, in conjunction with highly- or ultra-conserved elements [1,2]. The "50nt rule" is the prevailing model for how premature termination codons are defined in mammals, and requires a splice junction downstream of the stop codon. [3]. There is evidence that this rule holds in *Arabidopsis* [4] but not in other eukaryotes including *Drosophila* [5]. There is also evidence that a longer 3' UTR triggers NMD in yeast, plants, flies, and mammals [4,6,7].

To survey the targets of NMD genome-wide in human, zebrafish, and fly, we have performed RNA-Seq analysis on cells where NMD has been inhibited via knockdown of UPF1, a critical protein in the degradation pathway. We found that hundreds to thousands of genes produce alternative isoforms that are degraded by NMD in each of the three species, including over 20% of the genes alternatively spliced in human HeLa cells. These genes, potentially subject to regulation through NMD, are involved in many functional categories and, in human and fly, are significantly enriched for RNA splice factors, indicating that auto- and cross-regulation of splice factors through NMD is widespread. We also found a significant enrichment for ultraconserved elements in the human NMD targets, and usually these elements overlapped a poison cassette exon.

We were able to gain insight into what defines NMD targets from our RNA-Seq data. We found that the 50nt rule is a strong predictor of NMD degradation in human cells, and also seems to play a role in zebrafish and, surprisingly, in fly. In contrast, we found little correlation between the likelihood of degradation by NMD and 3' UTR length in any of the three species. In fly, we see no enrichment for longer 3' UTRs in isoforms degraded by NMD, unless they have an intron. Other features have also been associated with propensity for NMD. We also found that thousands of transcripts have uORFs that seem to affect their likelihood of degradation.

Ultimately, our findings demonstrate that gene expression regulation through NMD is widespread in human, zebrafish, and fly, and that NMD is strongly predicted by the 50nt rule but not by 3' UTR length.

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425 C Retroviral strategies for NMD evasion

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The nonsense-mediated mRNA decay pathway recognizes and degrades transcripts containing long 3' untranslated regions (3'UTRs). To counteract this activity and maintain proper gene expression, retroviruses have evolved RNA elements capable of protecting transcripts from 3'UTR length-dependent decay. The best-characterized retroviral NMD-antagonizing element, the RNA stability element (RSE) of the Rous Sarcoma Virus, stabilizes both retroviral and synthetic reporter mRNAs containing long 3'UTRs. This large (~ 400 nt) RNA segment sits immediately downstream of the viral *gag*termination codon, preventing it from being recognized as premature. Using an RNA-based affinity purification approach, we have identified a complex of proteins specifically recruited to the RSE. These proteins are currently under investigation for potential roles in stabilizing retroviral and cellular RNAs.

Second, we find that retroviral recoding elements that promote translational frameshifting or readthrough antagonize NMD at two distinct steps. Relatively frequent stop codon bypass can reduce steady-state accumulation of Upf1 in mRNPs, disrupting its ability to monitor 3'UTR length. In addition, using variants of the Moloney murine leukemia virus (M-MLV) recoding pseudoknot, we find that less frequent readthrough events permit recovery of Upf1 binding to mRNPs but remain able to inhibit degradation of mRNAs containing long 3'UTRs. Our data indicate that diverse viral and cellular recoding sequences can similarly inhibit NMD, suggesting that suppression of NMD is a general feature of translational readthrough. We are currently investigating the ability of translational readthrough to disrupt the molecular events preceding commitment to nonsense-mediated decay.

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426 A Poly(A)-specific ribonuclease (PARN): Mechanisms of processivity and catalysis

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Poly(A)-specific ribonuclease (PARN) is a divalent metal-ion dependent poly(A)-specific, processive and cap-interacting 3'-5' exoribonuclease that efficiently degrades poly(A) tails of eukaryotic mRNAs. PARN is unique among the poly(A) degrading nucleases, being the only one that has the capacity to directly interact during poly(A) hydrolysis with both the m^7G-cap structure and the poly(A) tail of the mRNA. On the basis of biochemical and structural evidence we present and discuss a working model for PARN action. The model defines two alternating reaction steps, a translocation event that pushes the scissile bond of the poly(A) substrate into the catalytic center of the active site followed by a hydrolytic event that cleaves the scissile bond. The two reaction steps are repeated and in each cycle one AMP residue is released. The reaction cycle depends on the coordinated movements of the RNA recognition motif (RRM), which pushes the substrate into the active site, and a catalytically essential His residue of the catalytic center. The model also provides a plausible mechanistic framework for how the m^7G-cap structure could allosterically affect the hydrolytic activity of PARN. Structural and functional data supporting the model will be presented. Significantly, we have found that divalent metal ions are required for both hydrolysis and substrate translocation in the active site. Our data imply that three divalent metal ions are required for proper action. Two ions participate in hydrolysis while the third plays a key role during translocation. The generality of this proposal in relationship to other processive enzymes participating in cleavage or formation of phosphodiester bonds will be discussed.

427 B DHX34 activates NMD by promoting the transition from the SURF to the DECID complex.

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Nonsense-mediated decay (NMD) is a surveillance mechanism that degrades aberrant mRNAs. A complex comprising the NMD factors SMG1 and UPF1 and the translation termination factors eRF1 and eRF3 (SURF) is assembled in the vicinity of a premature termination codon. Subsequently, an interaction with the exon junction complex induces the formation of the decay-inducing complex (DECID) and triggers NMD (1). We previously identified the DExD/H box protein DHX34 as a novel NMD factor in *C. elegans* and showed that it acts in the NMD pathway both in human cells and also in zebrafish (2,3). Here, we investigate the mechanism by which DHX34 activates NMD in human cells. We show that DHX34 is an RNA-binding protein that is recruited to the SURF complex via its preferential interaction with hypo-phosphorylated UPF1 and the kinase SMG-1. A series of molecular transitions induced by DHX34 include an enhanced recruitment of UPF2, increased UPF1 phosphorylation and finally dissociation of eRF3 from UPF1. These molecular transitions are dependent on the ATPase activity of DHX34. Altogether, these results show that DHX34 has a central role in NMD by triggering the conversion from the SURF into the DECID complex. Undergoing structural studies using cryo-EM with DHX34 in combination with NMD core factors will help establish the precise mechanism of DHX34 in the activation of NMD.

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428 C Identification of novel UPF1 target transcripts by direct determination of whole transcriptome stability.

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UPF1, an evolutionarily conserved protein, plays the central role in nonsense-mediated mRNA decay (NMD), which eliminates aberrant mRNAs harboring premature termination codon (PTC), preventing the accumulation of nonfunctional or potentially harmful truncated proteins. UPF1 is also involved in staufen1 (STAU1)-mediated mRNA decay (SMD) as well as replication-dependent histone mRNA decay. Thus, UPF1 is an important factor not only for the RNA quality control system but also for the regulation of physiological gene expression through regulation of RNA stability. In this work, we directly measured the stability of whole transcriptome in UPF1 knock-down HeLa cells by BRIC-seq method (5¢-bromo-uridine immunoprecipitation chase–deep sequencing analysis) that was developed by us. We successfully determined the half-lives of ~10,000 transcripts, and found that 785 transcripts were stabilized in UPF1 knock-down HeLa cells. Among 785 stabilized transcripts, the expressions of only 76 transcripts were increased and remaining 709 transcripts were not altered. RNA immunoprecipitation experiment showed that UPF1 bound to the transcripts whose decay was interfered but their expression was not changed, suggesting that UPF1 directly destabilizes 709 transcripts. Most of the transcripts identified as UPF1 targets in this study were not found in previous studies, therefore, we identify novel hundreds of UPF1 targets in HeLa cells. Moreover, we found that GC-rich sequence is statistically enriched among UPF1 target transcript, suggesting that UPF1 regulates the stability of mRNAs harboring GC-rich sequence.

429 A RT-qPCR Reference Genes and Potassium Stress Responses in the Haloarchaeon, Haloarcula marismortui

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Potassium ion transport is of interest in many systems as K+ concentrations are thought to be used to maintain or change a membrane potential. In halophiles, such as *Haloarcula marismortui*, intracellular potassium ion (K^+) concentration is postulated to be a mechanism for balancing the osmotic pressure experienced in a hyper-saline environment[1], and unlike most organisms, these concentrations have been reported to be in excess of 3M [2,3]. We are interested in the dependence of these halophiles upon the external and internal K+ concentration with respect to osmotic stress.

We demonstrate that cellular generation time for the halophile varies with extracellular K^+ concentration, and growth occurs on alternative monovalent ions including lithium, rubidium, and caesium at concentrations equivalent to the observed K^+ optima. We have also shown that intracellular ion concentrations correlate to ion concentrations in the media. Our results suggest *Har. marismortui* is both highly selective for, and an excellent scavenger of K^+.

In light of this information, we used qRT-PCR to analyze the change in expression of any postulated potassium transport systems. Genome analysis[5] indicates the possible existence of three separate potassium channel/transport systems in *Har. marismortui*, Trk, MthK and Pch. These are othologous to the *Esherichia coli* K-transport systems KcsA and Kch. TrkA and MthK are both KcsA orthologs, the is the constitutive K channel in *E. coli6*, while Pch is the ortholog of the Kch channel. Kch is similar to the voltage-gated K channels (Kv channels) in eukaryotic K-channels as it has a similar P-loop region, even though specific sequence identity is low, and is expressed under low K+ conditions in *E. coli* as it has an active transport mechanism[6]. As prior archaeal qRT-PCR studies appear to use only a single reference gene, we assessed the stability of several canadidate genes in order to meet the minimum of 3 reference genes as recommended by the MIQE Guidelines[4]. Using these novel references, expression of potassium channel genes was examined in cellular shock (5 min, 2 h) and stable growth (2 week) conditions at each of the analyzed K+ conditions.

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430 B Investigation of premature termination codon recognition in nonsense-mediated mRNA decay

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Nonsense-mediated mRNA decay (NMD) is best known for its role in quality control of mRNAs. Premature translation termination codons (PTCs) are recognized and the corresponding mRNA gets rapidly degraded. The basic mechanism of NMD appears to be conserved from yeast to mammals. According to the current working model, aberrant translation termination leads to NMD. It is thought that correct termination requires the interaction of the ribosome at the stop codon with the poly(A)-binding protein (PABP) mediated through eukaryotic release factor 3 (eRF3). The model predicts that in the absence of this interaction, the NMD core factor UPF1 binds to eRF3 instead and initiates the assembly of an NMD complex that ultimately leads to mRNA degradation. However, the exact mechanism that allows distinguishing between proper and aberrant (i.e. NMD-inducing) translation termination is not yet well understood.

We address this question using a tethering approach in which proteins of interest are bound to a reporter transcript into the vicinity of a PTC. Subsequently, the ability of the tethered proteins to inhibit NMD and thus to stabilize the reporter transcript is assessed. Preliminary results revealed that the C-terminal domain interacting with eRF3 seems not to be necessary for tethered PABP to suppress NMD. In contrast, the N-terminal part of PABP, consisting of 4 RNA recognition motifs (RRMs) and interacting with eukaryotic initiation factor 4G (eIF4G), retains the ability to inhibit NMD. Tethered eIF4G is also able to stabilize the reporter transcript, it is however not yet clear if this is a direct or indirect effect. There are indications that this stabilization might be due to endogenous PABP being recruited, but also that the interaction of eIF4G to initiation factor 3 (eIF3) might play a role. The results obtained so far suggest that PABP undoubtedly has an important function for the suppression of NMD, but that there might be other factors able to complement or circumvent its actions.

431 C Expression of nonsense-mediated decay factors is controlled by conserved feedback loops and responds to salt stress in plants

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Nonsense-mediated decay (NMD) is a conserved eukaryotic mRNA surveillance pathway that selectively recognizes aberrant transcripts and targets them for degradation. NMD target transcripts can be generated by faulty gene expression or selectively produced to serve gene regulation. NMD is triggered by c*is*-acting elements, comprising premature termination codons (PTCs), upstream open reading frames (uORFs), long 3' untranslated regions (UTRs), and 3' UTR-located introns. Recent studies indicated that more than 10% of all genes from *Arabidopsis thaliana* produce at least one NMD transcript variant, suggesting a major role of NMD in shaping the plant transcriptome. However, it is unclear whether NMD activity is constitutive or can be altered under certain developmental or stress conditions. Here we present data suggesting feedback regulation of NMD factor expression in *A. thaliana*. The transcripts from three of the core NMD factors (UPF1, UPF3, and SMG7) contain long 3' UTRs and/or 3' UTR-positioned introns and are upregulated in NMD-impaired seedlings. Furthermore, transcript levels of these NMD factors are elevated upon salt stress in seedlings, which might be explained by diminished NMD activity under these conditions. In line with this, the transcript from another core NMD factor, UPF2, that lacks any NMD eliciting feature display unchanged expression upon both NMD impairment and salt stress. Interestingly, we found that both feedback and salt stress regulation of NMD factors is also present in *Nicotiana benthamiana*, revealing evolutionary conservation of NMD control in different plant species. Our data suggest that NMD activity might be significantly altered under certain conditions, which we currently further investigate by use of an *in vivo* NMD reporter system.

432 A High-resolution characterization of regulatory sequences within a mammalian 3′ UTR

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Many important gene regulatory events occur post-transcriptionally, typically by regulation of mRNA translation and decay rates. Post-transcriptional regulatory events are usually controlled by *trans*-acting factors (such as microRNAs) that bind to *cis*-acting sequence elements (*cis*-elements), which are often located within the 3' untranslated regions (3' UTRs) of mRNAs. Importantly, previous analyses suggest that typical mammalian 3' UTRs contain many different *cis*-elements and that certain cis-elements can interact in synergistic or inhibitory ways. While many *cis*-elements have been identified, most of our current understanding of their function is based on analysis of elements in isolation rather than in combination with other elements. The work presented aims to better understand the range of regulatory sequences within a 3' UTR and how sequences within 3' UTRs interact with one another. Towards that goal, I have been studying in detail the 3' UTR of the mouse HMGA2 gene, which is known to direct substantial post-transcriptional regulation of its mRNA. The regulatory information within this 3′ UTR is particularly important, since HMGA2 over-expression due to 3' UTR truncations is found in many types of cancer and is linked with a poor prognosis.

To identify regions that confer regulation, I used reporter assays to assess the regulatory information within 100 nucleotide (nt) segments (100mers) tiled across the HMGA2 UTR, generating a high-resolution *cis*-regulation map of the UTR. This map shows several new regions of regulation beyond those previously described. For each strongly regulating 100mer, I am currently identifying the specific *cis*-elements responsible, which will be critical in identifying the *trans*-factors involved. To understand how interactions between *cis*-elements contribute to regulation, I also measured 200 nt segments (200mers) tiled across the 3' UTR. If elements within two adjacent 100mers do not interact, we expect the regulatory potential of the corresponding 200mer to be recapitulated by the regulatory potential of the 100mers. By looking for 200mers that fail to be recapitulated in this manner, I have been able to identify four regions that contain *cis*-elements that may be interacting. I am currently working on characterizing those interactions towards a mechanistic understanding of their function. Getting a complete picture of the *cis*-regulatory information within 3' UTRs, and how the *cis*-elements can interact to control the mRNA, is an important step towards a fuller understanding of post-transcriptional gene regulation.

433 B Direct Visualization of Alternative RNA Substrate Recruiting Pathways in Yeast Exosome

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The multi-subunit exosome complex plays crucial roles in RNA degradation and processing both in cytoplasm and nucleus by cleaving RNA substrates from their 3' end. From archaea to eukaryotes, exosome has a structurally conserved core complex consisting of nine subunits forming a ring-like structure with a channel in the center allowing single stranded RNA to go through. Eukaryotic core complex has lost its exonuclease activity but gains its RNase activity through two additional hydrolytic 3' to 5' exoribonucleases, Rrp44 and Rrp6, that both bind to the core. Rrp44 degrades RNA substrates processively while Rrp6 degrades RNA distributively.

Structural analysis revealed that Rrp44 protein situates at the bottom of the core facing the core channel exit. In tune with a similar function of the archaeal exosome, biochemical data indicate that RNA substrates with long single stranded 3' overhangs are first channeled through the eukaryotic exosome core before being degraded by Rrp44. This "through-core" route is supported by the most recent crystal structure of the yeast Rrp44-exosome (RE) in complex with an RNA substrate with a 5' hairpin and long 3' end single stranded overhang. On the other hand, recent transcriptom data suggested the presence of alternative route for RNA substrates with shorter 3' end single stranded overhangs to be processed by exosome. Our previous reconstruction of the apo-RE complex revealed multi-porous structure suggesting the potential for RNA substrates to take multiple routes including the "through-core" and some "direct access" routes to get access to Rrp44's exonuclease site.

In order to further probe the mechanism of RNA degradation by exosome especially the recruitment route of RNA substrates, we have performed biochemical and single particle electron microscopy analysis on yeast Rrp44-exosome in concert with RNA substrates. We used model structured RNA and linear RNA substrates with 3' end single stranded tails of various lengths as a molecular ruler to determine the length of RNA required for interaction with and degradation by the exosome. We exploited single particle reconstruction of the RE-RNA complexes to reveal the substrate induced conformational change of the complex. Single particle electron microscopy analysis of Rrp44-exosome in complex streptavidin-labeled RNA substrates provided us direct visual evidence of the alternative RNA recruiting pathways by Rrp44-exosome complex.

434 C SMG7 recruits the CCR4-NOT complex for degradation of NMD targets

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The nonsense-mediated mRNA decay (NMD) pathway triggers rapid degradation of aberrant mRNAs that contain premature translation termination codons (PTCs). In metazoans, NMD requires three 14-3-3-like proteins: SMG5, SMG6, and SMG7. These proteins are recruited to PTC-containing mRNAs through the interaction of their 14-3-3-like domains with phosphorylated UPF1, the central NMD effector. The recruitment of SMG5, SMG6, and SMG7 causes NMD target degradation. SMG6 possesses an active PIN domain at its C-terminus that cleaves the target mRNA in the vicinity of the PTC. On the other hand, tethered SMG7 has been shown to degrade mRNA efficiently through its Proline-rich C-terminus (PC) region, which is necessary and sufficient for this activity. Previous studies indicate that SMG7-mediated mRNA degradation requires the general mRNA decay enzymes. However, the mechanism by which these enzymes are recruited to the target mRNA has remained unclear. To determine how SMG7 elicits mRNA decay, we used the Tandem-Affinity Purification (TAP-tag) methodology to identify interacting partners. Amongst the SMG7-binding proteins, we found all subunits of the CCR4-NOT complex that are responsible for deadenylation of mRNAs. We further show that the PC-region of SMG7 is responsible for binding POP2, the catalytically active component of the CCR4-NOT complex. Additional mapping experiments of POP2 reveal that the catalytic domain alone is sufficient to bind the PC region of SMG7. Over-expression of catalytically inactive POP2 shows stabilization of SMG7-tethered RNA and PTC-containing reporter RNA. In addition, over-expression of a catalytically inactive DCP2, the decapping enzyme, also results in stabilization of NMD targets. Functional studies in human cells demonstrate that the PC region of SMG7 is required for NMD only in cells depleted of SMG6. Together with previously published data, our findings show that the 5' to 3' decay pathway is utilized in the SMG7-dependent degradation of NMD targets.

435 A The mRNA quality control factors Ski7 and Hbs1 evolved from an alternatively spliced gene that produced Ski7-like and Hbs1-like proteins.

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One major function of mRNA degradation is to maintain fidelity of gene expression by specifically targeting aberrant mRNAs for rapid degradation. Two *Saccharomyces cerevisiae* paralogs play central roles in this process. The recognition and degradation of mRNAs that lack a stop codon requires Ski7 and the RNA exosome, while the recognition and degradation of mRNAs with stalled ribosomes in the middle of an ORF requires Hbs1 and an unknown endonuclease. In addition to its role in recognizing nonstop mRNAs, Ski7 has a second role as a cytoplasmic exosome cofactor. Ski7 and Hbs1 are paralogs that arose in budding yeast about 100 million years ago, and thus most other eukaryotes only contain one corresponding gene. How other eukaryotes recognize and degrade nonstop and no-go mRNAs, and whether they contain a Ski7-like exosome cofactor was not fully understood.

Lachancea kluyveri is closely related to budding yeast, but like other eukaryotes has only one *SKI7/HBS1* gene. rt-PCR, RNA-seq and Western blot analysis show that this one gene encodes two distinct proteins through alternative splicing. Furthermore, the longer splice isoform functions as Ski7, while the shorter splice isoform functions as Hbs1. Thus, the expression of two distinct proteins to recognize nonstop and no-go mRNAs is more widespread than anticipated.

While alternative splicing is much less common in fungi than in mammals, the alternative splicing of *SKI7/HBS1* is conserved in both the ascomycetes and the basidiomycetes as shown by rt-PCR and bioinformatics analysis. Although alternative splicing of *SKI7/HBS1* is conserved, the exact mechanism has changed several times during fungal evolution, such that both alternative 3' splice sites, alternative first exons, and likely other mechanisms are used. Strikingly, other than *S. cerevisiae* and its close relatives, the only other fungi where we failed to detect alternative splicing are in the *Schizosaccharomyces* genus. While this genus is very distantly related to *S. cerevisiae*, it also contains an uncharacterized *SKI7* gene in addition to its canonical *HBS1* gene. This comparison of splicing strategies in diverse fungi provides a model to understand the evolutionary changes in alternative splicing.

The human genome also contains only a single ortholog whose major splicing isoform resembles Hbs1, both in sequence and biochemical activity. However, the human gene is also alternatively spliced, and our results suggest that the alternatively spliced isoform functions as the missing Ski7-like cytoplasmic exosome cofactor.

436 B Functional analysis of IMP3, a RNA-binding protein

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RNA-binding proteins regulate multiple steps of post-transcriptional gene expression, and it is important to know the target RNAs of RNA-binding proteins. The RNA binding protein IMP3 (Insulin-like growth factor 2 (IGF-2) mRNA-binding protein 3) is an oncofetal protein, emerging as a useful indicator of the progression and prognoses of several cancers. According to preceding study, IMP3 binds to IGF-2 mRNA thereby activating its translation, and stabilizes CD44 mRNA. IMP3 has a potential not to be simply a marker of prognosis in cancer cells, but also to regulate cancer progression. However, little is known about the function of IMP3 in cancer cells because transcripts regulated by IMP3 are unclear. In this study, we tried to identify RNA targets of IMP3 through genome-wide analysis.

Initially, we tried to identify RNAs bound to IMP3 by RNA immunoprecipitaion followed by deep sequencing (RIP-seq). We then determined 2201 RNAs enried in IMP3 immunoprecipitant as candidates of IMP3 binding RNAs. We also determined the expression levels of whole transcripts in IMP3-depleted HeLa TO cells by deep sequencing analysis. The expression levels of 65 transcripts were increased in IMP3-depleted cells. Among them, 15 transcripts were bound to IMP3, and we judged these 15 transcripts are regulated by IMP3. We measured the stability of the RNA targets of IMP3 in IMP3-depleted HeLa TO cells using BRIC-method, an inhibitor-free method for directly measuring RNA stability. CDK18 mRNA, SOCS2 mRNA, LRFN3 mRNA and CCDC92 mRNA were stabilized by depletion of IMP3. Our results suggest that IMP3 promotes RNA degradation of RNA targets, and might regulate cell growth through control of RNA stability of *CDK18* and *SOCS2*, negative regulators of cell growth. This is a first demonstration that IMP3 is a RNA destabilizing factor for a set of transcripts.

437 C Global analysis of exosome target introns

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U12-type introns are a class of rare introns in the genomes of diverse eukaryotes. They number over 800 introns in the human genome, making up less than 0.5 % of our introns (Turunen et al., 2013). These introns have earlier been shown to splice at a slower rate compared to the major U2-dependent pathway. This suggests a rate-limiting regulatory function for the minor spliceosome in the nuclear processing of transcripts containing U12-type introns (Patel et al. 2002). In support to this model, an elevated level of unspliced U12-type introns have been detected in the steady-state RNA populations in various organisms (Patel et al. 2002; Pessa et al. 2006; Pessa et al. 2010) However, both the mechanism of slower splicing kinetics and the fate of mRNAs containing unspliced U12-type introns remain unknown.

Here we have analyzed globally the effect of exosome processing on the nuclear pre-mRNA transcripts by inactivating either the Rrp41 or Dis3 subunit of the exosome. Using SOLiD RNA sequencing technology, we report 30-120 million mapped cellular compartment specific reads per sample allowing the detection of unspliced pre-mRNAs.

We show that Rrp41 and Dis3 knockdowns stabilize an overlapping set of U12-type introns, with a total of 132 stabilized introns using 2-fold upregulation as cutoff. At least 3-fold upregulated introns total 84, and at least 4-fold upregulated introns number 57, covering a remarkable fraction of the human U12 intron set (15 %, 9.5 % and 6.5 %, respectively). Collectively the median U12-type intron retention distribution shifts towards upregulation in the knockdown, contrary to U2-type introns, whose distribution shows no change. Validation of the top stabilized introns is performed using RT-PCR. Finally, we explore the characteristics of introns targeted by the exosome.

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438 A A Novel Role for the Arginine Methyltransferase CARM1 in Nonsense Mediated Decay: Implications for Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is amongst the leading genetic causes of infant deaths and is characterized by specific degeneration and loss of spinal cord motoneurons. It is caused by the disruption of the "survival of motor neurons" gene (SMN1), which plays an essential role in the cytoplasmic assembly of core snRNPs. To date, we still do not understand why the motoneurons are the most affected cell type in response to a deficiency in this protein. It has been proposed that motoneurons are more sensitive to low snRNPs levels and splicing defects. Alternatively, it has also been proposed that SMN may have a distinct function specifically in motoneurons. We recently uncovered that SMN normally represses CARM1 translation, thus leading to an increase of CARM1 expression in SMA. Since our results obtained for the past 4 years strongly suggest a key role of CARM1 in the etiology of SMA, we decided to investigate the impact of an increase of CARM1 in the disease. Amongst the different cellular roles of CARM1, we decided to focus on the relationship between the massive alteration of splicing in SMA and the fact that CARM1 can regulate this mechanism. Thus, our objective is to identify splicing targets that are misregulated by CARM1 in the pathology.

While pursuing splicing targets regulated by CARM1, we uncovered a completely novel function for CARM1 as a regulator of nonsense-mediated mRNA decay (NMD). Interestingly, in our preliminary results we identified USPL1, as a target regulated by CARM1. USPL1 is a gene which has been found to be mis-spliced in a number of difference SMA cell culture and in vivo models. This gene can generate two transcripts including or not alternative exon 2. In SMA, it was reported that the splicing variant containing exon 2 is enriched. Upon analysis of the sequences of these two USPL1 splicing isoforms, we observed that the transcript without the exon 2 in fact matched the criteria to be a NMD-regulated target. To test this hypothesis, we decided to inhibit the NMD pathway by using either drugs or a RNAi strategy against UPF1 (the main effector of NMD). Thanks to these strategies, we were able to confirm that this variant was a NMD target. Afterwards, to confirm a role of CARM1 in the NMD mechanism, we used a well-accepted NMD reporter assay which allowed us to validate our hypothesis. Furthermore, by using co-immunoprecipitation experiments, we observed an interaction between CARM1 and both UPF1 and UPF2, but not with UPF3 or components of the EJC, suggesting CARM1 is not recruited co-transcriptionally to NND transcripts and may actually play a direct role in the early activating steps of NMD. Finally, our preliminary screen has identified a few additional NMD targets regulated by CARM1, that are also misregulated in SMA. Altogether, identification of the full spectrum activities of CARM1 may provide crucial insights into our understanding of the etiology of SMA.

439 B Assessing differential susceptibility of mRNAs to NMD

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Nonsense-mediated mRNA decay (NMD) co-translationally reduces the steady state levels of many physiological mRNAs, hence acting as a post-transcriptional gene regulation mechanism. The fate of the mRNA is decided during translation termination. According to the „unified NMD model", the interaction of the poly(A)-binding protein (PABPC1) with the eukaryotic release factor 3 (eRF3), bound to the terminating ribosome, typifies normal translation termination. In contrast, if the distance from the termination codon (TC) to the poly(A) tail is too large for the PABPC1-eRF3 interaction to occur efficiently, the recruitment of the SURF complex to the ribosome, through the binding to eRF3 and eRF1, is facilitated instead. This step initiates the cascade of events that result in the rapid decay of the mRNA [1]. This model predicts that spatial rearrangements of the 3'-UTR altering the physical distance between TC and poly(A) tail could serve as a novel post-transcriptional mechanism for gene regulation. However, no physiological mRNAs being regulated by this putative mechanism has been identified so far [2,3]. Therefore, our goal is to identify endogenous mRNAs that are NMD-sensitive under certain conditions but NMD-resistant under other conditions. As an experimental model we have established a human neuroblastoma cell line that can be differentiated in culture into a neuronal-like state and in which we can inactivate NMD by induction of an RNAi-mediated depletion of UPF1. Whole transcriptome analysis was performed by RNA-Seq on undifferentiated and differentiated cells, either with or without UPF1 depletion and the bioinformatics analysis is ongoing. If such putative physiological mRNAs that are differentially regulated by NMD indeed exist, they should be revealed by comparing the transcriptomes under these four conditions. In addition, this analysis will provide new insights into the post-transcriptional regulation in a neuronal-like background.

440 C Structural insights into the Dhh1-Pat1 interaction

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Eukaryotic mRNA turnover starts with the shortening of the 3′ poly (A) tail and continues with either 3′-5′ degradation by the exosome complex or with decapping and 5′-3′ degradation by Xrn1. Decapping is catalyzed by the Dcp1-Dcp2 complex and is regulated by several co-activator proteins, including Dhh1, Pat1, Edc3 and the heptameric Lsm 1-7 complex. All these factors are conserved from yeast to humans.

Pat1 is a multidomain protein. It uses the N-terminal domain to interact with Dhh1 and the C-terminal domain to interact with Lsm1-7. As such, Pat1 is believed to act as a bridging factor between the 3′ end and the 5′ end of the message. Dhh1 (also known as Rck or DDX6 in metazoans) is a DEAD-box protein. Like all other members of the DEAD-box family, Dhh1 is expected to bind RNA via the two RecA domains. In addition, Dhh1 interacts with another enhancer of decapping, Edc3. How Dhh1 interacts with Pat1 and how this affects the RNA-binding and protein-protein binding properties of this DEAD-box protein is unclear.

We have determined the 2.8 Å crystal structure of yeast Dhh1 bound to the N-terminal domain of Pat1. The structure reveals an evolutionary conserved recognition mechanism that we have confirmed by testing mutations in *in vitro* assays with the yeast proteins and in co-immunoprecipitation assays with the corresponding human orthologues. Comparison with the known structures of human DDX6-Edc3 and of DEAD-box proteins bound to RNA reveals how Pat1 binding impacts on other macromolecular interactions mediated by Dhh1.

441 A Acetylation of CAF1a and BTG2 accelerates general mRNA degradation

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The half-life of most mRNAs is determined by the rate of poly(A) tail shortening. In metazoans, two deadenylases, CAF1a and CAF1b, play a major role in removing poly(A) tails in the cytoplasm, thereby initiating degradation of mRNAs. The CAF1 enzymes are integral components of the CCR4-CAF1-NOT complex, which is actively recruited to mRNAs destined for degradation. In addition, CAF1a and CAF1b interact with the TOB/BTG family of proteins, which promote the activity of CAF1 enzymes. Given the central importance of CAF1 deadenylases in mRNA turnover, we examined whether their activity is regulated.

Here we report that hyperacetylation strongly promotes the interaction between human CAF1a and BTG2 *in-vivo*. In accordance with this finding, hyperacetylation accelerated degradation of stable mRNAs. Hyperacetylation also caused a general loss of poly-A tails, pointing towards a general activation of deadenylation. Using an anti acetyl-lysine antibody on purified proteins, we found that both CAF1a and BTG2 are directly acetylated. By testing candidate enzymes, we were able to identify two acetyl transferases that promote acetylation of CAF1a and BTG2, as well as a deacetylase that removes lysine acetylation on both proteins.

Mass spectrometry revealed acetylated lysine residues on both CAF1a and BTG2. Mutation of two lysine residues in BTG2 was sufficient to abolish acetylation. Importantly, the same mutation also prevented proteasome-dependent degradation of BTG2, indicating that acetylation stabilizes BTG2. The analysis of reporter mRNA degradation further suggested that the activities of CAF1 and BTG2 are enhanced by hyperacetylation. Taken together, our data show for the first time that acetylation plays an important role in regulating posttranscriptional gene expression by promoting the general turnover of mRNAs.

442 B Mpn1, mutated in poikiloderma with neutropenia protein 1, unveils cellular surveillance of catalytic spliceosomal small nuclear RNAs.

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We have recently demonstrated that Mpn1 is an evolutionary conserved RNA exonuclease that trims the 3'-end polyuridine (oilgoU) tail of the spliceosomal small nuclear RNA (snRNA) U6 post-transcriptionally. Fission yeast strains deleted for the *mpn1+* gene and cells from human patients affected by the rare genodermatosis poikiloderma with neutropenia (PN), which is associated to mutation in the *hMPN1* gene, carry aberrant U6 molecules with oligoU tails that are longer than in normal cells and are devoid of their typical 2'-3' cyclic phosphate groups. We have also shown that lack of Mpn1 leads to increased U6 degradation rates, revealing that Mpn1 promotes U6 stability, and proposed the existence of a quality control of U6. Here we show that in *mpn1*Δ yeast cells, U6 oligoU tails are largely adenylated, indicating that 2'-3' cyclic phosphate groups may prevent adenylation. Adenylated U6 also accrues in cells deleted for the nuclear exosome component *rrp6+* and in double mutants *mpn1*Δ/*rrp6*Δ: thus Rrp6 is at least in part responsible for the degradation of U6 molecules that have not been processed by Mpn1. Ectopically expressed, catalytically inactive U6 molecules are not processed by Mpn1 confirming the existence of a cellular RNA surveillance pathway able to discriminate between catalytically active and inactive U6, and to communicate the outcome of the quality check to Mpn1. We also find that human Mpn1 processes not only U6 but also U6atac, an snRNA component of the minor U12-type spliceosome. Similarly to U6, in PN cells, U6atac lacks a 3' end cyclic phosphate and is degraded more rapidly than in cells from healthy individuals. Our data unveil an intricate cellular circuit dedicated to the quality control of major and minor spliceosome components, and pave the way for understanding the molecular bases of PN.

443 C MKT1: a hub in a post-transcriptional regulatory network

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Trypanosomes are uniquely reliant upon post-transcriptional mechanisms for the control of gene expression. We previously found that stabilisation of trypanosome mRNAs during heat shock depends on binding of the zinc finger protein ZC3H11 to the 3'-untranslated regions of target mRNAs; also, tethering of ZC3H11 to a reporter mRNA increases reporter expression, suggesting an active stabilisation mechanism. We have now found that ZC3H11interacts with trypanosome MKT1 and PBP1, and that interactions with both proteins are required for ZC3H11 activity in the tethering assay. *Saccharomyces cerevisiae* Mkt1p facilitates yeast survival during stress, while yeast Pbp1p interacts with Lsm12p and the poly(A) binding protein Pab1p. Both Pbp1p and its human homologue, Ataxin-2, are stress granule components.

Trypanosome MKT1, like Mkt1p, interacts with PBP1 and LSM12, and trypanosome PBP1 interacts with trypanosome poly(A) binding proteins. MKT1, PBP1 and LSM12 are all essential for parasite survival, and all increase reporter expression in the tethering assay, as does poly(A) binding protein. MKT1, like Mkt1p, is polysome associated. MKT1 and PBP1 are distributed throughout the cytoplasm at the normal growth temperature and after heat shock, but concentrated in stress granules during starvation. Stabliisation of heat-shock mRNAs could result from cooperative interactions between ZC3H11, MKT1, PBP1 and poly(A) binding proteins, resulting in enhanced protection of the poly(A) tail against deadenylation.

A two-hybrid assay, analysed by deep sequencing, revealed that MKT1 interacts with at least 10 proteins with RNA-binding domains, some of which were also active in a high throughput tethering assay. There were, however, also possible interactions with the mRNA degradation machinery. We suggest, therefore, that trypanosome MKT1 is at the centre of a post-transcriptional regulatory network.

444 A Structural basis for the multiple roles Edc3 plays in mRNA degradation

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The Dcp1:Dcp2 decapping complex catalyzes the removal of the protecting 5' cap structure from mRNA. Adaptor proteins, including Edc3 (enhancer of decapping 3), modulate this decapping process through multiple mechanisms. First, the Edc3 protein enhances the activity of the Dcp2 enzyme directly. Secondly, Edc3 is involved in the formation of cellular processing bodies. Finally, Edc3 is important for the deadenylation independent degradation of the Rps28b mRNA. In the latter case, cellular Rps28b protein levels are regulated through a unique auto-regulatory pathway, where Rps28b that is not in complex with the ribosome binds to a specific stem-loop in the 3'-UTR of its own messenger-RNA.

To understand how the Edc3 protein is able to preform these multiple functions, we solved the structure of the yeast Edc3 LSm domain in complex with a short helical leucine-rich motif (HLM) from Dcp2. Based on that structure, we identified additional HLMs in the disordered C-terminal extension of Dcp2 that can interact with Edc3. We show that these multiple HLMs in Dcp2, together with the dimeric nature of Edc3, can lead to the formation of a network of intermolecular interaction. Our experiments thus provide initial insights into one of the mechanisms that underlie processing body formation. Finally, we solved the structure of the Edc3 LSm domain in complex with the Rps28b protein. These data display how the dimeric Edc3 protein is able to bring the Rps28b mRNA and the Dcp1:Dcp2 decapping complex together, thereby targeting the mRNA for degradation.

In summary, we show that the Edc3 LSm domain is a plastic platform for multiple protein:protein interactions that are important for the regulation of mRNA degradation.

445 B CBP1 mRNA is cleaved and produces nonstop mRNA in a tRNA splicing endonuclease activity dependent manner

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Cells have surveillance systems that eliminate aberrant mRNAs to prevent the production of potentially harmful protein products. Non-Stop Decay (NSD) rapidly degrades an aberrant mRNA lacking a termination codon (nonstop mRNA) that is produced mainly by polyadenylation within an ORF. No-Go Decay (NGD) leads to an endonucleolytic cleavage of the mRNA when a ribosome is stalled on the ORF, and produces nonstop mRNA. We recently reported that the Dom34:Hbs1 stimulates degradation of the nonstop mRNA by dissociating the ribosome that is stalled at the 3' end of the mRNA. However, an endonuclease and an endogenous target of NGD have not been identified. tRNA splicing endonuclease is reported to localized to mitochondria outer membrane to play a role of itself, although there is a possibility that it cleaves a cytoplasmic mRNA.

Here, we report that *CBP1* mRNA is cleaved and produces nonstop mRNA in a tRNA splicing endonuclease activity dependent manner. CBP1 is a nuclear encoded protein that is imported into mitochondria. Deletion analyses revealed a mitochondria targeting signal and a 652-726nt sequence of *CBP1* are indispensable for the cleavage. Furthermore, a 643-738nt sequence of *CBP1* inserted between a *GFP* ORF and a *HIS3* ORF of a *GFP-HIS3* reporter sufficiently induced the endonucleolytic cleavage when a mitochondria targeting signal was also inserted at the amino terminus of GFP. Also, tRNA splicing endonuclease component *sen54* and *sen2*mutant cells lacking endonuclease activity eliminated the cleavage of *CBP1* and *GFP-HIS3* reporter mRNAs. These results suggest that *CBP1* mRNA is localized to the mitochondria to be cleaved by Sen splicing endonuclease complex. This is the first implication for the correlation between mRNA quality control and mRNA localization to organelle. Now, we are analyzing endogenous mRNAs encoding proteins localized to mitochondria whether to be endonucleolytically cleaved or not by using RNA-seq.

446 C Enriched density of UPF1 in 3′ untranslated regions results from its translation-dependent displacement from coding sequences

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The RNA helicase UPF1 is best known for its key function in mRNA nonsense-mediated mRNA decay (NMD), but has also been implicated in additional mRNA turnover mechanisms, telomere homeostasis, and DNA replication. In NMD, UPF1 recruitment to target mRNAs is thought to occur through interaction with release factors at terminating ribosomes, but evidence for translation-independent interaction of UPF1 with the 3' untranslated region (UTR) of mRNAs has also been reported. To map UPF1 binding sites transcriptome-wide, we performed individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) in human cells, untreated or after inhibiting translation by puromycin. Our results revealed a strongly enriched association of UPF1 with 3' UTRs in undisturbed, translationally active cells. After translation inhibition, a significant increase in UPF1 binding to coding sequence (CDS) was observed, indicating that UPF1 binds RNA before translation and gets displaced from the CDS by translating ribosomes. Our evidence for translation-independent UPF1-RNA interaction, which is corroborated by UPF1 crosslinking to long non-coding RNAs, suggests that the decision to trigger NMD occurs after association of UPF1 with mRNA, presumably through activation of RNA-bound UPF1 by aberrant translation termination.

447 A Activation of RNA cap methylation by CDK1

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Eukaryotic RNA polymerase II transcripts, such as mRNA, are modified by the addition of a 7-methylguanosine cap which is essential for gene expression and cell viability. The methyl cap stabilises mRNA by protecting it from 5' to 3' exoribonucleolytic degradation, and interacts with the cap-binding complex (CBC) and the eukaryotic initiation factor 4E (eIF4E), thus promoting transcription, splicing, polyadenylation, nuclear export and translation initiation. In mammals, 7-methylguanosine cap formation is catalysed by the capping enzyme (RNGTT) which adds a guanosine cap to the nascent mRNA and the RNA guanine-7 methyltransferase complex (RNMT-RAM) that methylates the cap. The catalytic domain of the methyltransferase complex resides on RNMT whereas RAM has a regulatory effect.

Recent work demonstrated that methyl cap formation is a regulated process. The transcription factors E2F1 and c-Myc can increase 7-methylguanosine levels of specific transcripts. Futhermore, methyl cap synthesis can be regulated under normal and stress conditions such as amino acid starvation.

In order to better understand the molecular mechanisms that regulate cap methylation in human cells we investigated RNMT post-translational modifications. Analysis by orthophosphate labelling and mass spectrometry revealed that RNMT is phosphorylated. We identified the predominant phosphorylation site and demonstrated that Cyclin-dependent kinase 1 (CDK1/Cyclin B) is responsible for RNMT phosphorylation during G2/M phase of the cell cycle. Interestingly, RNMT phosphorylation increases cap methyltransferase activity in vitro and loss of this phosphorylation site reduces cell proliferation. Preliminary data suggests that RNMT phosphorylation regulates gene expression in gene-specific manner.

Currently, we are investigating why RNMT is phosphorylated during G2/M phase and why RNMT phosphorylation regulates specific transcripts.

448 B An emerging role for double-stranded RNA binding domains: regulating the sub-cellular localization of proteins

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Double-stranded RNA binding domains (dsRBDs) are well characterized RNA binding domains that bind specifically to double-stranded RNA (dsRNA) [1]. DsRBD-containing proteins are therefore implicated in many biological processes involving dsRNA such as gene silencing through RNA interference pathways, regulation of translation, RNA processing and messenger-RNA editing. Strikingly, increasing examples of dsRBDs involved in the regulation of protein sub-cellular localization suggest a new important function for a sub-class of dsRBDs. For instance, the fission yeast dicer (Dcr1) contains a C-terminal dsRBD that has been shown to be essential for nuclear retention of the protein [2]. Additionally, the third dsRBD of the human mRNA deaminase enzyme ADAR1 has been shown to harbour a nuclear localization signal essential to target ADAR1 to its nuclear substrates [3].

We used solution NMR spectroscopy to get insights into the molecular determinants regulating the sub-cellular localization of these dsRBD-containing proteins. On the one hand, the structure of the C-terminal dsRBD of Dcr1 revealed an extended dsRBD fold embedding an unexpected zinc-binding motif [4]. This unconventional zinc-binding site, which is highly conserved among dicers in yeasts, extends the canonical dsRBD fold thereby generating a conserved and adjustable platform involved in the regulation of the subcellular localization of Dcr1 [5]. Strikingly, although the extended dsRBD of Dcr1 binds to dsRNA, this property is dispensable for proper functioning of Dcr1 in the RNAi pathway. This raises the attractive possibility that this new class of extended dsRBD might generally function in nucleo-cytoplasmic trafficking and not substrate binding. On the other hand, the structure of ADAR1 dsRBD3 also revealed an extended dsRBD fold with additional structured elements. As in the case of Dcr1, the extension to the canonical dsRBD fold is critical for targeting ADAR1 to the nucleus. Even though it lacks the well-established PY-NLS motif, this extension participates in the interaction with Transportin 1 (also referred to as Karyopherin-β2), thereby promoting ADAR1 nuclear import. As for Dcr1's dsRBD, ADAR1 dsRBD3 fully retains the capacity to bind to dsRNA, suggesting that extending the dsRBD fold would be a widespread strategy to acquire additional function without loosing the original dsRNA binding activity.

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449 C Revealing the full scope of Alu editing - over a hundred million genomic sites are subject to primate-specific A-to-I RNA editing

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RNA molecules carry the information encoded in the genome and reflect its content. Adenosine-to-inosine modification of RNA molecules (A-to-I RNA editing) by ADAR proteins converts a genomically encoded adenosine (A) into inosine (I). It is known that most RNA editing in human take place in the primate specific Alu sequences but the extent of this phenomenon and its effect on transcriptome diversity is not clear yet.

Analyzing large-scale RNA-seq data and by performing ultra-deep sequencing of selected Alu sequences, we show that the scope of editing is much larger than was anticipated. More than 700,000 Alu repeats can form dsRNA structures, and virtually all adenosines within these Alu repeats undergo A-to-I editing to some extent. Moreover, we observe editing of transcripts resulting from residual anti-sense expression, doubling the number of edited sites in the human genome. The total number is thus estimated to exceed a hundred million sites. Our ultra-high coverage enables us to probe editing levels that span a wide range, with few sites being fully converted while most sites exhibit low (<1%) levels.

We further studied the effect of Alu editing on transcriptome diversity. The number of different variants seem to grow with coverage, with no sign of saturation. Looking at the information included in each Alu repeat in terms of its editing pattern, Shanon's information entropy ranges from 5 to 9 bits per Alu. The average number of inosines per transcribed Alu also varies considerably among the randomly selected Alus, ranging between 1 and 19 inosines per transcript. Finally, we estimate the number of Alu-derived inosines to be roughly 100,000-fold higher than the number of inosines located in the Q/R of the glutamate receptor.

These finding naturally lead to the question of how this primate-specific diversification of the transcriptome is utilized.

450 A A Hundred million sites located in majority of human genes are subject to A-to-I RNA editing

Lily Bazak[1], Ami Haviv[1], Michal Barak[1], Jasmine Jacob-Hirsch[1], Karen Kesarcas[2], Gideon Rechavi[2], Jin Billy Li[3], Erez Y. Levanon[1], Eli Eisenberg[4]

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RNA molecules carry the information encoded in the genome and reflect its content. Adenosine-to-inosine modification of RNA molecules (A-to-I RNA editing) by ADAR proteins converts a genomically encoded adenosine (A) into inosine (I). It is known that most RNA editing in human take place in the primate specific Alu sequences but the scope of this phenomenon and its effect of transcriptome diversity is not clear yet. Here we show, by analyzing large-scale RNA-seq data and by performing ultra-deep

sequencing of selected Alu sequences, that the scope of editing is much larger than was anticipated. More than 700,000 Alu repeats can form dsRNA structures, and virtually all adenosines within these Alu repeats undergo A-to-I editing to some extent. Moreover, we observe editing of transcripts resulting from residual anti-sense expression, doubling the number of edited sites in the human genome. The total number is thus estimated to exceed 100 million sites. Our ultra-high coverage enables us to probe editing levels that span a wide range, with few sites being fully converted while most sites exhibit low levels (<1%). Our finding set the stage for exploring how this primate-specific diversification of the transcriptome is utilized.

451 B MODOMICS: a database of RNA modification pathways - new developments

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MODOMICS is a database of RNA modifications that provides comprehensive information concerning the chemical structures of modified ribonucleosides, their biosynthetic pathways, location of modified residues in RNA sequences, and RNA-modifying enzymes. In the current database version, accessible at http://modomics.genesilico.pl, we included new features: a census of human and yeast snoRNAs involved in RNA-guided RNA modification, a new section covering the 5′ end capping process, and a catalogue of "building blocks" for chemical synthesis of a large variety of modified nucleosides. The MODOMICS collections of RNA modifications, RNA-modifying enzymes and modified RNAs have been also updated. A number of newly identified modified ribonucleosides and more than one hundred functionally and structurally characterized proteins from various organisms have been added. In the RNA sequences section, snRNAs and snoRNAs with experimentally mapped modified nucleosides have been added and the current collection of rRNA and tRNA sequences has been substantially enlarged. To facilitate literature searches, each record in MODOMICS has been cross-referenced to other databases and to selected key publications. New options for database searching and querying have been implemented, including a BLAST search of protein sequences and a PARALIGN search of the collected nucleic acid sequences.Finally, we have developed a new nomenclature for nucleic acid modifications.

452 C Alu inverted repeats induce human specific site selective A-to-I RNA editing

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RNA editing by adenosine (A) deamination to inosine (I) is a common event in the human transcriptome due to the high frequency of *Alu* elements. *Alu* inverted repeats are targeted for A-to-I editing because of their ability to form hairpin structures of double stranded RNA, a requisite for ADAR editing. Recent high throughput sequencing (RNA-Seq) has revealed several hundred thousand edited sites within these *Alu* repeats.Nevertheless, the function of editing within repetitive elements is largely unknown. Using bioinformatic and experimental analyses we show that primate specific site selective editing in non-repetitive sequence often is found adjacent to *Alu* inverted repeats. We propose that *Alu* elements forming long, almost completely base paired structures can work as recruitment elements for the ADAR proteins and thereby induce editing in non-repetitive sequence. One primate specific site of editing is located in the transcript coding for the DNA repair enzyme NEIL1. Editing within exon 6 of this mRNA recodes a lysine for an arginine (K/R). We show that efficient editing at this site is dependent on the presence of a cis-acting *Alu* inverted repeat located 200 nucleotides upstream of the K/R site. We found this site to be efficiently edited also in rhesus monkey but not in mouse and rat. The upstream *Alu* inverted repeat in NEIL1 is conserved between rhesus and human. We propose that recruitment of ADAR to *Alu* elements increase the local concentration of the editing enzyme and thereby increase editing efficiency at other sites located in less stable duplex structures. We therefore suggest that *Alu* repeats can work as editing inducers, specifically increasing the variability in the human transcriptome.

453 A Identifying the function of highly conserved residues for pseudouridine formation through a combination of in silico and in vitro studies

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Pseudouridine is the most abundant post-transcriptional RNA modification and is formed through the isomerization of uridine to pseudouridine by enzymes known as pseudouridine synthases. These enzymes share a common catalytic domain and active site structure including a strictly conserved aspartate residue that is essential for catalysis. However, the exact mechanism of pseudouridine formation is still unknown. Here, a combination of *in silico* and *in vitro* studies was used to identify residues involved in catalysis in order to better understand the catalytic mechanism of pseudouridine synthases. First, molecular dynamics simulations of the *E. coli* pseudouridine synthase TruB, responsible for the formation of pseudouridine 55 in tRNA, were performed in the absence of substrate for 40 ns in two functionally different conformations. The simulations revealed two highly conserved residues, R181 and D90, that showed differential interaction patterns with the catalytic D48 residue. As known from crystallographic studies, R181 can form a hydrogen bond with the catalytic D48 residue. Interestingly however, R181 can also interact with the second-shell residue D90. This suggests that R181 may switch between two conformations, facilitated by D90. This switch might in turn be important for catalysis. To test this hypothesis, *in vitro* mutational studies on R181 and D90 were conducted to identify the precise roles of R181 and D90 for TruB's function. Although substrate binding was not affected by amino acid substitutions at these positions, catalysis was severely impaired. Upon changing D90 to glutamate, asparagine or alanine, the rate of pseudouridine formation decreased 30 to 600-fold, respectively. Likewise, substituting R181 with lysine reduced the catalytic rate over 2500-fold while substitutions with methionine or alanine caused a reduction of more than 5000-fold. Our findings suggest that R181 and D90 work together to position the catalytic residue D48 which is critical for efficient pseudouridine formation. Further experiments and molecular dynamics simulations are underway to identify in which step of TruB's mechanism R181 and D90 are involved. In summary, the use of molecular dynamics simulations in combination with *in vitro* assays has enabled us to identify two residues important for catalysis and will assist in determining the mechanism of pseudouridine formation that may be common to all pseudouridine synthases.

454 B Identification of the last rRNA methylase YhiR E.coli using a new technique

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The prokaryotic rRNA is highly modified, and one of two major types of these modifications is methylation. The role of methylated nucleotides in ribosome remains undiscovered yet but is still intriguing. The key obstacle arises at the first step of this study: the lack of information about the enzyme that modifies the concrete nucleotide renders the problem practically insoluble.

Here we suggest a very simple and useful method that allowed us to identify the last rRNA methylase YhiR (RlmJ). The proposed method is based on a melting of RNA-DNA duplexes formed by rRNA and two oligodeoxyribonucleotides with different length that are both complementary to rRNA in area of concrete modified nucleotide of rRNA (see figure 1). In our system the shorter oligonucleotide is modified by the Black Hole Quencher (BHQ1) at the 3'-terminus and hybridizes accurately with the nucleotide of interest and some neighboring nucleotides of rRNA. The longer oligonucleotide is modified by FAM at the 5'-terminus and hybridizes with the rRNA just near the shorter one.

We showed that the correct pair of such oligonucleotides allows us to get a remarkable difference in differential melting curves between methylated and non-methylated nucleotide in rRNA.

We proceeded to the search for the last rRNA methylase that modifies nucleotide A2030 of 23S rRNA. The set of annotated and putative rRNA methylases was chosen to be analyzed: YbiN, YecP, YjhP, YafS, SmtA, YmfD, LasT, YfiF, YfiC, YiiV, YecO, YgiQ, KsgA, YhiR, YhdJ and YafE. We compared rRNAs isolated from knockout strains lacking one of listed genes using our melting method and received differential melting curves that showed a great difference in its character for the rRNA from the knockout strain *ΔyhiR* (see figure 2).

Owing to these results we found a very short way to following demonstration of functional activity of the new enzyme. We showed that YhiR is actually SAM-dependent methylase that specifically methylates nucleotide A2030 of 23S rRNA. Moreover, it acts at the first stage of ribosome assembly, when the 23S rRNA is free from ribosomal proteins.

Thus, we demonstrate a very simple and convenient technique for rapid identification of rRNA modifying enzymes.

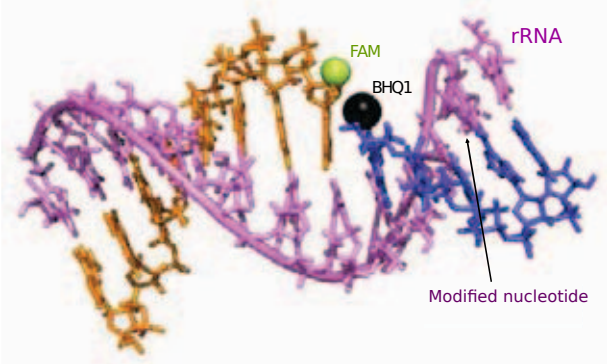

Figure 1. rRNA-DNA duplex. The long oligodeoxyribonucleotide modified by FAM is coloured by orange. The short one is modified by BHQ1 and coloured by blue.

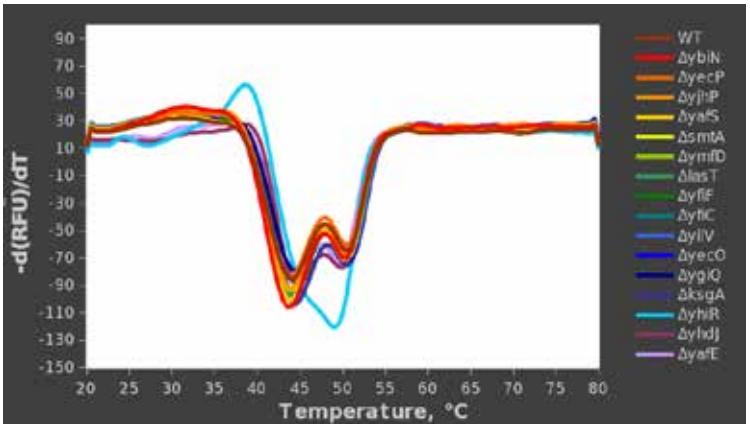

Figure 2. Differential curves for rRNA from corresponding knockout strains and wild type (RFU - relative fluorescence units).

455 C RAM: a novel and essential component of RNA cap methylation

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Eukaryotic gene expression is dependent on the 7-methylguanosine cap moiety, which is located at the 5' end of all RNA polymerase II primary transcripts. The 7-methylguanosine cap has a central role in most gene expression layers including transcription, splicing, nuclear export of both mRNA and snRNA, mRNA translation and decay and miRNA processing. The enzymes that catalyze the formation of the 7-methylguanosine cap are recruited to RNA polymerase II at the initial stages of transcription. The final step in this process, N-7 methylation of the guanosine cap, is catalyzed by the RNA guanine-7 methyltransferase, RNMT.

Recently, we demonstrated that RNMT does not function as a monomer but instead forms a heterodimer with a protein we designated as RAM (RNMT activating mini-protein), which is a novel component of the mammalian cap methyltransferase complex. The vast majority of cellular RNMT was found in a complex with RAM and *vice versa*. RAM is an RNA-binding protein, promoting recruitment of RNA to RNMT. RAM also increases recombinant and cellular RNMT cap methyltransferase activity and it is required for guanine-7 methylation *in vivo*. Therefore, we described RAM as an "obligate activator" of the human cap methyltransferase. As expected of a protein essential for cap methylation, RAM is required for mRNA translation, and loss of RAM results in loss of cell viability[1].

Here we dissect RAM functional domains and we demonstrate that RAM and RNMT can protect each other from proteasomal degradation. RAM N-terminus, which mediates RNMT interaction, is sufficient for stabilizing RNMT. However, the minimal RAM domain that is required for RNMT activation is more extensive than the minimal RAM domain that mediates the interaction with RNMT. This may suggest that upon RAM binding RNMT undergoes a major conformational change. Current studies focused on determining the crystal structure of RAM–RNMT complex will shed light on this hypothesis.

In addition, here we demonstrate that RAM C-terminus is essential for RAM nuclear accumulation. Although RNMT contains three classical nuclear localization signals (NLS) and utilizes the Importin-α/β pathway for its nuclear import, RAM does not contain a classical NLS. Therefore, it was speculated that RAM is imported into the nucleus *via* RNMT interaction. However, the observation that RAM–RNMT interaction is not sufficient for RAM nuclear localization indicates that RAM contains its own NLS. Preliminary results suggest that RAM nuclear localization is mediated by Transportin-1, which recognizes a PY-NLS motif at RAM C-terminus. These data propose that RNMT and RAM utilize distinct nuclear import pathways and that the cap methyltransferase complex is only formed in the nucleus.

1. Gonatopoulos-Pournatzis, T., et al., RAM/Fam103a1 is required for mRNA cap methylation. *Molecular cell* 44, 585–96 (2011).

456 A Conserved circuitry in eukaryotes for crucial Trm7 modifications of the tRNA anticodon loop

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Post-transcriptional modification of the anticodon loop of tRNA is critical for translation and proper cell growth. We recently reported that lack of 2'-*O*-methylated C_{32} (Cm_{32}) and Nm_{34} in *Saccharomyces cerevisiae trm7*-? mutants causes slow growth due to reduced function of $tRNA^{Phe}$, but not of the other Trm7 substrates $tRNA^{Trp}$ and $tRNA^{Leu(UAA)}$ (Guy M. P. *et al*., (2012) *RNA*. 10:1921-33). In addition, we described a complex circuitry for anticodon loop modification, in which Trm7 also requires its interacting partner Trm732 for Cm_{32} formation and its interacting partner Trm734 for Nm_{34} formation, and showed that Cm_{32} and Gm_{34} formation drives subsequent production of yW_{37} from m^1G_{37} on $tRNA^{Phe}$.

Available evidence suggests that the circuitry for Trm7 modification of tRNA is conserved and is important in other eukaryotes. 2'-*O*-methylation of the anticodon loop of tRNA is common to all characterized eukaryotic $tRNA^{Phe}$ and $tRNA^{Trp}$ species, and putative *TRM7*, *TRM732*, and *TRM734* orthologs are found in the vast majority of eukaryotes. Moreover, the putative human *TRM7* homolog *FTSJ1* is associated with X-linked mental retardation, and the putative *Schizosaccharomyces pombe* Trm7 homolog is reported to be essential in high throughput studies.

Consistent with a conserved circuitry and function, our results demonstrate that Trm7 and Trm732 orthologs from humans, *S. pombe*, and *Drosophila* complement the corresponding *S. cerevisiae* mutants, and in each case can function with the corresponding *S. cerevisiae* interacting partner. Furthermore, we find that *S. pombe trm732*-? and *trm734*-? mutants lack Cm_{32} and Gm_{34} respectively in their $tRNA^{Phe}$. Thus, we suggest that the circuitry of Trm7, Trm732, and Trm734 is conserved in eukaryotes for modification of the anticodon loop of substrate tRNAs.

457 B Structural and Functional Analysis of Archaeal ATP-dependent RNA Ligase.

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Archaea possess two different RNA ligases that catalyze the formation of 3'-5' phosphodiester bond; a monomeric RtcB/tRNA ligase that joins 2'-3' cyclic phosphate and 5'-hydroxyl RNA, and a homodimeric ATP-dependent RNA ligase, that joins a 3'-hydroxyl to a 5'-phosphate RNA. Here we report the structure and mechanism of ATP-dependent RNA ligase from *Methanobacterium thermoautotrophicum* (MthRnl). Crystal structure of MthRnl in complex with ATP, AMP-CPP-Mg, and ligase-AMP intermediate were solved. Analysis of mutational effects on individual steps of the ligation pathway underscored how different functional groups come into play during the ligase-adenylylation (step 1) reaction versus the subsequent steps of RNA-adenylylation (step 2) and phosphodiester formation (step 3), and how does the homodimic quaternary structure contributes to the ligation reaction. Putative RNA binding surface on the MthRnl was identified through mutagenesis. Mutational analysis within the dimer interface suggests that Phe272, Phe273, Ilu304 and Ilu305 contribute to the stability of the dimer, and contributes to the ligation activity. A proposed model on how MthRnl recognize the break in RNA strands will be discussed.

458 C Consequences of FilaminA editing

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RNA editing by ADARs (Adenosine deaminases that act on RNA) is a highly conserved phenomenon leading to diversification of the transcriptome. Of all RNA editing events, Adenosine to Inosine conversion is most common in metazoan, where it affects structured RNAs. A-I conversion occurs in many non coding sequences but is also found at highly conserved protein coding sites. One of such target encodes Filamin A (FLNA). Filamin A is an actin cross-linking protein known to be involved in a variety of functions including cell migration, cell adhesion and cell signaling. Filamin A is built of 24 Ig-fold repeats and editing at one site in repeat 22 leads to a single amino acid change (Glutamine to Arginine) in the protein. Although the function of Filamin A is quite well known, the significance of FLNA editing is poorly understood.

In order to understand the role of FLNA editing, we generated a mouse deficient in FLNA editing ($FlnA^{\Delta ECS}$). Homozygous null mice are viable, fertile and show normal development. The behavioral and physiological phenotyping in these mice highlighted a significant difference in social discrimination and acoustic startle response. Using deep sequencing method from various mouse organs, we found that FLNA editing is highest in the gastrointestinal tract, heart and dorsal aorta. In agreement with FLNA's function as an actin organizing protein, we observed disorganized actin in the epithelial surface of stomach and large intestine of $FLNA^{\Delta ECS}$ mice. Upon challenge with DSS (Dextran sodium sulfate) to induce colitis, our preliminary analyses demonstrate that $FlnA^{\Delta ECS}$ mice show more severe inflammation as compared to controls. This suggests that FLNA editing could function to give protection against epithelial inflammation. Further studies would test the role of FLNA editing in the pathophysiology of colitis and its underlying mechanisms.

Parallel studies focusing on the effect of FLNA editing on cellular phenotypes highlighted its role in cell migration. $FlnA^{\Delta ECS}$ mEFs show much reduced cell migration which was found to be matrix dependent as compared to controls. Currently, we are studying actin remodelling and Integrin signaling in these cells to understand the mechanism by which FLNA editing is involved in cell migration.

459 A 2´-SCF3 Modified Pyrimidine Nucleosides as Labels for Probing RNA Structure and Function by 19F NMR Spectroscopy

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The high natural abundance and intrinsic sensitivity of ^{19}F make it a perfect orthogonal NMR probe for investigating RNA and other biomolecules in *in vitro* and *in vivo* systems. Incorporation of three equivalent fluorine atoms into a biomolecule provides experimental performance at micromolar concentrations which is a significant improvement compared to the low millimolar range required for the single-atom labels. In this study, we present synthetic pathways to 2´-SCF_3 labeled uridine [1] and cytidine building blocks and their incorporation into oligoribonucleotides using the 2´-*O*-TOM-methodology for RNA solid-phase synthesis. Furthermore, the efficient synthetic access to 2′-SCF_3 modified RNA prompted us to evaluate the new label in ^{19}F NMR applications. Here, we demonstrate three examples: 1) probing of the secondary structure of bistable RNA; 2) verification of RNA–protein interactions; and 3) attesting a rationally designed riboswitch module.

1. Fauster, K., Kreutz, C., & Micura, R. (2012). 2′-SCF_3 Uridine –A Powerful Label for Probing Structure and Function of RNA by ^{19}F NMR Spectroscopy. Angewandte Chemie International Edition, 51(52), 13080-13084.

Financial support from the EU REA FP7 (Marie Curie ITN RNPnet 289007) and the Austrian Science Fund FWF (I317, P21641 to R.M.; I844 to C.K.) is gratefully acknowledged.

460 B A Quantitative Atlas of RNA Editing in Mammals Reveals Dynamic Spatiotemporal Regulation

Jin Billy Li[1], Meng How Tan[1], Robert Piskol[1]
[1]Stanford University Department of Genetics

RNA editing is as an important post-transcriptional mechanism that diversifies the transcriptome, but accurate, genome-wide measurements of editing levels are lacking, especially in mammals. Here, we build a comprehensive atlas of RNA editing sites in multiple human and mouse normal tissues, different developmental stages, as well as tissues from ADAR1, ADAR2, FMRP, and Pin1 knockout mice. We apply a high throughput microfluidic-based, targeted sequencing approach to capture thousands of human and mouse editing sites identified by us and others. The global patterns discovered in our accurate measurements reveal dynamic regulation of RNA editing. We are carrying out functional assays to interpret the functionality of several editing sites that are differentially edited. Our results are unprecedented at its scale, and will facilitate understanding of how RNA editing is regulated in different biological contexts.

461 C A single-molecule study on the molecular mechanism of microRNA uridylation

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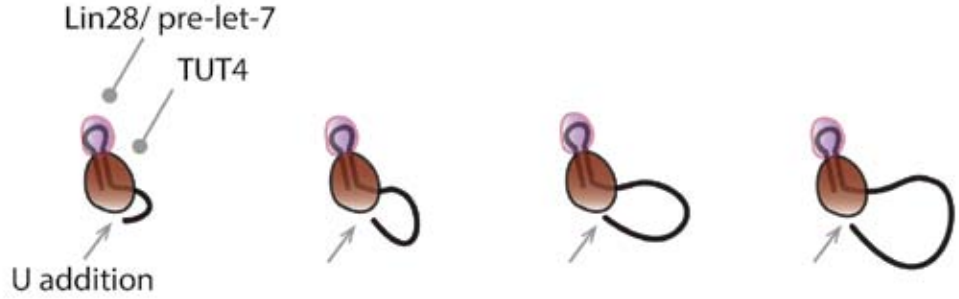
MicroRNAs are a class of short non-coding RNAs which play a key role in the regulation of gene expression in eukaryotes. Even though the birth and maturation of these small RNAs are well-characterized, the regulation and degradation pathways have only recently been explored. Previously, we and other groups showed that TUT4 (Terminal Uridylyl Transferase 4) uridylates precursor microRNA (pre-miRNA) in coordination with Lin28 and thus acts as a posttranscriptional repressor of microRNA maturation. Using single-molecule fluorescence spectroscopy, we show that Lin28 mediates a stable interaction between TUT4 and pre-miRNA [Ref-2011]. With FRET, we further show that TUT4 maintains the tight contact with pre-miRNA and Lin28, while it captures the 3' end of RNA and brings this to its catalytic domain. This mechanism leads to the formation of a unique closed loop of the U tail (see figure 1 below) [Ref-unpublished]. Our study provides insight into the molecular mechanism of Lin28-mediated oligo-uridylation. In addition, it may give a hint to a general mechanism of action of terminal uridylyl transferases.



462 A Structural features of Cbf5 and guide RNA involved in the functions of archaeal box H/ACA RNP complexes

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Pseudouridine (Ψ), the C-5 ribosyl isomer of uridine, is commonly found at several positions of stable RNAs of all organisms. In addition to single or multi-site specific protein-only Ψ synthases, Eukarya and Archaea have specific ribonucleoprotein (RNP) complexes that can also produce Ψ at many sites of different cellular RNAs. Each complex contains a distinct box H/ACA guide RNA and four core proteins, Cbf5 (NAP57 or dyskerin in mammals), Gar1, Nop10 and L7Ae (Nhp2 in Eukarya). Cbf5 is the Ψ synthase in these complexes. Here, we show that Ψs at 23S rRNA positions 1940, 1942 and 2605 of *Haloferax volcanii* (*E. coli* positions 1915, 1917 and 2572) are absent in both Cbf5 and a double stem-loop box H/ACA RNA deleted strains. Plasmid borne copies of *cbf5* and the RNA can rescue the syntheses of these Ψs. Based on *Pyrococcousfuriosus* crystal structure (PDB code 2EY4) we identified several potential residues and structures in *H. volcanii* Cbf5, which play important role in pseudouridylation. We mutated these structures and determined their in vivo effects towards Ψ production at the three rRNA positions. Mutations of some residues that are conserved in all Ψ synthases, and certain residues in the thumb loop resulted in absence of Ψs at all three positions. However, mutations of a few residues abolished one of the three Ψs, and some mutations showed partial Ψ production. Similarly, by changing a number of structures and mutating certain nucleotides in a single stem-loop box H/ACA RNA (the stem-loop responsible for Y2605 in *H. volcanii*) we show that the conserved 3' ACA (AUA here) trinucleotide is not essential for Y formation in vivo and also in vitro (using*Methanocaldococcous jannaschii* recombinant proteins). However, this trinucleotide is required for a stable binding of the Cbf5 to the guide RNA. Furthermore, a proper kink-turn with the two highly conserved G:A pairs (5' and 3') is essential for L7Ae binding as well as pseudouridylation. The guide-target pairings at both 5' and 3' sides of the Y pocket are needed for the modification. This study determines certain essential structures of both Cbf5 and box H/ACA guide RNA in Archaea that are necessary for a functional RNP formation.

463 B The role of ADAR1 in the innate immune response

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Adenosine deaminases acting on RNA (ADAR) catalyze the conversion of adenosine (A) to inosine (I) within double-stranded RNA (dsRNA). ADARs edit short duplex RNAs site selectively whereas longer dsRNA duplexes are promiscuously edited. A-to-I editing within exons results in recoding events that can alter the functional properties of the encoded proteins. However, the majority of editing events (?99% in humans) are promiscuous and occur within non-coding regions of RNA, in *Alu* and other repetitive elements. The biological function of this promiscuous editing is unknown.

In vertebrates there are two catalytically active ADARs; ADAR1 and ADAR2. *Adar1 null* mice die by day E12.5 due to haematopoietic defects resulting from liver disintegration which is associated with an aberrant rise in interferon (IFN). It is not known what unedited substrate(s) causes this lethality in the absence of *Adar1*.

Recently in collaboration with the group of Y. Crow we demonstrate that mutations in ADAR1 cause the human autoimmune disease Aicardi-Goutieres Syndrome (AGS) 1. Similar to the phenotype in the *Adar null* mice, AGS patients display heightened levels of IFN and increased expression of IFN stimulated genes (ISGs). The catalytic activity of the *ADAR1* mutants were assayed *ex vivo* on a known substrate for ADAR1 and revealed that all of the mutant proteins with the exception of the previously identified catalytically inactive *G1007R* mutation edited the transcript efficiently. The other mutations are in the deaminase domain of ADAR1 but how they cause a disease phenotype is unknown.

We have recently rescued the *Adar1 null* mouse to birth by generating a double homozygous for a gene encoding a key innate immune signalling protein. RNA sequencing and RT-qPCR of immune gene transcript was performed on E11.5 whole embryos and the results reveal that the aberrant rise in type I IFN, inflammatory cytokines and ISGs observed in *Adar1-/-* embryos are rescued in the *double knockout* embryos.

We propose that ADAR1 plays a major role in the regulation of endogenous cellular dsRNAs and in the absence of *ADAR1*, cellular RNAs aberrantly stimulate an innate immune response which leads to autoimmune disease phenotypes such as those seen in AGS patients.

1. Rice, G.I. et al. Mutations in ADAR1 cause Aicardi-Goutieres syndrome associated with a type I interferon signature. *Nat Genet* 44, 1243-8 (2012).

464 C Functional implications from the atomic model of the poly(U) polymerase Cid1

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In eukaryotes, mRNA degradation begins with poly(A) tail removal, followed by decapping, and finally mRNA digestion by exonucleases. In recent years, the major influence of 3'end uridylation as a regulatory step within several RNA degradation pathways has driven attention toward the poly(U) polymerase (PUP) enzymes. The protein Cid1 is the prototype of the PUP enzyme found in higher eukaryotes. We have determined the atomic structures of the Cid1 protein bound to its substrate and to its minimal product. Point mutations highlight key residues implicated in the catalytic cycle. Our study further underlines the RNA binding properties of Cid1, in particular for the stabilization of the substrate RNA molecule. Functional characterization of these features are critical for our understanding of miRNAs, histone mRNAs and, more generally, for cellular RNA degradation.

465 A tRNA wobble uridine hypomodification decreases the decoding efficiency of cognate codons in vivo

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Nucleotide modifications in tRNA are ubiquitous in all domains of life and those in the tRNA anticodon are important for accurate codon recognition during translation. Thiolation at the 2-carbon (s2) of the wobble uridine (U34) base is universally conserved in three tRNA species - tE(UUC), tK(UUU), and tQ(UUG). In the cytoplasm of eukaryotes, U34 also carries a 5-methoxycarbonylmethyl group (mcm5). The ELP complex is required for mcm5 formation, while the URM1 pathway mediates 2-thiolation. Aberrant U34 modification is associated with diverse phenotypes such as hypersensitivity to a wide range of chemical stresses in yeast, neurological dysfunction in nematodes, and cytokinesis defects in human cells. In yeast, the phenotypes can be suppressed by overexpressing hypomodified tK(UUU) and tQ(UUG), but the underlying molecular mechanisms are unknown. To delineate them, we analyzed the *in vivo* roles of U34 modification in *Saccharomyces cerevisiae*. We found that the stress sensitivities of cells lacking URM1 pathway or ELP complex genes are increased in strains deleted for chaperones linked to protein synthesis. Wild-type and U34 modification-deficient yeast exhibited similar polysome profiles, arguing against broad defects in translation elongation in the mutant strains. Thus, we explored potential codon-specific defects in translation by ribosome profiling. This approach allowed us to quantitatively compare transcriptome-wide ribosome occupancy in wild type and a U34 thiolation-deficient strain (*ncs2Δ*) grown in rich medium or subjected to diamide-induced oxidative stress. Diamide treatment led to widespread changes in gene expression with similar magnitude in both strains, suggesting that, despite the hypersensitivity of *ncs2Δ* to diamide, lack of U34 thiolation does not compromise the cellular response to this stress. The number of transcripts with altered ribosome loading in the deletion strain, however, was larger in the expression response elicited by diamide. Strikingly, we detected significantly higher ribosome occupancy for codons read by the hypomodified tRNAs in the putative A site within RPFs from *ncs2Δ* yeast. This effect was discernible in both unstressed and diamide-treated cells and was accompanied by smaller increases of cognate codon occupancy upstream of the A site. Our data thus suggests that U34 hypomodification is associated with translational slowdown at cognate codons *in vivo* that may be intensified by combinations of these codons. We are currently investigating whether this phenomenon is conserved in *Caenorhabditis elegans* and what its impact is on protein abundance and function.

466 B Localization of ADAR1 to cytoplasmic stress granules

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Adenosine Deaminases that act on RNA (ADARs) catalyze the conversion of adenosine (A) to inosine (I) within double stranded RNA (dsRNA). Of the three isoforms of ADARs that exist in mammalian cells, only ADAR1 and ADAR2 appear to have catalytic activity. Moreover, differences in subcellular localization suggest that the various ADARs may be involved in different pathways. Analysis of ADAR-null mutants has highlighted the importance of ADARs for normal cell function.

Various studies have demonstrated that ADAR1 p150 plays a role in stress pathways and is essential for survival. Deletion of ADAR1 p150 in mammalian cells results in an embryonic lethal phenotype due to widespread apoptosis. Moreover, a recent study revealed that the loss of ADAR1 p150 also led to global upregulation of interferon-stimulated genes. Apoptosis in the absence of ADAR1 was most likely a consequence of failing to keep the interferon response in check. In addition to these observations, we have recently demonstrated that ADAR1 localizes to cytoplasmic stress granules in mammalian cells following either oxidative stress or interferon induction[1].

We have now used immunofluorescence analyses to investigate which domain(s) of ADAR1 p150 are essential for localization to cytoplasmic stress granules. We have thus demonstrated that the first Z-DNA binding domain (Zα domain), which is uniquely found in ADAR1 p150, is sufficient for localization to stress granules. Furthermore, we have shown that mutation of several key amino acids involved in Z-DNA or Z-RNA binding, as determined from structural analyses, significantly impairs localization. In contrast, the second Z-DNA binding domain (zβ domain), which is unable to independently bind Z-DNA or Z-RNA, is insufficient for localization to stress granules. Importantly, we have shown that editing activity is not required for localization. While unexpected, these observations are in keeping with a previous study that showed that the Z-DNA binding protein 1 (ZBP1) localized to stress granules during various stress conditions. Moreover, we have gone on to show that the Zα domain from ZBP1 is also sufficient for localization to stress granules. We are now further characterizing the requirements for localization of proteins containing Z-DNA (or Z-RNA) binding domains to cytoplasmic stress granules.

[1]Weissbach, R. and Scadden, A.D.J. (2012). Tudor-SN and ADAR1 are components of cytoplasmic stress granules. RNA, 18, 462–471.

467 C A Comprehensive Analysis of RNA Modifying Enzymes in Zebrafish

Marion Pesch[1], Erez Raz[2], Jana Pfeiffer[2], Ursula Jordan[2], Sebastian Leidel[1]
[1]Max Planck Institute for Molecular Biomedicine; [2]Westfaelische Wilhelms-Universitaet Muenster

The chemical modification of RNA nucleosides is a widespread phenomenon that occurs in all species analyzed so far. Some of these modifications are highly conserved throughout evolution and most enzymes required for their generation have been identified and characterized. Nevertheless, we are still lacking insights into the in vivo functions of most of these modifications. Interestingly, in lower eukaryotes, deletion of most RNA modification pathways is not essential. In contrast, deletion of RNA modifying enzymes in higher eukaryotes can lead to severe phenotypes. While most research has been performed in single cell organisms, we know only little about RNA modification pathways in vertebrates. Do RNA modification defects generally lead to more severe phenotypes in higher eukaryotes and are RNA modification enzymes differentially regulated during development and in different tissues?

To gain insights into the spatio-temporal regulation of RNA modification pathways, we undertook a comprehensive analysis of the expression patterns of RNA modification genes in zebrafish (Danio rerio) using in situhybridization. To this end, we identified homologues of all known RNA modification genes in yeast and analyzed their expression patterns throughout zebrafish embryogenesis using embryos at 4h, 10h, 24h, 48h and 72h post fertilization (hpf).

Here we present the expression patterns of 56 putative RNA modification genes and knockdown experiments of selected candidates in zebrafish. We found, that expression levels of RNA modification genes differ. Furthermore, we observed an upregulation of RNA modifying genes during later embryogenesis (24hpf, 48hpf, 72hpf) in particular tissues: brain, eyes, branchial and pharyngeal arches, pectoral fin buds and myomeres. Our results provide insights into the developmental regulation of RNA modification genes in zebrafish and lay the basis for future investigations of the in vivo functions of RNA modifying genes in vertebrates.

468 A Differential expression of Human ADAT subunits

Adrián Gabriel Torres[1], Adélaïde Saint-Leger[1], Francisco Miguel Torres[1], Eva Maria Novoa[1], Anna Tor[1], Lluís Ribas de Pouplana[2]

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We have reported that two specific tRNA wobble base modifications contributed to genome evolution and extant codon usage biases. We showed that, contrary to prior observations, genomic codon usage and tRNA gene frequencies correlate in Bacteria and Eukarya if these two modifications are taken into account, and that presence or absence of these modifications explains patterns of gene expression observed in previous studies. We experimentally demonstrated that human gene expression levels correlate well with genomic codon composition if these identified modifications are considered [1].

As a continuation to this work we have started to characterize the biological role of ADAT (adenosine deaminases acting on tRNA) in mammalian cells, and in particular the potential effects of variations in the levels of ADAT activity upon the human proteome. Our initial results indicate that ADAT levels do indeed vary, and that this variation is due to changes in the levels of one of the enzyme's subunits. We will discuss these results at the meeting.

469 B RNA methylation: a mechanism for post-transcriptional regulation that is deregulated in cancer?

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Internal methylation of eukaryotic RNA in the form of N6-methyladenosine (m6A) and 5-methylcytosine (m5C) have been known to exist for decades, however, laborious detection methods have limited the understanding of their role. With the availability of high-throughput sequencing techniques, these drawbacks have been overcome, revealing non-random distribution of internal methylation in a wide variety of RNA biotypes. Early investigation into the prevalence of 5-methylcytosine (m5C) in RNA has largely been confined to tRNA and rRNA. Recently, we implemented a bisulfite sequencing-based technique for transcriptome-wide as well as locus-specific detection of m5C and mapped thousands of m5C sites in the human transcriptome including in mRNA and non-coding RNA. Biased distribution of m5C between and within mRNAs, e.g. enrichment in the untranslated regions, is consistent with roles in post-transcriptional regulation of gene expression. m5C is a key DNA modification associated with epigenetic gene regulation in mammalian cells and is also known to be deregulated in cancer. We have now begun to investigate the enzymes responsible for modifying mRNA by an RNAi approach as well as a role of RNA methylation in cancer by comparing the m5C profiles of normal prostate cells (PrEC) and metastatic prostate cancer cells (LNCaP). Analysis of the recorded patterns of m5C sites in mRNA show many transcripts are differentially methylated between each cell line. Currently, we are consolidating and extending the potential link of m5C to post-transcriptional regulation and cancer, as well as addressing the molecular function of methylation in mRNA.

470 C Engineered guideRNA-Dependent Deaminases - A Tool to Modify mRNA

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RNA modification is an important mechanism in higher organisms to alter gene expression and to diversify the gene products.[1] Modifications include pseudo-uridinylation, 2'-hydroxymethylation, and adenosine-to-inosine (A-to-I) editing. In contrast to other modifications, the effect of A-to-I editing is readily predictable since inosine is read as guanosine in biochemical reactions. Thus, A-to-I editing formally introduces an A-to-G point mutation on the RNA-level and results, for instance, in 1) the highly specific reprogramming of single amino acid codons, and 2) the alteration of RNA splice patterns.[1,2] Consulting the table of the genetic code one finds that 12 out of the 20 canonical amino acids could be targeted including Asp, Glu, Asn, Gln, His, Lys, Arg, Ser, Thr, Tyr, Ile, and Met/Start, and all three Stop codons, a striking accumulation of residues that are essential for enzyme catalysis, posttranslational modification (signaling), and general protein function. Consequently, harnessing enzymatic A-to-I deamination would make it possible to manipulate RNAs and their protein products in a currently unprecedented manner.

A-to-I editing normally operates via a protein-guided mechanism, thus its re-direction towards new targets has not been achieved yet. In our study,[3] we now present a simple way to steer deaminase activity towards user-defined sites on mRNA in order to introduce point mutations. To achieve the most rational and arbitrary target selection, we turned hADAR1 into a guide-RNA-dependent enzyme by covalently attaching a guide-RNA to its deaminase domain (see Figure). The potential and limitations of re-directing RNA-editing for application in biochemistry and medicine will be discussed.

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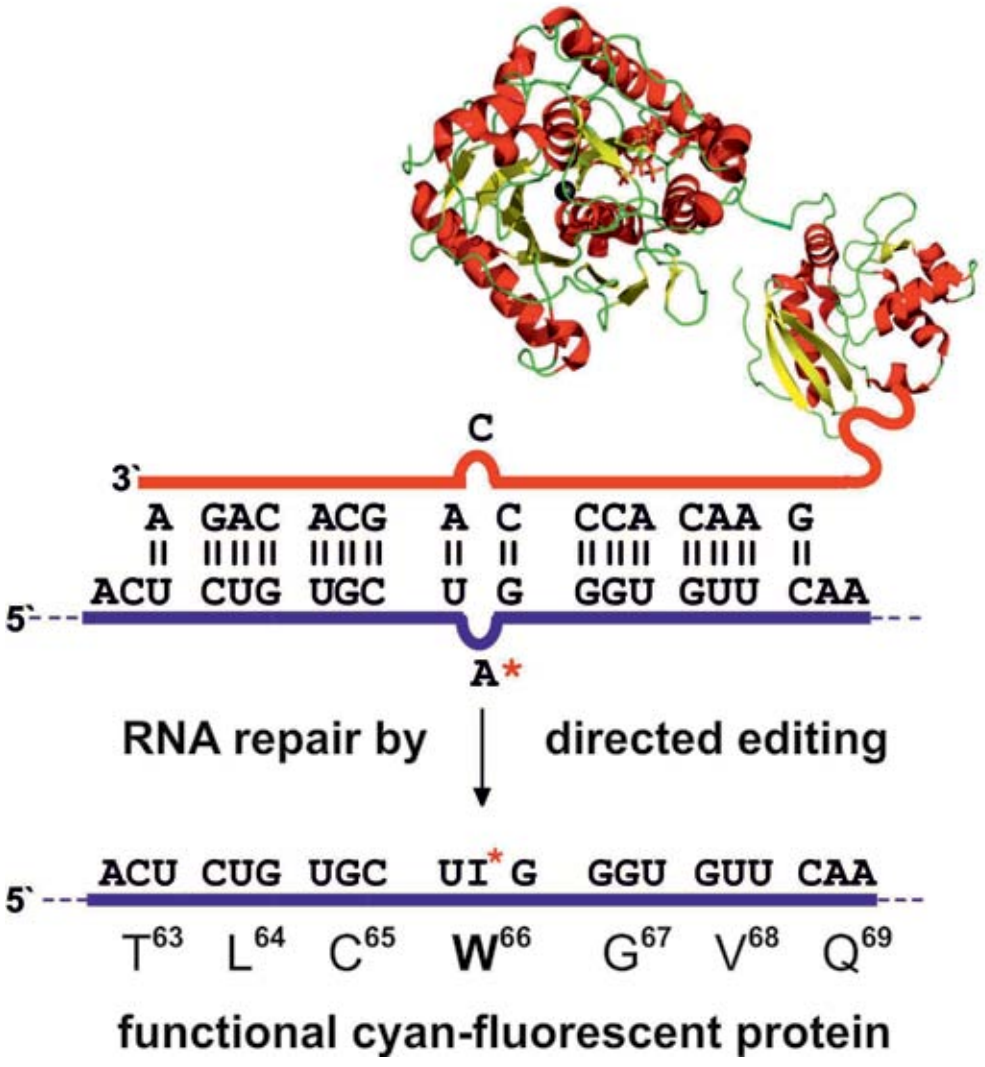

471 A Impact of inverted SINEs on gene expression

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The short interspersed elements (SINEs) comprise the largest family of repetitive elements in the mammalian genome and are enriched in gene rich regions. SINEs frequently occur within genes where they are mostly located in introns and UTRs. SINEs can have a dramatic impact on the transcriptome by several means such as repressing global transcription by impairing polymerase II activity, affecting folding and splicing, or by triggering Staufen mediated decay. In addition, SINE elements in inverted orientation can basepair and have been reported to control gene expression. As underlying mechanisms, RNA-editing, sequence specific degradation, or translational control has been discussed.

Here we aim at determining the impact of inverted, basepaired SINEs located in 3' UTRs on gene expression. So far we could show that the presence of inverted SINE in 3' UTRs can repress gene expression and reduce mRNA levels. Using knock out cells we could show that the reduced gene expression is sequence-independent and does not rely on known double-stranded RNA-dependent pathways, such as Dicer, Staufen, PKR, or ADAR.

The reduced RNA levels measured for RNAs containing basepaired double-stranded SINEs could not be correlated with an increase in mRNA decay. It therefore seems possible that inverted SINES can control the rate of RNA synthesis.

Thus, besides the previously reported nuclear retention and translational repression induced by inverted SINEs, a third mechanism may be triggered. The signals and pathways required for this repression are currently being investigated.

472 B RNA-binding proteins regulate substrate-specific changes in A to I editing patterns

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RNA-editing by adenosine deaminases that act on RNA (ADARs) converts adenosines to inosines in structured or double-stranded RNAs. As inosines are interpreted as guanosines by most cellular machineries, this type of RNA-editing greatly diversifies the transcriptome, by altering splicing, processing, localization, and translation of coding and non-coding RNAs. Consistently, RNA-editing by ADARs is an essential process in mammals.

Interestingly, editing levels vary in different tissues, during development, and also in pathogenic conditions. Frequently, editing levels do not reflect the levels of ADARs found in a particular tissue or cell type. Hence it appears, as if additional factors may stimulate or repress editing in specific tissues. To isolate such factors we have performed a yeast screen that allows to detect changes in editing via the expression of a reporter gene.

From this screen we have isolated both repressors and enhancers of editing. Three proteins that repress ADAR2 mediated RNA-editing are the RNA-binding proteins RPS14, SFRS9, and DDX15. Overexpression or depletion of these proteins in mammalian cells can decrease or increase editing levels by 15% thus allowing a modulation of RNA editing up to 30%. Interestingly, the three proteins alter RNA-editing in a substrate-specific manner, changing the RNA editing levels of some, but not all editing targets. This substrate specificity correlates well with the RNA-binding preferences of the three proteins.

In mammalian cells, SFRS9 significantly affects editing of the two substrates CFLAR and cyFIP2, while the ribosomal protein RPS14 mostly inhibits editing of cyFIP2 mRNA. The helicase DDX15, in turn, has only a minor effect on mammalian editing substrates. However, lack of DDX15 has a strong effect on the editing of 3' UTRs in C. elegans.

Expression of the three factors decreases during mouse brain development nicely showing an inverse correlation with the increase in global editing observed during brain development.

Most interestingly, expression levels of SFRS9 and DDX15 respond strongly to neuronal stimulation or repression of rat brain cultures. The fluctuation of these two factors again correlates nicely with the observed changes in editing levels in repressed or stimulated brain slices.

Colocalization and immunoprecipitation studies demonstrate a direct interaction of SFRS9 and RPS14 with ADAR2, while DDX15 associates with other helicases and splicing factors. Our data show that different editing sites can be specifically altered in their editing pattern by changing the local RNP landscape.

473 C Enzymes involved in human cap structure formation: their structure and function

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The 5′ cap of human messenger RNA consists of an inverted 7-methylguanosine linked to the first transcribed nucleotide by a unique 5′ - 5′ triphosphate bond followed by 2′-O-ribose methylation of the first and often the second transcribed nucleotides, serving to modify efficiency of transcript processing, translation and stability. Enzymes that methylate ribose moieties of the first and the second nucleotide of the transcript, named hMTr1 and hMTr2 respectively, have been recently identified. Both enzymes contain related catalytic domains with a Rossmann-like fold characteristic for the RFM superfamily of methyltransferases (MTases), as well as additional domains. However their structures and mechanisms of substrate recognition and methylation remain unknown. We report the crystal structure of methyltransferase (MTase) domain of hMTr1 in an unliganded form, as a ternary complex with a cofactor S-adenosyl methionine (SAM) and a 5′ mRNA cap analogue, and a complex with SAM and a short capped RNA. These structures reveal that with the exception of the methylated guanosine, the interactions occur between the protein and the phosphodiester backbone of the RNA molecule. This suggests that substrate binding and methylation are sequence–independent. Based on the crystal structure of hMTr1 catalytic domain as a template we generated a comparative model of the hMTr2 catalytic domain and carried out mutational analysis of hMTr2, which disclosed residues important for RNA and SAM binding. Inability to establish human somatic cells lacking *HMTR2* coding sequence argues for an essential function of its protein product in cell metabolism.

474 A Genetic analysis of the Prp28-bypass mutant reveals further insights on U1 snRNP/5′ splice site interaction

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Essential genes are mostly ancient, conserved, and indispensable components in the genome. Most of them are involved in critical cellular processes, representing the “core genome” of an organism. However, recent studies have shown that a number of essential genes can be made dispensable in the presence of specific “bypass mutations”, providing nonconventional routes to understand the functions of essential genes. We have previously shown that the otherwise essential Prp28 DExD/H-box splicing factor, known to facilitate the U1/U6 switch at the 5’ splice site (5’ss) during spliceosomal assembly, can be bypassed by specific alterations in U1-snRNP-related components. These alterations include specific changes in U1C, Snu71, Prp42, Cbp80, and Ynl187. Detailed analyses on one of bypass mutants, i.e., the *prp28Δ U1C-[L13F]* strain, suggested that weakening the U1-5’ss base pairing is likely to account for dispensing the essential Prp28. These results indicated that Prp28 counteracts the stabilizing effect of U1C. To dissect the intricate U1 snRNP/5’ss interactions, we sought to isolate dominant mutations capable of rescuing the cold-sensitive (cs) phenotype of the Prp28-bypass strain (see above), which reflects the stalling of U1 snRNP dissociation from the 5’ss at low temperature in the absence of Prp28. The isolated 24 mutations are mapped to at least three linkage groups. Cloning of the suppressor genes from two linkage groups revealed specific mutations, often locate at the conserved positions, in genes encoding U1C and Snu71. Identification of the third linkage group is underway. These data suggest U1C and Snu71 may engage specific protein-protein or RNA-protein interactions on U1 snRNP/5’ss during pre-mRNA splicing. Ongoing biochemical analyses via 4-thioU and psoralen crosslinking shall provide mechanistic insights into the roles of these U1 snRNP components, which remain largely unclear. The described approach for probing complex mechanism should be applicable to other systems in which an essential gene can be bypassed.

475 B Chemical tools for investigating alternative RNA splicing

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Splicing is the processing of pre-mRNA introns and exons to give mRNA for protein biosynthesis. Most eukaryotic genes contain multiple introns and exons. Alternative RNA splicing determines which combinations of exons are spliced, and therefore which protein isoform is synthesised. The variety of the possible isoforms gives rise to the diversity of the proteome; ca. 20k protein-coding genes in the human genome give rise to ca.100k transcripts.

Although splicing effects protein diversity and is critical in many genetic diseases, the molecular mechanism of regulation is not well understood. Pre-mRNA contains regions which act as exonic splicing enhancers (ESEs) which bind to SR proteins and enhance splicing. Knowledge of the mechanism of ESEs can help understanding of splice site selection at the molecular level, as well as in the development of therapeutic strategies for the treatment of splicing-related genetic diseases such as spinal muscular atrophy (SMA). Our approach to investigation of these mechanisms focuses on the development and application of novel chemical tools for probing these complex biological systems. Through the development of a new chemical approach, we have recently shown evidence that ESEs do not act via RNA looping mechanisms. Recent results in this area will be presented.

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476 C Biophysical characterization of the recombinant S. cerevisiae Lsm2-8 complex

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Biochemical and biophysical studies of macromolecules have historically focused on simple systems, frequently monomers or dimers of proteins. Many of the most important cellular processes, however, are carried out by complicated, multicomponent assemblies. A prime example of this is the spliceosome, the nuclear complex that carries out pre-mRNA splicing, which in S. cerevisiae contains five small, nuclear RNAs (snRNAs) and nearly 100 proteins. While the study of such complex systems has taken on increased urgency as their importance has become clearer, their recombinant expression and purification remain challenging.

In this poster, we report the development of a method to express and purify the hetero-heptameric LSm complex of proteins that associates with U6 snRNA. U6 is the most highly conserved snRNA, and substantial evidence suggests that it is intimately involved in the catalytic steps of splicing. The investigation of U6 function is complicated by its highly dynamic nature: it exists by itself as the U6 small, nuclear ribonucleoprotein (U6 snRNP), as a base-paired complex with U4 in several particles, and as a base-paired complex with U2 in the active spliceosome. The U6-associated Lsm proteins are known to play a role in base pair formation with U4, and, intriguingly, they have been shown to dissociate from U6 prior to spliceosome activation, suggesting they may have additional functions.

We present a system for simultaneously expressing all seven U6-associated Lsm proteins recombinantly in E. coli. The proteins appear to express at approximately stoichiometric levels, and co-purify as a single peak by gel filtration chromatography. Mass spectrometry confirms the presence of all seven proteins in the complex, and electron microscopy demonstrates that they assemble into toruses, as predicted from previous studies. Ab initio models of the molecular envelope based on SAXS data are consistent with crystal structures of homologous protein complexes. We are currently characterizing the ability of the Lsm complex to associate with U6 snRNA and Prp24. Interestingly, the complex appears to dramatically promote the association of U4 and U6, even in the absence of any other proteins. We anticipate that this system will be useful for the expression and analysis of numerous other protein complexes.

477 A smFRET studies of U6 during spliceosome activation in budding yeast

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Both splicing fidelity and chemistry rely on a defined series of snRNA structural rearrangements catalyzed by DExD/H helicases and regulated via other trans-acting factors. The highly conserved snRNA U6 plays a direct role in splicing catalysis. Catalysis requires the presence of a 3' U6 internal stem loop (ISL) structure that coordinates catalytically essential Mg2+ ions analogously to domain V of group II introns. To prevent aberrant ISL formation U6 conformation is very highly regulated. U6 arrives at pre-mRNA base-paired with U4 in the U4/U6-U5 tri-snRNP, which sequesters the U6 ISL, blocking premature U6 activity. U4/U6 unwinding allows for the mutually exclusive U2/U6 interactions required for catalysis to form. In yeast U4/U6 unwinding is performed by DExD/H box helicase Brr2, a component of the U5 snRNP. Intriguingly, it has been shown that, in the presence of ATP and Mg2+, Brr2 will unwind U4/U6 duplexes in purified tri-snRNP in the absence of pre-mRNA, which indicates that Brr2 activity must be carefully regulated in vivo. Previous experiments indicate that Brr2 activity is modulated by U5 component Prp8 and Snu114, however Prp8, and Snu114 are tri-snRNP components as well and must also be tightly temporally and spatially regulated. Despite extensive knowledge of the players involved, the kinetics and fundamental molecular mechanisms of U4/U6 unwinding by Brr2 and its regulation are currently unknown. This deficiency results from inherent limits to bulk experimental systems wherein the multiple reversible steps in spliceosome assembly proceed asynchronously, and the splicing reaction itself is inefficient. We are currently generating and testing reagents required to perform smFRET. We have developed a Cy3/Cy5 intramolecular U6 labeling scheme to monitor the formation of the U6 ISL, which assembles into U6 snRNPs and is capable of in vitro splicing. Using this system, we can provide fundamental kinetic and molecular information on U4/U6 unwinding, and how unwinding is regulated during spliceosome activation.

478 B Role of U2 stem IIb in splicing progression

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The highly dynamic properties of the spliceosome complicate its structural analysis. Previous studies have reported U2 snRNA conformations representative of different stages in the splicing process. The branch site loop (BSL) is formed at the BS-recognition step, U2 stem IIc and IIa alternate between more stable steps, and U2/6 helices I and II are present during the catalytic steps. Here we study the role of U2 stem IIb. We combined deletions of stem IIb with ATPase/helicase mutants to test for roles at different times and with splicing-defective reporters to detect consequences on the progression of splicing. U2 stem IIb deletion interacted genetically with Prp5 (which is thought to open the BSL), with Prp2 (which activates the catalytic spliceosome) and with Prp22 (which disassembles spliced mRNA from the spliceosome). U2 stem IIb mutants also exacerbated defects of most of the reporters tested.

We also show that there is a complex network of tertiary interactions among stem IIb, nts 26-30 of U2 snRNA, and Prp5, and that stem IIb integrity is needed for Prp5 recruitment. These results indicate a functional relationship among these structures. One hypothesis is that base-pairing interactions between U2 stem IIb and nts 26-30 of U2 compete with other interactions to help disrupt the BSL or U2/6 helix Ia. To better understand this, we designed point mutants to hyperstabilize or destabilize putative alternative conformations formed between stem IIb and nucleotides 26-30 of U2 and analyzed suboptimal splicing reporters. We show that some reporters improve when the alternative structures are stabilized, suggesting a role in opening the catalytic core, whereas others are improved when the alternative structures are destabilized, suggesting roles in promoting stabilization of the complex.

We conclude that U2 stem IIb facilitates opening of the BSL during the BS-recognition step, opening of the catalytic core during transitions between stable conformation, and spliceosome disassembly. This role can be by recruitment of ATPases/helicases that promote the changes, by competition between alternative base-pairing interactions, or both.

479 C Conserved Slu7 motif confers the preference for distal splice sites in yeast

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Mechanisms governing proper 3' splice site (3' ss) selection for the 2nd catalytic reaction of splicing, which are important for alternative 3' ss usage in Metazoans, are only incompletely understood. In S. cerevisiae, sequence specific (re)positioning of 3' ss for catalysis occurs only after lariat formation.

Splicing in *S. cerevisiae* represents a unique system to examine the evolutionarily conserved splicing factors in a simplified setting. One of the key factors that is necessary for 3´ ss positioning is the essential 2nd step factor Slu7, which associates with the spliceosome only immediately before or during the 1st step. Slu7 was found in vitro to be required for splicing of introns with 3´ ss located >9 bp from BP (Brys and Schwer, 1996). Allele slu7-1, isolated through its synthetic lethality with U5 mutants, suppressed the usage of distal splice sites in recombinant splicing substrates (Frank and Guthrie, 1992). Slu7 contains several highly conserved regions, but its N-terminal 199 amino acids, including a putative zinc knuckle motif (aa 122-135), are dispensable for the essential function (Zhang and Schwer, 1997).

Using the screen for synthetic lethality and subsequent direct testing, we identified genetic interactions between the allele of the splicing factor PRP45 (*prp45*(1-169)) and several mutations of *SLU7* (Gahura et al. 2009). The mutations mapped between arginines 247 and 271 within the essential part of *SLU7*. Analyses of splicing in vivo revealed second step splicing defects and impaired utilization of suboptimal splicing substrates. Importantly, several point mutations suppressed the utilization of distant splice site when two competing sites were present on a recombinant substrate. This feature was most prominent when the proximal splice site was close to BP (~10 bp). The novel *SLU7* mutations also shifted the utilization of cryptic splice sites on an endogenous gene in favor of the proximal AG. The newly identified Slu7 motif, unlike, e.g., the zinc knuckle motif, is part of the structure that is responsible for efficient positioning of distal splice sites for catalysis. More extensive mutational analysis of this region is under way to elucidate the role that this part of the protein plays in pre-mRNA splicing.

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480 A Prp8-substrate interactions in yeast spliceosomes

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The U5 snRNP protein Prp8 interacts with all 3 sites of chemistry in pre-mRNA substrates and with crucial residues in U5 and U6 snRNAs. These contacts have been mapped to the central region of yeast Prp8 by site-specific RNA-protein cross-linking and cleavage at engineered protease sites [Turner et al., RNA 12: 375-386 (2006)]. We have now used cleavage at natural and engineered Methionine residues by Cyanogen Bromide to map Prp8 contacts with the spliceosome's catalytic RNA core in more detail. Spliceosomes were stalled between catalytic steps 1 and 2 by 3' splice site mutations AG>AC or AG>AdG and captured via an epitope tag on Prp19 or one of the step 2-specific splicing factors [Slu7 or Prp18]. These techniques enabled us to monitor and physically map Prp8 contacts with the intron Branch Point [BP] and the 3' Splice Site [3'-SS].

Branch Point and 3' Splice Site cross-links map between residues 1585 and 1598 in yeast Prp8. In our recent structure of Prp8 [Galej et al., Nature 493: 638-643 (2013)] this sequence is part of a highly conserved linker region between the Reverse Transcriptase [RT] and Endonuclease [En] domains. Alanine-scanning mutagenesis of this region of Prp8 produced mutants in or near the cross-linked peptide with slow growth or lethal phenotypes. Moreover splicing assays in vitro revealed that these mutations caused accumulation of splicing intermediates. The cross-links from 2 of the sites of splicing chemistry [BP and 3'-SS] together with the positions of numerous splice site suppressor mutations unambiguously locate the spliceosome's catalytic RNA core to a cavity formed by the RT, En and RNaseH-like domains of Prp8.

481 B Identification of new natural compounds that modulate splicing in vitro and in cells

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Pre-mRNA splicing is an important step in gene expression. However in contrast to the other steps involved in gene expression, such as transcription and translation, few specific chemical inhibitors have been characterized that block specific steps in the splicing mechanism and which can be used to dissect the splicing process. Therefore, the identification of specific and selective splicing inhibitors/modifiers would not only be extremely valuable for research purposes as tool compounds, but also potentially useful for therapeutic applications.

To date compounds that have been described to be general splicing inhibitors include: Spliceostatin A (1), Isoginketin (2) and Pladienolide B (3), which are all natural products. In addition, several other natural compounds derived either from extracts of plants, or microbes, have also been reported either to inhibit splicing in vitro, or to change splicing of certain transcripts in cells.

We have screened a restricted set of compounds and identified a new group of closely related natural products, typified by the structure we term GRE010, which not only inhibit human pre-mRNA splicing in vitro but also alter the splicing of a subset of pre-mRNAs in vivo in a variety of different human cells lines. Interestingly, treatment of cells with GRE010 also leads to the specific relocation of early assembly splicing factors within the nucleus, which correlates with their inhibition of splicing and spliceosome assembly in vitro at a step after an A complex is formed. We are currently investigating the effect of GRE010 on splicing and cell growth in greater detail.

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482 C A new role for U2 snRNA in alternate 3' ss selection

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We have documented how several sequence features in the intronic region downstream from the BS act together to define the 3' ss. Briefly, the yeast spliceosome will pick any HAG (H = A, C, G) present in a window of ~35 nt, starting at position 10 from the BS; and because stems, loops, and bulges are not included in this window, pre-mRNA folding plays a critical role in 3'ss selection. Interestingly, this does not apply to the first nt after the BS; and instead our results are more consistent with the requirement to have this region unfolded for efficient splicing. They show that in a reporter with two 3'ss, one blocked by a stem and the other within spliceosomal reach, a switch in 3'ss selection can be induced by placing the stem at 10 nt or less from the BS. According to our model, a stem near the BS will not be formed, and this would both make available the occluded AG and place the downstream AG outside the spliceosomal window. The "exclusion zone" downstream from the BS would be also consistent with the proposed interaction of a number of essential spliceosomal factors with positions downstream the BS. However the molecular reasons for this remain unclear.

A genetic screen has revealed a role for U2 snRNA in modulating this 3'ss selection switch. Thus, in cells expressing U2 U40C the requirement for the unfolded region downstream from the BS is reduced and the 3'ss switch described above takes place closer to the BS. Position U40 is part of the U2 BSL stem-loop, which has been shown to play a role in BS identification (Perriman & Ares (2010) Mol. Cell). U40C stabilizes the BSL and promotes BS recognition as reported. However, other mutations that also stabilize the BSL, such as U44A, and U40G G32C, do not reproduce the U40C effect on 3' ss selection.

We have analyzed the intronic requirements for the U2-U40C phenotype and our data are consistent with the shifted recognition of the BS, by three nucleotides, for the first step of splicing. This shift occurs as well with the wt U2, albeit to a lesser extent. Surprisingly, with either U2 wt or U40C the shift is independent of having a stem near the BS. We are investigating the role of the regular BS in this process, as well as the molecular reasons behind the U2-U40C phenotype, distinct from other mutations that stabilize the BSL.

483 A BRAF branch point mutation confers Vemurafenib resistance

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The serine/threonine kinase BRAF is a proto-oncogene that acts in the MAP kinase pathway, connecting mitogen signals to transcription of proliferation genes. Constitutively activating mutations of BRAF are the cause of more than 60% of melanomas. The most prevalent BRAF melanoma mutation is V600E which constitutively activates downstream signaling pathways. Vemurafenib is a potent inhibitor of BRAF (V600E), however, patients rapidly develop resistance. One Vemurafenib resistance mechanism is the emergence BRAF (V600E) splicing isoform lacking exons 4 to 8 (3-9 isoform), harboring the RAS-binding domain. Here we show that a branch point (BP) mutation in intron 8 of BRAF (V600E) is the cause for the emergence of the BRAF 3-9 isoform in a subset of melanoma cells. We show that impairing BP recognition by either silencing SF3b155 or by the use of spliceostatin A promotes 3-9 usage. Using a BRAF minigene, we show that the BP mutation changes the ratio of splicing of the reporter minigene in favor of the 3-9 isoform. Similarly, increased intron 3 length favors 3-9 splicing, suggesting slower removal of intron 3 as the reason for mis-splicing. In support, slower removal of intron 3 compared to intron 4 was also found in the BP mutated melanoma cells. We identified SRp55 binding sites in the vicinity of the mutated BP and silencing of SRp55 in the BP mutant cells reduced the 3-9 splicing. These observations represent the first steps in characterizing aberrant BRAF splicing responsible for Vemurafenib resistance and they might point to a therapeutic strategy to eliminate Vemurafenib resistance.

484 B Conservation of U2 protein – branch site interactions between yeast and human, as investigated by UV crosslinking

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The multiple structural rearrangements occurring during catalytic activation of the spliceosome are only poorly understood at the molecular level. In the early assembly of human spliceosomes the U2 SF3a and SF3b proteins help to recruit the U2 snRNP to the branchsite by engaging in multiple contacts with a region upstream of the branchsite (the so-called "anchoring site"), the branchsite itself and a region downstream of it (Gozani et al., Genes and Dev. 2000). While most of the U2 proteins in the yeast S. cerevisiae are evolutionarily conserved, it is not clear whether they interact in a similar way with the extended branchsite region in yeast spliceosomes and whether an equivalent to the human anchoring site exists in yeast pre-mRNAs. While it is clear that the affinity of the U2 proteins for the spliceosome is significantly lowered during catalytic activation, it is not known whether this leads to a remodeling of U2 protein-pre-mRNA interactions. To address these questions we have started to investigate protein-RNA interactions by UV crosslinking of purified yeast spliceosomes stalled at specific assembly stages such as the Bact, B* or C complex, using our recently developed, purified yeast splicing system (Warkocki et al., NSMB 2009). For this purpose we have assembled spliceosomes onto actin-pre-mRNA constructs that harbored either site-specifically 32P -labeled nucleotides or defined 32P-labeled RNA stretches, focusing initially on the intronic region in and around the branchsite. Crosslinked spliceosomes were digested with ribonucleases and crosslinked 32P-labeled proteins were initially identified by 2D gel electrophoresis (Agafonov et al., MCB 2011) and by immunoprecipitation. Our results indicate that the region directly upstream of the branchsite is a major interaction platform of the U2 snRNP proteins in yeast Bact complexes. Some proteins also crosslink within the branchsite, as well as to the intronic region downstream of it, suggesting that interaction of U2 proteins with the extended branchsite region of the pre-mRNA is conserved in eukaryotes. During catalytic activation and the first step of splicing, we do not observe any significant changes in the interaction pattern. This suggests that the U2 proteins remain in close contact with the extended branchsite region even after the first step of splicing. In addition to U2 SF3a and SF3b proteins, several additional proteins, which are currently being characterized, are crosslinked to the branchsite region in a dynamic manner. We are currently extending our crosslinking approach to study protein interactions with other regions of the pre-mRNA within purified yeast spliceosomes.

485 C Identification of small molecule pre-mRNA splicing inhibitors using a stage-specific, high-throughput in vitro splicing assay

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Disruption/misregulation of alternative and constitutive splicing are the cause or a severity modulator of many human diseases, including among others cancer and, neurodegenerative and autoimmune diseases, making the spliceosome a highly attractive drug target. Small molecule inhibitors that block discrete steps of the extremely dynamic functional cycle of the spliceosome would not only be of potential therapeutic value, but also be highly useful for the detailed investigation of the structure and function of the spliceosome. However, only a limited number of small molecule inhibitors that specifically target the pre-mRNA splicing machinery have been identified to date. We previously established a robust, rapid and sensitive high throughput in vitro splicing assay, which monitors in the wells of a microplate the formation of step I spliceosomes (i.e. the spliceosomal C complex) by measuring the association of a FLAG-tagged version of the DEAD box ATPase, Abstrakt, which is incorporated into the spliceosome first upon C complex formation[1]. Using this assay, we screened a chemical library of approximately 160,000 compounds at 50 μM concentration. All compounds showing at least a 50% decrease in the signal intensity of bound Abstrakt were subjected to a second test, and reproducible hits were finally tested in an in vitro splicing assay using ^{32}P-labeled adenovirus-derived MINX-MS2 pre-mRNA as substrate. Ten compounds were confirmed to be inhibitors of pre-mRNA splicing in vitro, and exhibited IC_{50} values ranging from 3 to 50 μM. To determine at precisely which stage they inhibit splicing, we performed a splicing time course and analysed the spliceosomal complexes formed by native agarose gel electrophoresis. At least one compound (hereafter designated 28), led to an accumulation of A complexes and a complex migrating slightly faster than the B complex. To characterize in more detail spliceosomal complexes formed in the presence of compound 28, we subjected the stalled splicing reactions to glycerol-gradient centrifugation and purified the complexes in a given peak by MS2-MBP affinity selection. Initial analyses of the RNA and protein composition of complexes affinity-purified from the "B-like" peak, suggest that compound 28 may stall splicing at a novel stage of the spliceosome activation step. This apparently unique property may allow us to obtain a novel snapshot of the spliceosome assembly pathway for detailed structural and functional investigations, and thus to improve our limited understanding of the dynamic rearrangement of spliceosomal components during spliceosome activation.

[1]Samatov et al., Chembiochem. 2012, 13:640-644

486 A Localization of the pre-mRNA path in the activated yeast spliceosome by immuno-EM

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The spliceosome is a highly dynamic molecular machine whose composition is not static and whose structure undergoes several rearrangements during each cycle of splicing. A particularly dramatic structural rearrangement of the spliceosome occurs during the transition from the pre-catalytic B complex, which contains all of the spliceosomal snRNPs U1, U2, U4/U6 and U5, to the activated B^{act} complex. This remodeling step involves the dissociation of the U1 and U4 snRNPs, allowing the U6 snRNA to basepair with both the branchsite-bound U2 snRNA and the 5'end of the intron, forming a network of RNA-RNA base pair interactions that is thought to be at the heart of the spliceosome's catalytic center. While the biochemical composition of the various assembly stages of the yeast spliceosome is relatively well known, the ultrastructural study of isolated yeast spliceosomes is at a relatively early stage. Recently, we presented initial, high-quality electron microscopy images of yeast B and B^{act} spliceosomes (Mol Cell, 2009, 36:593). 2D images of the B^{act} complex exhibit a mushroom-like appearance, consisting of a main, slightly asymmetric body (the "mushroom cap"), from which a "mushroom stalk" is seen to emerge as a slightly tapered, ca. 15 nm-long protuberance.

A first step in understanding the spliceosome's architecture is to localize the position of individual spliceosomal components and functional centers. To topographically locate the emerging catalytic center of the purified yeast B^{act} complex, we first determined by DNA oligonucleotide-directed RNAse H digestion, accessible regions of the pre-mRNA closest to nucleotides of the 5' splice site and branch site. For EM localization studies, biotinylated 2'-OMe-RNA oligonucleotides were annealed to these accessible regions. The position of the bound oligonucleotides was then visualized by EM at the surface of the B^{act} complex using anti-biotin antibodies. We also inserted MS2 hairpin loops at defined positions of the exons and the intron, and mapped their positions in the B^{act} complex as well. The identified positions in the B^{act} EM map give first hints as to the path of the pre-mRNA in the yeast B^{act} complex and further define the possible location of the emerging catalytic core at the center of the mushroom cap.

487 B Functional and structural analysis of Cwc25 required for first-step splicing

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Cwc25 is a first-step splicing factor that acts after Prp2 and is recruited to the spliceosome in the presence of Yju2. Previous study shows that Cwc25 with four copies of V5 tagged at the N-terminus has better first-step splicing activity, but binds to the spliceosome more stably when tagged at the C-terminus. These results indicate that tags at different positions of the protein would affect the function of the protein. We therefore systematically analyzed positional and length effects of the tag on Yju2 and Cwc25 on the splicing activity. Splicing was carried out in extracts depleted of both Yju2 and Cwc25, and complemented with different versions of tagged Yju2 and Cwc25. It was found that while splicing was moderately affected when only one protein contained additional residues at the N-terminus, splicing was impaired when both Yju2 and Cwc25 contained additional residues at the N-terminus. We also examined how the structure of Cwc25 affects its function. The N-terminus of Cwc25 is conserved while the C-terminus is highly diverse. A series of C-terminally truncated forms of Cwc25 were constructed. Analysis of the splicing reaction and spliceosome association revealed that deletions of Cwc25 in the C-terminus resulted in destabilization of Cwc25 binding to the spliceosome, suggesting that the C-terminal region of Cwc25 may play a role in stabilizing the association of Cwc25 with the spliceosome.

488 C The G patch protein Spp2 couples Prp2-mediated ATP hydrolysis to catalytic activation of the yeast spliceosome

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The spliceosome is a dynamic molecular machine that is continuously remodeled during its assembly and catalytic cycle. A major structural rearrangement occurs during the transition from the activated B^{act} complex to the catalytically competent B* complex, which entails the loss and destabilization of numerous spliceosomal proteins. This structural rearrangement is driven by the DEAH-box ATPase Prp2 in cooperation with its essential co-factor Spp2. Basic questions concerning the requirements for triggering Prp2's ATPase activity and how Prp2-mediated ATP hydrolysis is coupled to the B^{act} to B* complex transition have not been answered yet. The role of the G patch protein Spp2 in this process is also not well understood. It has been shown, however, that Spp2 may interact in solution with the C-terminal DUF domain of Prp2 via its G patch region (MCB 24, 2004,10101). Here we have addressed these questions using a purified yeast splicing system (NSMB 16, 2009, 1237).

The major findings are as follows: (1) Spp2 significantly enhances the oligo(U)30-stimulated, but not the unstimulated, intrinsic ATPase activity of Prp2 in solution. (2) Prp2 binds stably to purified B^{act} spliceosomes in the absence of Spp2 and vice versa. The stability of Spp2 binding to the B^{act} complex is enhanced in the presence of Prp2, suggesting that they interact with each other in the spliceosome. (3) We have measured for the first time the spliceosome-dependent NTPase activity of Prp2 (using UTP instead of ATP as exogenous source of energy to avoid measuring possible ATPase activities of other endogenous ATPases such as Brr2) and show that B^{act} spliceosomes efficiently trigger Prp2-mediated UTP hydrolysis in the absence of Spp2, with an initial hydrolysis rate of about 120 UTP•Prp2-spliceosome^{-1}•min^{-1}, which does not significantly level off after 10 min. However, B^{act} spliceosomes do not undergo catalytic activation under these conditions. (4) In the presence of Spp2, Prp2 in the spliceosome exhibits a similar initial rate of UTP hydrolysis, which levels off after 5-7 min. This effect is even more pronounced in the presence of both Spp2 and Cwc25, suggesting that in the presence of the latter factors, the spliceosome is remodeled and looses its binding site for Prp2.

In conclusion, our data suggest that certain structural elements of the B^{act} spliceosome can efficiently trigger the NTPase activity of Prp2 even in the absence of Spp2. UTP hydrolysis by Prp2 in the spliceosome observed in the absence of Spp2 is, however, unproductive in that it does not lead to catalytic activation of the B^{act} spliceosome. The energy of Prp2-mediated UTP hydrolysis can only be coupled to structural rearrangements of the spliceosome that yield the B* complex when Spp2 is simultaneously present in the spliceosome.

489 A Chromatin affects the splicing efficiency by regulating the U2snRNP activity

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A number of studies propose a role of the chromatin in the regulation of splicing. Many of these observations are based on in vivo experiments therefore a caveat of these studies is their correlative nature. Here, we present an in vitro system that reproduces the coupling of transcription/splicing, chromatin remodeling/transcription, and, for the first time, chromatin remodeling/splicing. Using this system, we demonstrate that the chromatin regulates the splicing efficiency in a co-transcriptional manner, independently of the recruitment of SR proteins by Pol II. Thus, we hypothesized that chromatin factors could have a structural role in the regulation of splicing. Using a high-throughput siRNA screen, we identified several chromatin factors affecting splicing, among which are regulators of acetylation and methylation of histones, chromatin remodelers, transcription regulators and nucleosome components. Using the U2snRNP to purify spliceosome, we show that several of these chromatin factors are physically associated to the splicing machinery, suggesting a functional coupling between the chromatin and the U2snRNP. Consistent with this proposal, alternative exons controlled by the active U2snRNP1 are miss-regulated in absence of specific chromatin factors. These findings allowed us to propose a model in which chromatin modulates efficiency of splicing through the co-transcriptional regulation of the U2snRNP activity.

1. Nuclear Matrix Factor hnRNP U/SAF-A Exerts a Global Control of Alternative Splicing by Regulating U2 snRNP Maturation.
2. Xiao R. et al., Mol. Cell., 2012

490 B The Evolutionary Landscape of Alternative Splicing and its Regulation in Vertebrate Species

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How species with similar repertoires of protein coding genes differ so dramatically at the phenotypic level is poorly understood. Alternative splicing (AS) has been proposed to play an important role in phenotypic differences, because it is a widespread process by which diverse mRNA and protein isoforms can be produced from individual genes. By comparing the transcriptomes of multiple organs from ten vertebrate species spanning ~350 million years of evolution, we observe significant increases in the frequency of "cassette" exon events associated with proximity to the primate lineage [1]. Moreover, in species separated by at least six million years, the exon-skipping profiles of physiologically equivalent organs have diverged to the extent that they are more strongly related to the species identity than to organ type. These species-dependent AS patterns are controlled by a largely conserved cis-regulatory code, together with specific changes in trans-acting factors. In particular, we have identified and characterized species and lineage -classifying "cassette" exon AS events that are predicted to remodel protein-protein interactions involved in gene regulation and other processes. Our recent results indicate that these AS events have further contribute to the major transcriptomic differences underlying phenotypic differences between vertebrate species (refer to abstract by Serge Gueroussov et al.). We have also expanded our evolutionary comparisons to the analysis of other classes of AS events, including alternative retained introns. Intron retention is known to play important roles in the control of mRNA export, localization and turnover by nonsense-mediated decay. Our initial results suggest widespread roles for intron retention throughout vertebrate evolution, and also that this type of AS regulation has impacted the same biological processes and pathways in multiple species. Finally, we are also using our datasets to investigate possible roles of intergenic and antisense non-coding RNAs (ncRNAs) in the regulation of AS. Results from these analyses will be presented.

1. Barbosa-Morais, N.L. et al (2012). *Science* 338(6114):1587-93

491 C TNF influences alternative stop codon usage in DAPK mRNA

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Death-associated protein kinase (DAPK) pre-mRNA is undergoing alternative splicing leading to the production of two isoforms, which elicit antagonistic functions. DAPKα mediates induction of apoptosis whereas the DAPKβ isoform, extended by ten amino acids on the C-terminus, inhibits cell death. Until now nothing is known about the splicing elements regulating the introduction of an alternative stop-codon in the DAPK pre-mRNA. External signals leading to disproportion of DAPK transcripts are unknown as well. Therefore, this study aims to initiate the investigation of the complex regulatory mechanisms of DAPK splicing. We thus started to determine the regulatory mechanism of DAPK splicing.

To investigate the functional role of these isoforms in inflammation process we generated U937T macrophage cell lines stably expressing human DAPKα or DAPKβ. DAPKα over-expression in differentiated macrophages led to a significant increase (up to 3-fold) in apoptosis accompanied by a significant decrease in TNF secretion. Although DAPKβ over-expression supressed TNF secretion by macrophages the β-isoform does not enforce apoptosis. Interestingly, preliminary work demonstrated different pattern of α and β DAPK mRNA expression in normal and malignant intestinal epithelial cells. From these findings we suggest that a change in alternative splice site usage seems to be caused by cellular stress induced by cytokine over-production. To further study the role of TNF in DAPK alternative splicing we generated a DAPK minigene containing a retained intron inside of exon 26. TNF treated HEK293 cells transfected with the DAPK minigene showed a promotion of DAPKα isoform containing an intron retained sequence. Screening of splicing factors potentially involved in alternative stop-codon usage is ongoing.

This study will provide important information about possible triggering of DAPK isoforms in inflammatory and apoptotic pathways.

492 A PTB regulates the alternative splicing of the apoptotic gene *BCL-X*

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Alternative splicing is a key cellular mechanism to control gene expression, which is frequently altered in cancer cells. Mutations in splice sites, or regulatory sequences, as well as aberrant expression of splicing factors can contribute to defective splicing in cancer cells. Interestingly, changes in splicing regulation were shown to correlate with disease progression, whereas the specific splicing signature of cancer cells was employed to stratify patients with high accuracy.

An example of splicing-regulated gene with strong relevance for cancer is *BCL-X*. Alternative usage of the 5' splice sites located in exon 2 of *BCL-X* promotes the generation of two splice variants, the anti-apoptotic long variant (*BCL-X*L) and the pro-apoptotic short variant (*BCL-X*s). Here, we identified the hnRNP I (PTB) protein as a novel regulator of *BCL-X* splicing. PTB was isolated by RNA chromatography as one of the splicing factors that associate with the alternatively spliced region of exon 2. Overexpression of PTB in HEK293T cells modulates 5' splice site selection in *BCL-X* exon 2, thereby favouring the pro-apoptotic *BCL-X*svariant. Conversely, depletion of PTB promotes the *BCL-X*L variant. Crosslink immunoprecipitation (CLIP) experiments showed that PTB directly binds *BCL-X* exon 2 RNA in a sequence-dependent manner.

PTB is up-regulated in human glioblastoma and ovarian cancer. The PTB gene encodes at least three splice variants, named PTB1, PTB2 and PTB4. Interestingly, we observed that PTB1 was more efficient than PTB4 in promoting *BCL-X*s. Moreover, by analysing several glioblastoma cell lines originated from tumours at different stages, we observed a positive correlation between the *BCL-X* S/L and the PTB1/PTB4 ratio. This finding suggests that a switch in PTB variants may favour the reduced expression of the pro-apoptotic form of *BCL-X* in glioblastoma cells. We are currently addressing this issue by evaluating apoptosis and cell survival in glioblastoma cells selectively silenced for PTB4 or for both variants.

Thus, our results identify *BCL-X* as a new splicing target for PTB and uncover a potential novel role for this RNA binding protein in the regulation of apoptosis.

493 B The many lives of Co-transcriptional Splicing

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Researchers working in multiple model organisms – notably yeast, insects and mammalian cells – have shown that pre-mRNA can be spliced during the process of transcription (i.e. co-transcriptionally), as well as after transcription termination (i.e. post-transcriptionally). Since 2010, eight studies have used global datasets as counting tools, in order to quantify co-transcriptional intron removal. The consensus view, based on four organisms, is that the majority of splicing events takes place co-transcriptionally in most cells and tissues. We present a summary of the various global datasets and how bioinformatic analyses were conducted. The agreement between budding yeast and higher metazoans indicates that budding yeast is an excellent model for investigating mechanisms of coupling between transcription and splicing. We have previously reported the phenomenon of Terminal Exon Pausing (TEP), in which RNA polymerase II pausing within short last exons correlates with a high degree of co-transcriptional splicing (Carrillo Oesterreich et al 2010 Mol Cell, 40(4):571-581). We have initially focused on potential roles for nucleosome positioning, elongation factors and selected RNA binding proteins. Data on their contribution to TEP and co-transcriptional splicing will be presented.

494 C Alternative splicing in the regulation of the barley circadian clock

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The circadian clock is a cellular mechanism able to organize several physiological processes in anticipation/preparation to daily changes. As sessile organisms, plants strongly rely on the circadian clock to match processes such as leaf movement, immune responses and flowering according to the right time of the day/season. This 'internal clock' is maintained and run by clock genes and most of what is known about them in plants comes from studies in *Arabidopsis*. Regulation of the circadian clock in plants is complex involving interlocked expression feedback loops, control of protein phosphorylation and degradation, and chromatin remodelling. Recently, extensive alternative splicing (AS) was shown to regulate clock genes through dynamic changes in AS transcripts, some of which are temperature-dependent and altered levels of productive mRNAs through AS/NMD [1]. In particular, low temperatures reduced the levels of LHY transcript and protein without affecting the promoter strength, which suggests that its expression is regulated post-transcriptionally by AS events that produce nonfunctional transcripts. In addition, temperature-dependent AS had opposite effects on pairs of partially redundant clock components (LHY/CCA1; PRR7/PRR9). This raised the question of whether clock genes and their modes of regulation are conserved in other higher plants, such as crop species. We have now carried out a robust *in silico* analysis using 27 *Arabidopsis* core clock/clock-associated genes and have identified 21 barley genes, 60% of which are true *Arabidopsis* orthologues. Most of the barley clock genes have a clear daily rhythm which is maintained in constant light conditions. Mutations of the barley clock genes *HvPpd-H1* (a putative orthologue of *AtPRR7*) and *HvElf3* strongly affect flowering time (earliness) and have extended the geographic range where barley is grown. We show that both mutations affect expression of clock genes involved in flowering time: the *Hvppd-H1* mutation affects expression levels and phase while the *Hvelf3* mutation also causes arhythmicity of some of these genes which helps to explain their earliness. We have identified AS in some of the core orthologues and are addressing temperature-specific AS in barley. This novel layer of fine clock control observed in two different species, a model plant and a crop species, might help our understanding of plant adaptation in different environments and ultimately may offer a new range of targets for plant improvement.

1. James et al (2012) Plant Cell 24: 961-981

495 A The Nuclear Matrix Protein Matrin 3 is a Regulator of Alternative Splicing

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Polypyrimidine Tract Binding protein (PTB) regulates an extensive range of alternative splicing events (ASE), and is composed of four RRM domains. In an MS2-tethering assay the second RRM and the following linker are sufficient to promote exon skipping. Short linear peptide motifs of the form [S/G][I/L]LGxfP, known as PTB RRM Interacting motifs (PRI), which are present in multiple copies in the PTB-coregulator Raver1, bind to the "dorsal" surface of RRM2. A key Tyr247 residue in RRM2 is critical for this interaction. Seeking to better understand the mechanism of splicing regulation by PTB, we used proteomics to identify proteins that bind to the PTB minimal repressor domain and are sensitive to mutation of Tyr247. The two strongest interactors were Raver1 and the nuclear matrix protein Matrin 3. The interaction with Matrin3 was mediated by a conserved GILGPPP motif, which is both necessary and sufficient for interaction with PTB. Matrin3 is composed of two DNA binding zinc-finger domains as well as two tandem RRMs. It is known to interact with RNA processing and transcription factors, and a mutation in this protein is associated with a type of distal myopathy, although its precise molecular function is unclear. We tested the consequences of Matrin3 knockdown in HeLa cells using splice-sensitive microarrays. Multiple ASEs were strongly affected, suggesting the activity of Matrin3 both as a splicing repressor and activator. There was a significant overlap between ASEs regulated by Matrin3 and PTB, but strikingly the majority of Matrin3 events were not co-regulated by PTB. Matrin3 targeted events showed a significant enrichment for chromatin proteins. Structure-function analyses indicated that the ZF domains are dispensable but Matrin3 requires its RRMs for splicing activity suggesting it binds directly to RNA. A number of 5-mer motifs are significantly enriched around Matrin3 repressed exons and adjacent to the downstream constitutive exon, including pyrimidine-rich motifs similar to optimal PTB sites. These motifs were also enriched among Matrin3 target exons that are not regulated by PTB. Strikingly, we found that Matrin3 activity was abolished by mutations of its GILGPPP motif for both PTB co-regulated and PTB-independent events, suggesting that this motif can mediate interactions with other splicing regulators. Our data indicate that Matrin 3 is not only functional as a nuclear matrix component but also as an active splicing regulator in the nucleoplasm.

496 B Damage-induced alternative splicing in MDM2: Identifying cis elements and trans factors

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The *MDM2* oncogene encodes a protein that negatively regulates p53 by targeting it for proteasome-mediated degradation. Through the induction of DNA damage and in cancer, *MDM2* is alternatively spliced into a variety of isoforms. The *MDM2-ALT1* isoform, comprised of exons 3 and 12 is generated in cells in response to genotoxic stress. MDM2-ALT1 lacks a p53-binding domain and abrogates full-length MDM2 from binding p53 by sequestering it. This leads to the stabilization of p53, causing cell cycle arrest and/or apoptosis. However, as mutations accumulate to uncouple the p53 pathway, *MDM2-ALT1* becomes an oncogenic driver. For instance, *MDM2-ALT1* is observed in numerous cancers including over 85% of rhabdomyosarcomas and is correlative with high-grade metastatic disease. It is therefore critical to understand the regulation of stress-induced MDM2 alternative splicing to identify novel targets for anticancer therapies.

In order to study the alternative splicing of *MDM2* we have developed an *in vitro* splicing system using *MDM2* minigenes and normal and cisplatin-treated HeLa S3 nuclear extracts. The *MDM2* 3-11-12 minigene predominantly excludes exon 11 under UV and cisplatin treatment both *in vivo* and *in vitro*, recapitulating the behavior of the endogenous gene. Using ESEfinder 3.0 we identified predicted binding sites for splicing regulators SC35 and SF2/ASF in exon 11 of the *MDM2* minigene and made mutations in their predicted binding sites to uncover their roles in splicing of *MDM2*.

We confirmed the affinity of these regulatory proteins for their predicted targets through RNA oligonucleotide pull downs using the wild-type and mutant sequences of each binding site. We then performed *in vitro* splicing and demonstrated that disrupting SC35 sites led to exclusion of exon 11 and disrupting the SF2/ASF site promoted inclusion of exon 11. Additionally, overexpression of SF2/ASF in MCF-7 cells led to skipping of exon 11 in our wild-type *MDM2* minigene, while the corresponding site mutant was impervious to SF2/ASF expression. When we knocked down expression of SC35 in SC35 Tet-Off mouse embryonic fibroblasts our wild-type *MDM2* minigene caused exclusion of exon 11 and the corresponding SC35 site mutants did not. We are currently performing splicing assembly assays to determine whether SC35 and SF2/ASF interact with core splicing machinery to facilitate skipping of exon 11 under damage.

To summarize, we have found that SC35 promotes the inclusion of *MDM2* exon 11 under normal conditions, whereas SF2/ASF causes the exclusion of *MDM2* exon 11 under damaged conditions. This is consistent with the canonical role of SC35 as a positive regulator of splicing, but suggests a negative regulatory role for SF2/ASF. Taken together, these data provide insight into the regulation of damage-induced *MDM2* alternative splicing by SC35 and SF2/ASF and present potential targets to modulate *MDM2* alternative splicing in cancer.

497 C H3K9me3 and its role in pre-mRNA splicing.

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Splicing of the pre-mRNA occurs mainly co-transcriptionally. Close relationship between transcription and splicing was recently extended to chromatin modifications, which affect splicing outcome. Our bioinformatics analysis revealed specific enrichment of H3K9me3 across splicing sites on actively transcribed genes. To study the impact of H3K9me3 on co-transcriptional splicing we prepared inducible stable cell lines expressing methyltransferase and demethylase. We monitor changes of H3K9me3 on selected gene and we analyze splicing efficiency. Our results suggest role of H3K9me3 in definition of splice sites and efficiency of co-transcriptional splicing.

498 A Is Prp16 remodelling of helix I during the two steps of pre-mRNA splicing carried out through the Nineteen Complex protein Cwc2?

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The DEAH box ATPase Prp16 has dual functions during the two catalytically steps of splicing. In the first catalytically step, in the absence of ATP, Prp16 stabilizes the binding of Cwc25 to the spliceosome and proofreads 5'splice site cleavage. To allow the second step of splicing Prp16, in an ATP-dependent manner, releases Cwc25 to allow for the binding of Prp22, Prp18 and Slu7. Because mutations in U2/U6 helix I are able to suppress a mutation in PRP16, it is thought that helix I is destabilized/remodelled by Prp16 between the two steps of splicing.

Cwc2 is a splicing factor that is part of the Prp19 complex (NTC). Cwc2 contains a Torus domain, an RNA recognition motif (RRM) and interacts directly with Prp19. Previously we showed that Cwc2 interacts directly with the U6 snRNA. We have now performed a large scale genetic analysis of Cwc2 mutants and over 80 snRNA mutants and found only one synthetic lethal interaction between Cwc2 and U6 in helix I. Mutations in the Cwc2 N-terminal Torus domain combined with the U6 mutation A56C, U57C in U2/U6 helix I leads to lethality because this U6 mutation destabilizes U2/U6 base-pairing. This lethality can however be suppressed by mutations that restore helix I base pairing between U2 and U6, suggesting that Cwc2 participates in U2/U6 helix I formation and/or stabilization.

We hypothesize that Cwc2 may be the target of Prp16 helix I destabilization/remodelling as Cwc2 directly interacts with helix I through the whole splicing cycle. Mutation in a conserved region of the Cwc2 Torus domain is able to suppress the cold sensitive *prp16-302* phenotype indicating a functional interaction between Cwc2 and Prp16. Initial crosslinking analysis suggests that Cwc2-U6 interaction is altered with a Prp16 first step mutant but is not altered with a dominant negative Prp16 second step mutant. As Prp16 does not directly interact with U2/U6 helix I, we propose that the destabilization/remodelling of U2/U6 helix I by Prp16 is carried out through Cwc2. We are currently investigating whether other spliceosomal ATPases exert their activity through the NTC.

499 B Selective constraint on mRNA splicing pattern by protein structural requirement

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Alternative splicing (AS) of messenger RNA can significantly increase transcriptome diversity in complex organisms. Different AS transcript isoforms can be translated into peptide sequences of different lengths andfunctions. In mRNA splicing events, whether the splicing patterns are constrained by protein structural requirements remains unclear. Here we address this issue by examining whether the intactness of three-dimensional protein structural units (compact units in protein structures, namely Protein Units (PUs)) tends to be preserved in AS events in human. We show that PUs tend to occur in constitutively spliced exons (CSEs) and to overlap constitutive exon boundaries. In addition, when PUs are located at the boundaries between two alternatively spliced exons (ASEs), these neighboring ASEs tend to co-occur in different transcript isoforms. Moreover, the PU-spanned ASE pairs tend to have a higher frequency of being included in transcript isoforms. ASE regions that overlap with PUs also have lower nonsynonymous-to-synonymous substitution rate ratios than those that do not overlap with PUs, indicating stronger negative selection pressure in PU-overlapped ASE regions. Notably, we found that PUs have protein domain- and structural orderness-independent effects on mRNA splicing. Overall, our results suggest that fine-scale protein structural requirements have significant influences on the splicing patterns of human mRNAs.

1. Gelly J-C, Lin HY, de Brevern A.G.*, Chuang TJ*, and Chen FC*. 2012. Selective Constraint on Human Pre-mRNA Splicing by Protein Structural Properties. Genome Biology and Evolution 4(9):966-75.

500 C Alternative splicing and gene expression in cardiomyocytes and cardiac fibroblasts during development

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Alternative splicing (AS) expands the proteomic complexity in mammals. During postnatal development the fetal heart converts to adult function through transcriptional and posttranscriptional mechanisms, including AS regulation. The RNA binding proteins CELF1 and MBNL1 coordinately regulate AS during heart development affecting >40% of the developmental transitions: CELF1 is down regulated 10-fold after birth; MBNL1 is up regulated 4-fold. Heart tissue is ~20% cardiomyocytes (CMs), ~66% cardiac fibroblasts (CFs), epithelial and vascular cells. CMs provide contractility through excitation contraction coupling (ECC) and CFs provide a mechanical scaffold, electrophysiological conduction and signaling through paracrine function and direct CF-CM communication.

AS and gene expression transitions were studied by RNA-seq from total hearts of embryonic day 17, postnatal day 1 (PN1), 10, 28 and adult mice (>150 million paired-end reads, >80% genome alignment per sample). AS events identified by RNA-seq correlated well with RT-PCR validation (r2=0.8). Gene ontology (GO) analysis revealed different enriched categories between developmental stages: protein transport and endocytosis between PN1-PN28; transcription and chromatin modifications between PN28-adult. Among CELF1 / MBNL1 dependent AS, endocytosis was an enriched functional target.

We also isolated CMs and CFs for RNA preparation within 3 hours from PN1-3, PN28-30, and adult mice for RNA-seq (>160 million paired-end reads, >84% genome alignment per sample). While CM-up regulated genes were enriched in mitochondrial metabolism categories, similar processes were enriched in CF-down regulated genes. A similar connection was found between CM-down regulation and CF-up regulation (cytokines, adhesion, JAK-stat and MAPK signaling) consistent with CMs-CFs communication *in vivo.* In terms of AS, more transitions were found in CMs (999) than in CFs (409). Interestingly in CMs 30% responded to MBNL1, 27% to CELF1 and between these 2 groups there was >50% overlap. Similarly with total heart, endocytosis was enriched in CMs suggesting a role of AS in vesicular traffic, membrane organization and invagination. Possibly this enrichment reflects: *i)* ligands/growth factors uptake changes, *ii)* surface density of ion channels changes, *iii)* the deep architecture reorganization involved in the postnatal maturation of sarcoplasmic reticulum and T-tubules which are crucial for ECC.

501 A Assessing influence of mutations in first nucleotides of exons on splicing of the BTK and SERPING1 genes.

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Mutations in the first nucleotide of exons (E+1) mostly affect splicing when found in AG-dependent 3' splice sites (3' ss), whereas exons with AG-independent splice sites are resistant to this type of mutation. In introns, AG-dependency is determined by the quality of the polypyrimidine tract, its length, and degeneracy. Because of this complex interplay between interdependent factors the mRNA splicing outcome of substitutions in 3' ss may be difficult to assess just from primary nucleotide sequence data. Recent study showed that mutations in the +1 G impaired splicing in exons with preceding polypyrimidine stretches (PPS) from 4 nt to 10 nt long whereas those with PPS from 9 to 16 nt long were normally spliced.1

In order to test this rule, we have used a minigene system to analyze the influence of five +1 G substitutions found in *BTK* and *SERPING1* genes on pre-mRNA splicing. Two of the mutations with the PPS length of 8 and 10 nts led to splicing aberration, while the other three with PPS length of 6; 6 and 8 nts did not affect the splicing. The two groups of sequences were slightly better distinguishable from each other according to the number of pyrimidines in 25 nts from the 3' ss, although we detected one outlier in the non-aberrant group.

Next, we examined how the most commonly used *in silico* splicing prediction tools would cope with the effect of these mutations on splicing. Both predictions of polypyrimidine tracts according to Kol and Schwartz failed in the discrimination between the AG-dependent and AG-independent 3' ss. Much better were the outcomes of splice site predictors. In the NNSPLICE program the border value discerning between the two groups of sequences was 7 % difference of the splice site score. One of the aberrant and one of the non-aberrant species showed that value. Analogous border value in the MaxEnt predictor was 20 % score difference, which clearly discerned the sequences according to their AG-dependency. Excellent results gave the PSSM predictions that showed 1 % score difference for all splicing non-affecting variants and 3 % difference for both splicing-affecting variants. We conclude that these *in silico* tools may be helpful for assessing the effect of a +1 G mutation on splicing. However, rather than generally recommended minimum of 10 % score difference for splicing affecting mutations, one should use values specific for each prediction tool.

1. Fu Y, Masuda A, Ito M, Shinmi J, Ohno K, Nucleic Acids Res 39 (2011) 4396-4404.

502 B Investigating the role of PTBP1 alternative exon 9 in the evolution of lineage-specific alternative splicing in vertebrates

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Through the use of comparative transcriptomics we and others have recently shown that alternative splicing (AS) patterns have diverged dramatically over the course of vertebrate evolution [1,2] (refer to abstract by Nuno Barbosa-Morais et al.). While the majority of species-specific AS differences appear to be governed by changes in the use of a largely conserved cis-regulatory splicing code, a subset of pronounced species- and lineage-specific AS events are found in trans-acting splicing regulators. These AS events are enriched in disordered regions of proteins and are expected to modulate surface interactions. One such AS event involves mammalian-specific skipping of exon 9 of the splicing regulator PTBP1. This exon encodes a 26 amino acid sequence located within a highly disordered linker region that connects RNA Recognition Motifs (RRMs) 2 and 3. To investigate the functional significance of this lineage-dependent AS event, we generated 293 cell lines that selectively express PTBP1 isoforms with and without exon 9. RNA-Seq profiling of these lines revealed over 100 cassette alternative exons that are differentially-regulated by the two isoforms. Remarkably, the level of splicing of these exons is significantly correlated with the relative expression of the two PTBP1 isoforms across diverse organs and vertebrate species. To investigate the mechanism by which differential inclusion of PTBP1 exon 9 affects lineage-specific AS patterns, we employed isoform-specific immunoprecipitation and quantitative mass spectrometry. Interestingly, exon 9 exclusion results in a several-fold increase in the interaction between PTBP1 and its RRM-containing co-regulator, RAVER1. In summary, a subset of AS differences between vertebrate species can be linked to the differential inclusion of a single alternative exon in the splicing regulator PTBP1. This exon appears to function, at least in part, by modulating the interaction between PTBP1 and its co-regulator RAVER1.

1. Barbosa-Morais, N. et al. 2012. The evolutionary landscape of alternative splicing in vertebrate species. *Science* 338: 1587–93.
2. Merkin, J., Russell, C., Chen, P., and Burge, C. 2012. Evolutionary dynamics of gene and isoform regulation in Mammalian tissues. *Science* 338: 1593–9.

503 C Prp45 affects early stage of spliceosome assembly and pre-mRNA abundance in yeast

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Prp45 is an evolutionarily conserved essential splicing factor that was found to be part of B, C, and post catalytic spliceosomal complexes; Prp45 is believed to enter the splicing cycle as part of the Prp19 complex (NTC). Previously, we reported that the C-terminal part of Prp45 regulates the partition of the second step helicase Prp22 in Cwc2 pull-downs and affects the fidelity of 3' splice site choice in *S. cerevisiae*. Using substrates non-conforming to the consensus, we also found that branch site mutations decrease 1st step efficiency in *prp45*(1-169) cells relative to WT (Gahura et al., 2009).

Our recent data indicate that Prp45, besides its role in the 2nd splicing step, affects spliceosome function also before the first transesterification. We found that truncation of Prp45 (*prp45*(1-169)) resulted in pre-mRNA accumulation of intron containing genes but that the corresponding mRNA levels remained unchanged with the exceptions of long intron genes such as *COF1* and *IMD4*. We employed Mer1-dependent reporter substrates, SpR and ExR, which yielded translated products when either spliced or not spliced, respectively. Cells expressing truncated Prp45 displayed SpR/ExR ratios which were consistent with a defect before the first transesterification. We also examined Mer1-dependent pre-mRNA and mRNA accumulation of *MER2* and other endogenous meiotic genes. *prp45*(1-169) cells accumulated ~three times higher levels of *MER2* pre-mRNA than WT cells only when Mer1 was present (i.e., spliceosome was assembling on the pre-mRNA). ChIP experiments also revealed the impaired recruitment of Prp45(1-169)-HA to intron containing genes. Co-transcriptional association of other splicing factors with intron containing genes in prp45(1-169) cells was differentially affected, suggesting that Prp45 plays role before the B complex formation. Notably, we found earlier that *S. pombe* U2AF35, which forms part of early spliceosome and pre-dates NTC in the splicing cycle, interacts with Prp45 homolog SNW1 (Ambrozkova et al., 2001).

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504 A SR proteins regulate from cellular environment dependent splicing

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Oxygen (O2) is essential for the life of all aerobic organisms. In mammals, at the whole body level, oxygen supply is optimized by tight regulation of ventilation, arterial blood hemoglobin saturation and systemic oxygen transport. The oxygen tension has been demonstrated to be a key regulator to optimize specific organ functions. Hypoxia-inducible factors (HIF-1α; HIF-2α; HIF-3α) are a transcriptional complex that plays a central role in oxygen-regulated gene expression. HIFs DNA binding complex consists of a heterodimer of HIF-α and HIF-β - identical to the previously identified arylhydrocarbon receptor (AhR) nuclear translocator (ARNT). All these proteins belong to the family of basic helix-loop-helix (bHLH) proteins that contain a PAS domain. HIFs bind to hypoxia responsive elements (HRE) and activate the transcription of a variety of genes involved in the regulation of erythropoiesis, angiogenesis, vasomotor control and energy metabolism.

In mice a dominant negative regulator of hypoxia-inducible gene expression (IPAS) is generated in hypoxic cells by alternative splicing from HIF-3α pre-mRNA. In this study, we aim to understand the molecular mechanisms, regulation of which underlay the alternative HIF-3α/IPAS pre-mRNA splicing process.

We report that: 1) HIF-3α splice sites are efficiently used in HeLa cell nuclear extracts prepared under normoxic conditions, and very inefficiently in HeLa cell nuclear extracts prepared under hypoxic conditions; 2) IPAS splice sites used very inefficiently in HeLa extracts prepared under normoxic conditions, and used in HeLa cell nuclear extracts prepared under hypoxic conditions; 3) UV crosslinking results revealed that SR proteins isolated from normoxic and hypoxic cells interact with RNA differentially.Regulation of HIF-3α/IPAS pre-mRNA splicing dependent from oxygen tension is under further investigation.

505 B High-resolution Rbfox2 binding patterns predict widespread splicing regulation in mouse embryonic stem cells

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The Rbfox family is unique among splicing regulators, which generally recognize degenerate sequence elements, in its recognition of a well-defined motif, UGCAUG. Yet recent observations imply the existence of additional determinants in defining a functional binding event. Notably, RBFOX2 CLIP(cross-linking immunoprecipitation)-seq in human embryonic stem cells suggested that RBFOX2 occupied only a subset of UGCAUG motifs in expressed transcripts and that not all binding events were motif-dependent (Yeo et al., 2009). Using complementary CLIP-seq and RNA-seq approaches, we explored the relationship between binding and splicing regulation by Rbfox2 in mouse embryonic stem cells (mESCs). To determine Rbfox2-dependent splicing changes in mESCs, we performed RNA-seq upon shRNA-mediated depletion of Rbfox2. Analysis of splicing changes between control and Rbfox2 knockdown using the MISO algorithm (Katz et al., 2010) revealed hundreds of putative Rbfox2-regulated splicing events in all major modes of alternative splicing. Expression of a human RBFOX2 transgene in an Rbfox2 knockdown background resulted in titratable rescue of Rbfox2-dependent splicing changes. We identified direct targets of Rbfox2 regulation in mESCs using a variant of iCLIP (individual-nucleotide resolution CLIP) (Konig et al., 2011; Ule et al., 2005) for epitope-tagged RBFOX2 in mESCs. RBFOX2 binding was enriched in cassette-associated and mutually exclusive exon-associated introns relative to constitutively-spliced introns. Sixty percent of crosslink clusters harbored a motif closely related to the canonical UGCAUG motif. By overlaying RBFOX2 CLIP signal onto Rbfox2-enhanced and –repressed exons determined by MISO, we generated an Rbfox2 RNA map largely consistent with prior observations of position-dependent regulation. We applied the binding criteria inferred from this RNA map to all mouse exons and predicted the regulation of hundreds of additional exons bound by RBFOX2 in flanking introns. Surprisingly, many of these putative Rbfox2-regulated splicing events were predicted to be associated with splicing-coupled nonsense-mediated mRNA decay (NMD). Rbfox2-dependent splicing changes that resulted in unstable mature transcripts may have been underrepresented in our RNA-seq data; however, these events were identifiable in the RBFOX2 CLIP-seq because the crosslink signal originated from pre-mRNA prior to the generation of an NMD isoform. We are currently investigating the extent to which Rbfox2 and the NMD machinery coordinately control isoform expression. We propose that Rbfox2, in part through the regulation of unstable isoforms, orchestrates a more dynamic and widespread network of splicing decisions than previously appreciated.

506 C Elucidation of UP1 binding to RNA substrates: Does RNA structure matter?

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The RNA binding protein hnRNP A1 performs numerous functions: mRNA transportation, splicing regulation, translation initiation, and telomere biogenesis. Many infectious agents utilize hnRNP A1 for its RNA binding capabilities. Two such agents are HIV-1 and Enterovirus 71 (EV71). In HIV-1 infection, hnRNP A1 acts as a trans splicing regulator. Meanwhile, in EV71, hnRNP A1 stimulates IRES-mediated translation. These functions of hnRNP A1 could provide targets for small molecules which block the binding of hnRNP A1 to viral RNA.

In our studies of hnRNP A1 binding to RNA, sub-domains of larger constructs are investigated. The DNA unwinding protein UP1, which consists of the two RRM domains of hnRNP A1, is being used in all our experiments. The RNA constructs used are SL3 (ESS3) of HIV-1 splice site A7 and SLII of the EV71 IRES. Previous experiments have revealed both stem loops bind UP1.

By working with RNA constructs of different structure, we hope to determine if RNA structure is a significant factor for UP1 recognition. Previous studies have shown UP1 binds UAG sequences in single stranded RNA. Our studies have shown UP1 binds ESS3 at its hairpin loop. SLII has two binding sites, a hairpin loop and a 5 nucleotide bulge which contains a UAG sequence. Examination of UP1 binding to its RNA substrates has been done by NMR and ITC. NMR has shown residues in UP1 potentially involved in RNA binding. ITC experiments have revealed the thermodynamic profile of UP1 binding to SLII and ESS3.

507 A Thailanstatins: New Pre-mRNA Splicing Inhibitors and Potent Antiproliferative Agents Discovered from Burkholderia thailandensis MSMB43

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More than 90% of human genes undergo alternative slicing, which results in protein variants far greater than the number of the encoding genes. While this processing inherently provides for transcriptome diversity, aberrant alternative splicing has been implicated in numerous diseases conditions such as cancer and neurodegeneration. The cellular machinery of alternative splicing has thus become a valid drug target, and dozens of small molecule effectors interrogating the alternative splicing process have been identified and evaluated as drug candidates. The aim of our research is to discover new natural products from rare bacterial species that target eukaryotic epigenetics and gene transcriptional regulation including alternative splicing. Mining the genome sequence of *Burkholderia thailandensis* MSMB43 revealed a cryptic biosynthetic gene cluster highly resembling that of FR901464, a prototype pre-mRNA splicing inhibitor produced by *Pseudomonas* sp. No. 2663. Transcriptioal analysis identified a cultivation condition in which a key gene of the cryptic gene cluster is adequately expressed. Consequently, three new compounds, named thailanstatins A, B and C, have been isolated from the fermentation broth of *B. thailandensis* MSMB43 through natural product chemistry (see Figure 1 below). Thailanstatins belong to the FR901464-family of microbial products biosynthesized by a hybrid polyketide synthease-nonribosomal peptide synthetase pathway. They have an overall structural similarity with FR901464, but differ by lacking an unstable hydroxyl group and by having a carboxyl moiety which together endow the compouds with a significantly greater stability than FR901464 under physiologically relevant conditions. *In vitro* assays showed that thailanstatins inhibit pre-mRNA splicing as potently as FR901464, with half-maximal inhibitory concentrations in the single to sub mM range, causing pre-mRNA to accumulate and preventing the production of mRNA and splicing intermediates. *In vitro* cell culture assays indicated that thailanstatins also possess potent antiproliferative activities in representative human cancer cell lines, with half-maximal growth inhibitory concentrations in the single nM range (see Table 1 below). This work provides new chemical entities as reagents for research and as drug candidates for development, and validates the*Burkholderia* species as an exciting new source of bioactive natural products.

Table 1. Properties of Thailanstatins in comparison with FR901464

Natural Product	Half-life ($t_{1/2}$, hr)	Pre-mRNA splicing inhibitory activity (IC_{50} in μM)	Antiproliferative activity (GI_{50} in nM)			
			DU-145 (prostate cancer cell line)	NCI-H232A (non-small cell lung cancer cell line)	MDA-MB-231 (triple-negative breast cancer cell line)	SKOV-3 (ovarian cancer cell line)
Thailanstatin A	>78	0.65±0.36	1.11±0.02	2.26±0.17	2.58±0.11	2.69±0.37
Thailanstatin B	19	6.18±2.47	3.00±0.92	2.50±0.06	6.22±1.67	4.94±1.76
Thailanstatin C	25	6.84±2.90	2.98±0.90	3.67±0.53	8.82±2.20	5.57±2.01
FR901464	10	0.58±0.07	1.05±0.02	1.94±0.24	2.10±0.19	1.06±0.01

508 B Effects of SR Protein Expression on HIV-1 Splicing

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From a single 9kb polycistronic transcript, HIV-1 generates more than 40 mRNAs, which encode the full viral protein complement. This process is largely regulated by the host protein factors that belong to hnRNP and SR protein families. Generally, hnRNP proteins function to suppress splicing by binding the RNA silencer elements; counteracting hnRNP proteins, SR proteins up-regulate splicing through interaction with RNA enhancer elements. Here, we show that the expression levels of SR proteins modulate the relative abundance of HIV transcript levels. In particular, changes in SRp20 levels (up or down) negatively affect HIV-1 RNA metabolism, resulting in altered balance of RNA splicing that decreases unspliced viral RNA abundance as well as altered splice site selection that modulates Tat expression. Overexpression of 9G8 enhances HIV-1 gene expression while its depletion suppresses synthesis of HIV-1 Gag and, to a lesser extent, Env production. To test whether SRp20 directly or indirectly affects HIV transcript levels, viral RNA IP analysis was performed using a U2OS cell line that stably express non-infectious HIV provirus along with Myc-tagged SRp20 protein. The preliminary result indicates SRp20 specifically binds to HIV transcript. Quantitative experiments are underway to determine the binding preference of SRp20 to the HIV unspliced, singly spliced, and multiply spliced RNA classes.

509 C Real-time kinetics of human pre-mRNA splicing

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We have developed a system to visualize splicing in real-time in living human cells. We combined genomic integration of a single reporter gene in human cells, intron labelling with the MS2 technique and spinning disk confocal microscopy to directly image the kinetics of intron excision from pre-mRNA. The fluorescence intensity associated with a single transcription site, which appears as a diffraction-limited object, is quantified as a function of time. Increments in the fluorescence signal result from de novo transcription of MS2-binding sites, and its disappearance reflects intron excision. Fluctuations in fluorescence intensity are used to determine the intron lifetime. We also determined the number of introns present at each individual transcription site at any given time point based on the number of GFP molecules bound to intronic MS2-stem loops. The results reveal different splicing kinetics depending on intron length and splice site strength. These results have important implications for mechanistic understanding of splicing regulation in the live-cell context.

510 A Sex-specific alternative splicing in the head of Drosophila melanogaster and its underlying regulatory mechanisms

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Drosophila sex-determination is a prime example of an alternative splicing cascade where Sex-lethal (SXL) as a master regulator controls alternative splicing of the key sex-determination genes *Sxl, transformer (tra),male-specific-lethal (mls-2), doublesex (dsx) and fruitless (fru)*.

The recent discovery that the number of sex-specifically spliced genes is magnitudes higher than expected, suggests a more extensive post-transcriptional regulation program, which may act together with transcriptional changes to shape sex determination in *Drosophila melanogaster*. The question arises of how these events are regulated. Interestingly, some of the sex-specific alternative splicing (AS) changes in adult fly heads are lost in genetically manipulated flies lacking a germline, suggesting that signals from the germline control sex-specific AS in distant tissues. A similar scenario might be true for male-derived accessory gland proteins that are transferred via the seminal fluid during copulation from males to females. We use the *Drosophila* model system to identify new regulatory concepts in sex-determination and take advantage of the genetic accessibility of *Drosophila* to study alternative splicing directly *in vivo*.

To determine to what extent sex-specific AS is present in the head, and what influence the germline and mating process have on the AS pattern, we analyzed the transcriptome of adult wild type, tud, and virgin female and male fly heads, using Next Generation Sequencing (NGS). Significant sex-specific expression changes were observed in 218 genes and AS changes in 230 genes (corresponding to 407 AS events). In addition, the experiments showed that AS is highly regulated in the heads of germline-less and virgin flies. Indeed, first analysis of the data suggests that appr. 30-40 % of the AS events in the head depend on the germline and mating process, respectively.

Dual fluorescence minigene reporters are excellent tools to monitor AS, but have not been used in *Drosophila* so far. As tools for cell culture and *in vivo* experiments, these reporters can be used to identify trans-acting and cis-acting regulatory elements of the splicing process. For large scale analysis, AS of these reporters in cell culture can be analyzed using FACS. For different promising candidate genes of the NGS experiments, AS reporters are being established and the data will be presented. For example, we constructed a dual fluorescence reporter minigene to monitor *sqd* AS, using its endogenous promoter. Differences in AS of *sqd* were recapitulated in *Drosophila* S2 cells. Differential isoform expression, using transgenic flies carrying this reporter was detected in the cortex of the nervous system with one isoform showing higher levels in the mushroom body.

This established system enables to monitor AS directly *in vivo* and allows to identify trans-acting and cis-acting regulatory elements of the splicing process.

511 B Structural investigation of hnRNP G interaction with SMN RNA

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The protein hnRNP G is an important regulator of gene expression in human cells. This factor modulates splicing of several pre-mRNAs including Survival of Motor Neuron (SMN). SMN is encoded by two genes SMN1 and SMN2, which differ by five nucleotides. As a result, a different set of splicing factors is recruited on SMN2 resulting in exon 7 skipping in most of its transcripts. Inclusion of this exon is essential for the production of functional SMN proteins(1). A homozygous loss of SMN1 gene results in the insufficient production of active proteins from the SMN2 gene causing the Spinal Muscular Atrophy (SMA) disease. As the SMN2 gene is systematically present in the genome of SMA patients, a promising strategy to cure SMA is to target the splicing of SMN2 transcripts to increase the percentage of exon 7 inclusion and consequently the production of functional SMN proteins(2).

HnRNP G was previously proposed to be recruited by Tra2-β1 upstream its binding site on SMN exon 7(3) and together, they activate the inclusion of this exon(1). The specificity of RNA recognition by hnRNP G remains elusive. Based on high sequence identity between the RNA Recognition Motifs (RRMs) of hnRNPG and RBMY, its paralogue in testis that binds CAA containing RNA motifs(4), it was suggested that hnRNP G could bind similar sequences on SMN exon 7(3). In this study, we use NMR spectroscopy to better characterise the mode of RNA recognition of this protein. We solved the structure of hnRNP G RRM in complex with the SMN exon 7 derived 5'-AUCAAA-3' RNA. The structure reveals that the RRM recognises specifically two successive adenines utilizing its β-sheet surface and, more surprisingly, its C-terminal extremity. This mode of recognition differs from what was previously reported for RBMY which, unlike hnRNP G, recognises an additional cytosine 5' to the two adenines(4). We could show that the recognition of this additional cytosine by RBMY RRM occurs only in the context of interaction with a stem-loop RNA. Interestingly, two stretches of successive adenines are present in the putative hnRNP G binding site on SMN and we tested the importance of hnRNP G binding to them for exon 7 splicing both in vitro and in vivo.

In conclusion, this study reveals the mode of RNA recognition of hnRNP G and brings novel information about regulation of SMN exon7 alternative splicing by this protein.

512 C A highly conserved GC-rich element regulates alternative splicing of mRNA for the variant thyroid hormone receptor TRa2

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In mammals the THRA gene is alternatively spliced and encodes two proteins: TRα1, the α-thyroid hormone receptor, which is widely expressed in all vertebrates; and TRα2, a non-hormone binding variant present only in eutherian mammals. Coding sequences unique to TRα2 share an antisense overlap with those of Rev-erbα (NR1D1), a nuclear receptor and core component of the mammalian circadian clock. Intronic and exonic splicing enhancers for TRα2 have evolved in the context of sequences required for expression of Rev-erbα mRNA. Of particular interest is a GC-rich region (designated G30) at the 5' end of the terminal exon that encodes the unique C-terminal sequence of TRα2 mRNA. This sequence is antisense to the 3'UTR of Rev-erbα and tightly conserved in all eutherian Rev-erbα mRNAs, but not in those from marsupials or monotremes that do not express TRα2. Closely spaced deletions and substitutions within G30, differing by as little as a single nucleotide, have dramatically different effects on TRα2 splicing depending on their precise position. For example, deletion of 12 nucleotides can lead to >95% inhibition or >2.5-fold enhancement of TRα2 splicing. Substitutions of 2 or more nucleotides within G30 also display a range of effects. Our results suggest that G30 is a highly structured sequence, possibly a G-quadruplex, and part of a larger complex splicing regulatory element that exerts both positive and negative effects on TRα2 expression. Factors bound to G30 may interact directly with the 3'splice site and polyadenylation site that define the terminal exon. Alternatively, this element may affect splicing via effects on transcription within this bidirectionally transcribed region. Further experiments to distinguish between these possibilities are underway.

513 A The centrosomal kinase NEK2 is a novel splicing factor kinase

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NEK2 is a serine/threonine kinase belonging to the family of the NIMA kinases, which are well known as key regulators of the cell cycle. In particular, NEK2 promotes centrosomes splitting and insures correct chromosomes segregation during the G2/M phase of the cell cycle through the phosphorylation of specific substrates, such as the centrosomal protein c-Nap1. Aberrant expression and activity of NEK2 leads to dysregulation of the centrosome cycle and aneuploidy. Thus, a tight regulation of NEK2 activity and expression is needed during cell cycle progression.

NEK2, as other centrosomal kinases, is up-regulated in several human cancers, such as breast carcinoma, testicular seminoma, and myeloma, and its expression levels have been proposed as an accurate prognostic marker.

In both testicular seminomas and myelomas, NEK2 overexpression correlates with its nuclear localization. This observation suggests the existence of unknown nuclear functions for NEK2 in cancer cells, which have been object of our further investigation.

We found that NEK2 localizes in the nucleus of cancer cells derived from several different tissue and in particular it localizes in their splicing speckles. Moreover, NEK2 interacts with several splicing factors and phosphorylates some of them, like the proto-oncogene SRSF1. Overexpression of NEK2 exerts the same effect of the SR protein kinase SRPK1 on phosphorylation of endogenous SR-proteins. Moreover, NEK2 and SRPK1 similarly affect the splicing activity of SRSF1 towards reporter minigenes and its endogenous targets.

Our results identify NEK2 as a novel kinase involved in splicing regulation, suggesting that part of its oncogenic activity may be ascribed to its ability to modulate this key step in the regulation of gene expression, which is frequently altered in cancer cells.

514 B Reconstructing alternative splicing of SMN exon 7 by NMR, SRM-Mass-Spectrometry and mathematical modeling

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Spinal Muscular Atrophy (SMA) is a lethal neurodegenerative disease affecting human infants with an incidence rate of 1 in 6000 live births. SMA phenotype is strongly connected to skipping of exon 7 in the Survival Motor Neuron 2 (SMN2) gene, which appears to be primarily driven by a single silent mutation in +6 position of the exon (c.840C>T). According to available data this mutation converts binding site of the positive SMN splicing regulator SRSF1 into the site of the negative regulator hnRNP A1 (A1). Despite substantial research efforts the exact molecular mechanisms leading to exon 7 skipping are still poorly understood. Among the reasons for such limited progress is the complexity of exon 7 splicing regulation, which involves interplay of at least 5 regulator proteins.

In this project we aim to explain the mechanism of SMN exon 7 skipping by building a holistic model of this system using Nuclear Magnetic Resonance Spectroscopy (NMR), Selected-Reaction-Monitoring Mass-Spectrometry (SRM-MS) and mathematical modeling. We use SRM-MS to measure the exact *in vivo* concentrations of five regulatory proteins (hnRNPs A1 and G, SRSF1, Tra2b1, SRp30c). Next, we are constructing an *in vitro* model of the system by mixing selectively labelled protein regulators and target SMN RNAs, aiming to visualize all protein-RNA and protein-protein interactions in NMR tube under conditions matching those found in vivo. Third, we are integrating the obtained MS, NMR and other biophysical data into a predictive mathematical model to explain the mechanism of exon 7 alternative splicing. Finally, we plan to validate the predictions of the resulting models against exon 7 splicing assays in cell extracts.

Preliminary *in vitro* reconstructions involving three core system components (SRSF1, A1 and target 24nt SMN ESE/ESS RNA) have shown promising results. NMR data indicates that SRSF1 is not completely displaced from SMN2 ESE/ESS sequence under equimolar A1:SRSF1 ratio (100 μM each). SRM-MS analyses show that nuclear concentration of A1 is 4.5-fold higher than that of SRSF1 (90 μM A1 vs 20 μM SRSF1). These facts suggest that increased nuclear concentrations of A1 shall be important for it to drive the exclusion of SMN2 exon 7.

To evaluate possible co-transcriptional effects we performed time-resolved NMR analysis of *in vitro* transcription of the target SMN sequences in the presence of A1 and SRSF1 factors. This analysis shows that folding rate of the target RNA may be slowing down in the presence of regulator proteins, suggesting additional kinetic control in exon 7 alternative splicing.

We expect that extending the core *in vitro* and mathematical models to include remaining regulator proteins will eventually allow predicting the outcome of SMN exon 7 splicing based solely on the knowledge of concentrations of involved regulators.

515 C Comprehensive Mapping of the Splicing Regulatory Circuitry Involved in Cell Proliferation and Apoptosis

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We present an integrated experimental and computational approach aimed to derive a comprehensive functional interactions map of the Splicing Regulators involved in cell proliferation and apoptosis. Our strategy is based on screening the effects of knock down (KD) of 300 individual genes encoding factors implicated in the splicing process and its regulation on 37 selected targets. The target list encompasses alternative splicing events implicated in tumor progression including apoptotic regulators, signaling molecules and metabolic enzymes. Splicing output of the targets upon KD is robustly quantified by high-throughput capillary electrophoresis. In turn this information is used as input for state-of-the-art methods for Graphical Model Selection and Analysis in order to recover the structure of the underlying splicing regulatory circuitry and to identify distinct modules within its topology.

Our method accurately captures well-established functional associations demonstrating its validity as a general tool for mapping out the splicing regulatory landscape. We are further able to identify several novel associations and to differentiate between those critical for general or alternative splicing regulation. One compelling feature of our approach is the possibility to extend the screening to physiological / pharmacological treatments in order to link their effects to the splicing regulatory circuitry that underlies cell proliferation and apoptosis. As a proof of principle we map the associations of splicing-arresting drugs and iron-homeostasis to the functional network of splicing regulators.

516 A Functional characterization of the RNA-binding protein Acinus: its role in pre-mRNA processing and apoptosis

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Acinus (Apoptotic Chromatin Condensation Inducer in the Nucleus) is an RNA-binding protein originally identified for its role in inducing nuclear changes during apoptosis (1). This protein has also been found to be an auxiliary component of the Exon Junction Complex (EJC), which is deposited at exon junctions as a consequence of pre-mRNA splicing (2). A role for Acinus in splicing is also suggested by its association with RNPS1 and SAP18 proteins in the ASAP (apoptosis- and splicing-associated protein) complex.

In order to uncover the cellular functions of Acinus, we searched for endogenous RNA targets using the Cross-Linking Immunoprecipitation protocol (iCLIP) that allows the mapping of protein-RNA interactions at an individual nucleotide resolution. We found that Acinus is mostly associated with constitutively expressed exons of protein-coding transcripts. Interestingly, Acinus binding is excluded from the region where the core EJC is deposited (20/24nt before the exon-exon junction). We also identified non-coding RNAs targets of Acinus.

An exon-junction array was used to investigate changes in gene expression and alternative splicing following siRNA-mediated depletion of Acinus in HeLa cells. This analysis revealed changes in expression levels of around 450 genes as well as changes in 250 splicing events. A large number of genes presenting a change in alternative splicing were associated with an Acinus binding site, suggesting a direct role of this RNA-binding protein in the event. We are also investigating whether the role of Acinus during apoptosis involves its binding to specific RNAs. The combination of these approaches will help us to uncover the role of Acinus in pre-mRNA splicing, apoptosis and other cellular processes.

1. Sahara et al. (1999) Nature, 401, 168-73.
2. Tange et al. (2005) RNA, 11, 1869-83.

517 B Widespread regulatory functions of Polypyrimidine Tract-Binding Proteins in splicing and development of Arabidopsis thaliana

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The process of alternative splicing (AS) is not only common in mammalian systems, but also a widespread phenomenon in plants, with currently ~60% of all multiexon genes from *Arabidopsis thaliana* being reported to be associated with AS events. Among the regulators of AS are polypyrimidine tract-binding proteins (PTBs), of which three homologues have been identified in *Arabidopsis thaliana*. While the proteins encoded by *At3g01150* (PTB1) and *At5g53180* (PTB2) are closely related, the protein encoded by *At1g43190* (PTB3) exhibits a quite low level of sequence similarity to the other two. Using a misexpression approach for all *Arabidopsis* PTBs to investigate PTB-dependent changes in global splicing patterns, we have identified ~450 putative At-PTB regulation targets, thus providing the first evidence of a widespread AS regulatory role of plant PTBs. Interestingly, a major role in splicing control was only observed for At-PTB1 and At-PTB2, but not At-PTB3. The observed reciprocal changes of AS ratios upon up- and downregulation of At-PTBs indicated a direct regulation of the identified targets by these proteins. Using electrophoretic mobility shift assays (EMSAs), direct RNA/protein interactions could be demonstrated for the target candidate phytochrome interacting factor *PIF6* with At-PTB2. Furthermore, PTB binding motifs within the *PIF6* pre-mRNA have been identified. To address a possible correlation of PTB binding positions and the splicing outcome, the role of polypyrimidine stretches located in different positions around a regulated cassette exon is being examined using a mutational approach.

With respect to the biological functions of plant PTBs, we have reported several flowering time regulators as well as *PIF6*, to exhibit a PTB-dependent alteration of splicing and/or expression patterns. Single T-DNA mutants in *At-PTB1* and *At-PTB2* did not exhibit a visible phenotype, whereas the respective double mutant has been reported to be nonviable. To further elucidate the functional implications of At-PTB1/2, we are aiming to generate plants having the lowest tolerable levels of these splicing factors. Therefore, we are using an approach based on the combination of T-DNA insertion lines and artificial microRNAs (amiRNA). Interestingly, first phenotypical analyses indicated an earlier onset of senescence, as well as stunted growth and a serrated leaf phenotype upon combining the *At-PTB2* knockout with an *At-PTB1* targeting amiRNA.

518 C hnRNP A1 promotes exon 6 inclusion of apoptotic Fas gene.

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Fas is a transmembrane cell surface protein recognized by Fas ligand (FasL). When FasL binds to Fas, the target cells undergo apoptosis. A soluble Fas molecule that lacks the transmembrane domain is produced from skipping of exon 6 encoding this region in alternative splicing procedure. The soluble Fas molecule has the opposite function of intact Fas molecule, protecting cells from apoptosis. Here we show that knockdown of hnRNP A1 promotes exon 6 skipping of Fas pre-mRNA, whereas overexpression of hnRNP A1 reduces exon 6 skipping. Based on the bioinformatics approach, we have hypothesized that hnRNP A1 functions through interrupting 5' splice site selection of exon 5 by interacting with its potential binding site close to 5' splice site of exon 5. Consistent with our hypothesis, we demonstrate that mutations of the hnRNP A1 binding site on exon 5 disrupted the effects of hnRNP A1 on exon 6 inclusion. RNA pull-down assay and then western blot analysis with hnRNP A1 antibody prove that hnRNP A1 contacts the potential binding site RNA sequence on exon 5 but not the mutant sequence. In addition, we show that the mutation of 5' splice site on exon 5 to a less conserved sequence destructed the effects of hnRNP A1 on exon 6 inclusion. Therefore we conclude that hnRNP A1 interacts with exon 5 to promote distal exon 6 inclusion of Fas pre-mRNA. Our study reveals a novel alternative splicing mechanism of Fas pre-mRNA.

519 A Unusual evolutionary insertion of G-tracts creates splice variants of distinct localization and function in human cells

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Alternative splicing contributes greatly to the proteomic complexity in higher eukaryotes particularly humans. However, the underlying molecular mechanisms for the evolvement of alternative exons during evolution and its functional consequences remain largely unknown. The polypyrimidine tract (Py) and 3′ AG are often close to each other within a consensus sequence (Y)nNYAG at the 3' splice site. In contrast to this arrangement for exon inclusion, here we report an unusually evolved G-tract insertion between the Py and AG for exon skipping, and consequently a variant protein of distinct localization and function in human cells.

We identified 130 3' splice sites containing G-tracts between the Py and 3' AG in a human genome search. Interestingly, examination of several such elements indicates that they are inserted evolutionarily in higher species. Particularly G-tracts upstream of the exon 3 of the PRMT5 (protein arginine methyl transferase 5) gene are gradually evolved from none in fish to one in certain lower and two in higher mammals. Contrary to the intronic G-tracts at other locations that are often splicing enhancers, these G-tracts are splicing repressors in mini-gene splicing reporter assays. Moreover, the repression is strong in humans but barely detectable in fish for both mini-gene reporter and endogeneous exons. In *in vitro* UV crosslinking and immunoprecipitation assays, the G-tract element is bound by hnRNP H and inhibitory of U2AF65 binding to Py. Consistently, hnRNP F/H knockdown enhances the inclusion of endogenous exon 3 in HeLa cells. Together these suggest that the unusually localized G tracts are evolutionarily inserted splicing repressors bound by a regulatory protein in human cells.

To understand the consequences of the G-tract-mediated skipping of exon 3 in humans, we examined the expression profiles and expressed each of the splice variant separately in cells. The variants are differentially expressed among cells or during differentiation. With exon 3 included, a full length protein is produced but restricted to discrete Golgi-like structures while as with the exon skipped, a shorter protein is produced and spread all over the cell. Consistent result is obtained when each variant was knocked down by siRNA interference. Importantly, the full length but not the shorter isoform greatly increases the fragmentation of the Golgi apparatus in co-immunostaining assays with an antibody against the Golgi marker Giantin. Therefore, the two protein isoforms have distinct localization and function.

These data demonstrate that the unusual evolutionary insertion of G-tracts creates an alternative exon of distinct localization and function in human cells. This is likely a mechanism common to the emergence of a group of alternative exons that contribute to the proteomic complexity in humans.

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520 B Mechanisms regulating alternative splicing of Dscam

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The *Drosophila* Dscam gene encodes a cell adhesion molecule of the immunoglobulin superfamily and is required for neuronal wiring and phagocytosis in the immune system. A hallmark of the Dscam gene is the extraordinary molecular diversity that can be generated by mutually exclusive alternative splicing in four exon clusters resulting in 38'016 different isoforms. The Dscam splicing pattern has to be different in neighboring mushroom body neurons for normal neuronal development and is altered upon pathogen exposure in immune cells.

To study the mechanisms regulating Dscam mutually exclusive splicing we focused on the exon 9 cluster containing 33 variable exons. We have developed a transgenic fly model containing a reporter gene recapitulating alternative splicing of the endogenous gene. Current models for mutually exclusive splicing in the Dscam exon 6 cluster propose that the variable cluster is kept in a repressed state until one variable exon is chosen. Key to the release of the chosen exon from repression seems to be RNA base pairing between evolutionary conserved sequences in the vicinity of 5' and 3' splice sites of the proximal intron resulting in inclusion of the chosen exon. In the exon 4 and 9 clusters, RNA secondary structure has also been postulated to play an essential role, yet in the distal intron to bring its 5' and 3' splice sites together. Using transgenic flies we are currently testing these models and present our analysis characterizing sequence requirements for exon selection and for mutually exclusive splicing in the exon 9 cluster. Our results will provide important insights into the regulatory mechanisms governing Dscam mutually exclusive splicing.

521 C A Role for the Polyadenosine Binding Protein, Nab2, in Splicing and Quality Control

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Gene expression is temporally and spatially regulated to produce a precise protein expression profile that dictates the function of each cell. From the onset of transcription, RNA binding proteins immediately associate with a nascent mRNA transcript and guide it through post-transcriptional processing events like 5'-capping, splicing, and 3'-end processing. Many messenger ribonucleoproteins (mRNP) that contact the mRNA transcript throughout its post-transcriptional journey have more than one role in mRNA biogenesis to ensure proper processing of an mRNA transcript and avoid production of faulty proteins. These processing steps are highly conserved with much of the mechanistic information gleaned from studies in budding yeast. The essential *S. cerevisiae* zinc-finger nuclear polyadenosine RNA binding protein, Nab2, has been implicated in control of poly(A) tail length and mRNA export; however, a role for this protein in splicing has not been explored. To globally examine splicing of endogenous intron-containing transcripts, we performed a splicing microarray usingnab2 *mutant cells.* A mild constitutive splicing defect was detected in these *nab2* mutant cells. Consistent with a role for Nab2 in modulating splicing, we also detected defects in splicing in an *in vitro* splicing assay. To begin to understand which splicing step depends on Nab2, we tested for genetic interactions between *NAB2* alleles and splicing factors required at different steps in splicing. We observed synthetic lethality with splicing factors required early in splicing. A physical interaction was confirmed between Nab2 and the commitment complex proteins, Mud2 and Msl5, which are critical for both splicing and nuclear retention of unspliced transcripts. We then utilized a novel GFP reporter to track splicing and retention in Nab2 and Mud2 double mutants and found an exacerbated splicing defect as well as a reduction in pre-mRNA leakage. Alleles of *NAB2*also genetically interact with the mRNA decay machinery, suggesting that this atypical polyadenosine RNA binding protein acts as a nuclear watchdog to prevent accumulation and export of aberrant unspliced mRNAs. Our findings identify a role for Nab2 in regulating splicing and offer insights into how splicing and quality control are coupled in S. cerevisiae.

522 A Prp40p WW Domain is Critical for Splicing of Introns Containing Non-canonical Branch Site Sequences in Saccharomyces cerevisiae

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The WW domain is a protein domain with two highly conserved tryptophans that binds proline-rich peptide motifs. Prp40p is a yeast-specific and essential U1-snRNP protein that harbors two WW repeats at its N-terminus, which were thought to interact with the proline-rich region of BBP (branch-site binding protein) for promoting the formation of commitment complexes. Surprisingly, earlier studies by others showed independence of the conserved WW repeats in splicing and contranscriptional spliceosome assembly. We have carefully approached the same issue and discovered that WW repeats are in fact critical for splicing of introns containing non-canonical branch site sequences (e.g. GAUUAAC vs. canonical UACUAAC) in vivo. To exclude the possibility of gene-specific bias, we employed a splicing reporter system and found that when the branch-site sequence is changed into GAUUAAC, splicing became sensitive to the loss of either WW repeats and, most apparently in particular, the loss of both WW repeats. This sensitivity appears most dramatic when cell growth was assayed at 37°C. To further determine in detail the WW repeats' function, and therefore Prp40p's, in splicing, we used splicing-sensitive microarray to identify intron-containing genes that are susceptible to the loss of Prp40p WW repeats. To enhance our understanding of the function of the Prp40p-WW repeats from an entirely different angle, we searched for gene deletions that cause synthetic lethality or sickness in combination with the *prp40-?N* mutation in the Synthetic Genetic Array (SGA) platform. Finally, we applied the BPA chemical cross-linking approach to directly test the hypothesis that the WW repeats indeed interact with the proline-rich region in BBP in vivo. In summary, we uncovered a hitherto hidden splicing role for the WW domain of Prp40p in the light of their relationships to the role of BBP. Because the branch site sequences is less conserved in mammals than that in yeast, the WW-domain containing proteins in mammals may play regulating roles at the branch site recognition in splicing.

523 B Muscleblind and Fox proteins cooperate to change a splicing program involved in stem cell differentiation and maintenance.

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Reprogramming somatic cells into induced pluripotent stem cells (iPS cells) types that bear much similarity to Embryonic Stem cells has provided a vast amount of insight into the pathways, mechanisms, and key transcription factors involved in pluripotency. Here we have used human iPS cells derived from normal and senescent fibroblasts to uncover key splicing regulators instrumental in establishing an alternative splicing profile that characterizes pluripotency. Our approach was based on the use of 47 alternative splice events in genes involved in cancer and apoptosis to identify robust alternative changes induced by knock down of selected 49 splicing factors in 5 different cell lines and compare them to similar changes occurring during reversible stem cells induction and re-differentiation. We discovered that two RNA-binding proteins, namely MBNL1 and RBFOX2, when knocked down, accounted for more than 90% of the splicing changes. Strikingly, MBNL1 expression was completely abrogated in stem cells and re-expressed late during mesoderm redifferentiation. We speculate that MBNL1 sequestration by target RNA repeats in cases of Myotonic Dystrophy may impair differentiation of stem cells and contribute to skeletal muscle wasting.

524 C SWI/SNF regulates alternative trans-splicing of the mod(mdg4) gene

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Trans-splicing is a pre-mRNA maturation event by which two pre-mRNA molecules are spliced together forming a processed mRNA. In this study, we show that Brahma, the ATPase of the SWI/SNF chromatin-remodeling complex, modulates the abundance of trans-spliced transcripts derived from the *mod(mdg4)* locus of *Drosophila melanogaster*. We have characterized the expression of anti-sense mod(mdg4) transcripts in S2 cells, mapped transcription start sites and cleavage sites, identified and quantified cis-spliced and trans-spliced transcripts, and got insight into the regulation of the *mod(mdg4)* trans-splicing. Using RNA interference and over-expression of recombinant Brahma proteins, we show that the levels of Brahma affect the levels of a trans-spliced *mod(mdg4)* mRNA isoform in S2 cells. The trans-splicing effect is independent of the ATPase activity of Brahma, which suggests that the mechanism by which Brahma modulates trans-splicing is independent of its chromatin remodeling activity. Interestingly, we also observed a similar effect on trans-splicing *in vivo* when reducing the levels of Brahma in larvae.

525 A Splice-sensitive array profiling suggests a role for STAR proteins and PTB in control of smooth muscle cell alternative splicing.

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Tissue-specific alternative splicing has been extensively investigated in striated muscles (heart and skeletal muscle), and a large amount is known about the relevant RNA sequence elements and RNA binding proteins involved. By contrast, the regulation of alternative splicing in smooth muscle cells (SMCs) has been relatively neglected. Vascular SMCs show phenotypic plasticity and can interconvert between a differentiated 'contractile' phenotype and a more proliferative 'synthetic' phenotype marked by increased synthesis of extracellular matrix proteins. This phenotypic modulation process plays a significant pathophysiological role in various cardiovascular diseases. While the transcriptional changes during phenotypic modulation have been well investigated, knowledge about the global changes in alternative splicing has been very limited.

We have used splice-sensitive microarrays to interrogate global changes in both transcript levels and alternative splicing during phenotypic modulation of mouse aorta and bladder smooth muscle. Genes affected by alternative splicing showed distinct functional enrichments from those that were transcriptionally up or down-regulated. Splicing particularly affected cytoskeletal proteins, while ion channels and receptors were transcriptionally down-regulated and receptor binding and extracellular matrix proteins were upregulated. Nucleic acid binding proteins were significantly depleted among the transcriptionally regulated genes. We identified sets of cassette exons that were substantially up or down-regulated during phenotypic modulation. Computational analysis showed that exons that are included in contractile SMCs are associated with PTB-binding motifs on the upstream side, where PTB represses splicing. Downstream of these exons there was a substantial enrichment of motifs resembling the binding sites for members of the signal transduction and activation of RNA (STAR) protein family (UUAAC, UAACC, ACUAA, CUAAC), which have not previously been associated with regulation of splicing in SMCs. Candidate STAR-regulated exons with potential binding sites in downstream intron were manually identified from 50 top-ranked events and the splicing pattern changes of 7 events (*Ncam1 exon 2, Atp2b4 exon 20, Cacna2d1 exon 23, Ppp4r1 exon 3, Sfrs10 exon 2, and Bnip2exon 10, Ppp1r12a exon 24*) were validated by RT-PCR. In preliminary experiments using an *Ncam1* exon 2 minigene reporter in proliferative rat PAC1 cells, overexpression of Sam68, SLM1 and SF1 significantly increased *Ncam1* exon 2 inclusion, with Sam68 being the strongest regulator. These initial observations suggest that STAR proteins might act widely to promote inclusion of cassette exons in contractile SMCs, while many of the same exons might be repressed by PTB in de-differentiated cells.

526 B EJC can regulate alternative splicing in mammalian cells

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The exon junction complex (EJC) is a dynamic multi-protein complex deposited onto nuclear spliced mRNAs 20-24 nucleotides upstream of exon-exon junctions. The four core proteins, eIF4A3, Magoh, Y14 and MLN51 are stably bound to mRNAs during their lifecycle in the cells, serving as a binding platform for other nuclear and cytoplasmic proteins. Therefore, the EJC plays an important role in connecting splicing to downstream post-transcriptional events including mRNA transport, translation and stability. Despite EJCs are deposited onto mRNAs after splicing, recent evidences have shown that the EJC is also involved in splicing regulation of specific events both in Drosophila and mammalian cells. However, the mechanism for this new function of EJC in splicing remains largely unknown. To study whether EJC is directly involved in alternative splicing regulation in a genome-wide manner, we performed RNA-seq experiments in HeLa cells with siRNA against EJC components as well as the NMD factor Upf1. Differential expression analysis showed that EJCs affect only a specific set of gene expression changes. Differential exon usage were analysed using MISO and Diffsplice programs, and these identified all types of alternative splicing changes on a global scale in EJC knockdown cells. These splicing changes are specific to EJC core proteins, as knockdown of eIF4A3, Y14 and MLN51 showed the same splicing changes. The splicing changes can be rescued by a siRNA-resistant form of eIF4A3, indicating a direct involvement of EJC core proteins in regulating alternative splicing. Taken together, these data indicate that EJC core proteins are involved directly in alternative splicing regulation on a global scale.

527 C The Ribosome-OME II: Alternative Splicing for Ribosomal Proteins?

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"Transcripts from ~95% of multiexon (human) *genes undergo alternative splicing."*[1]

"92-94% of human genes undergo alternative splicing. (sic)" [2]

Ribosomal proteins (RPs) are highly conserved across diverged species, a necessary their positioning in the compact, complex structure of the ribosome. To what degree does alternative splicing affect the nature and the structure of the ribosome? Could alternative splicing of transcripts of RP genes lead to production of proteins with non-ribosomal functions?

To approach these questions we have examined the splice junctions of RP gene transcripts from several multi-Gbyte RNA-seq databases. The results are as follows:

Abundant alternative splicing is rare. In no case did we observe tissue-dependent alternative splicing. In only one case, RPS24, have we observed substantial tissue-specific three-way alternative splicing, leading to S24 proteins whose C-termini: …VGAGKKPKE vary in the last three amino acids.

There are many cases where one or even two exons are skipped, usually in less than 2% of observed transcripts. This usually leads to a truncated protein due to a nearby stop codon. Since this could result in nonsense-mediated decay, the actual frequency of such intron skipping could be much greater. For several genes, in a few % of cases an intron is mis-spliced (?), leading to the deletion of one to 19 aa from the middle of the RP. Are such RPs incorporated into the ribosome?

Finally there are several cases in which 0.1 to 5% of the mRNAs are mis-spliced (?) to encode a protein with the N-terminal portion of a RP followed by 10 to 100 additional AA. Since RPs are very abundant proteins, these fusion proteins have the potential to play as yet unknown, non-ribosomal functions in the cell. In none of these cases were the appropriate tryptic peptides observed in the Mass Spec database GPMDB.

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RNA-seq data for multiple tissues was kindly provided by the Gene Expression Applications research group at Illumina, Inc.

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528 A hnRNP A1 and Secondary Structure Coordinate Alternative Splicing of Mag

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Myelin is a lipid-rich structure that protects neurons from degeneration and facilitates the propagation of electrical impulses along axons. In the central nervous system, it is formed by a specialized glial cell called an oligodendrocyte, which extends hundreds of spiraling processes to nearby axons and ensheathes them. A major protein component of myelin in the vertebrate central nervous system is myelin-associated glycoprotein (MAG). MAG is positioned at the periaxonal interface of the myelin structure, where its position allows it to mediate communication between the neuron and the oligodendrocyte, inhibiting axon outgrowth and protecting neurons from degeneration. The Mag pre-mRNA is alternatively spliced to produce two developmentally regulated transcripts. The longer mRNA contains an alternative cassette exon that has a stop codon, yielding a protein with a truncated C-terminus. How Mag alternative splicing is regulated is not clear. Here, we describe an evolutionarily conserved stem loop structure that overlaps the Mag exon 12 5′ splice site. The non-consensus 5′ splice site occupies the loop of the stem and is able to interact with the splicing repressor hnRNP A1. Analysis of a series of splicing reporters shows that both the sequence and the structure of the element regulate Mag alternative splicing.

529 B Regulation of alternative splicing by QKI protein in lung cancer

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Lung cancer is the leading cause of cancer-related death worldwide. Changes in alternative splicing have been implicated in lung tumorigenesis. However, the functional links between alternative splicing and lung cancer are not well studied. In this study, we observed that RNA-binding protein QKI is down-regulated in non-small cell lung cancer (NSCLC) tissues. Overexpression of QKI in lung cancer cells inhibits cell proliferation and transformation. Using a combined RNAi and RNA-Seq analysis, we identified several hundreds of alternatively spliced genes regulated by QKI and validated at least 24 lung cancer-related events in lung cancer tissues. We have obtained evidence that QKI inhibits cell proliferation through isoform-switch of its targets. To understand the mechanism of splicing regulation by QKI, we generated an RNA map of QKI and revealed that QKI can positively and negatively control exon inclusion in a binding-site position-dependent manner. Additionally, we showed that QKI affects splice site selection by competing with core splicing factors. Our findingsdemonstrate that QKI regulates a number of splicing events in lung cancer cells and contributes to lung tumorigenesis by modulating alternative splicing of its targets.

530 C Site-Specific Intercalation of Doxorubicin Disrupts the Iron-Responsive Element RNA – Iron Regulatory Protein Interaction

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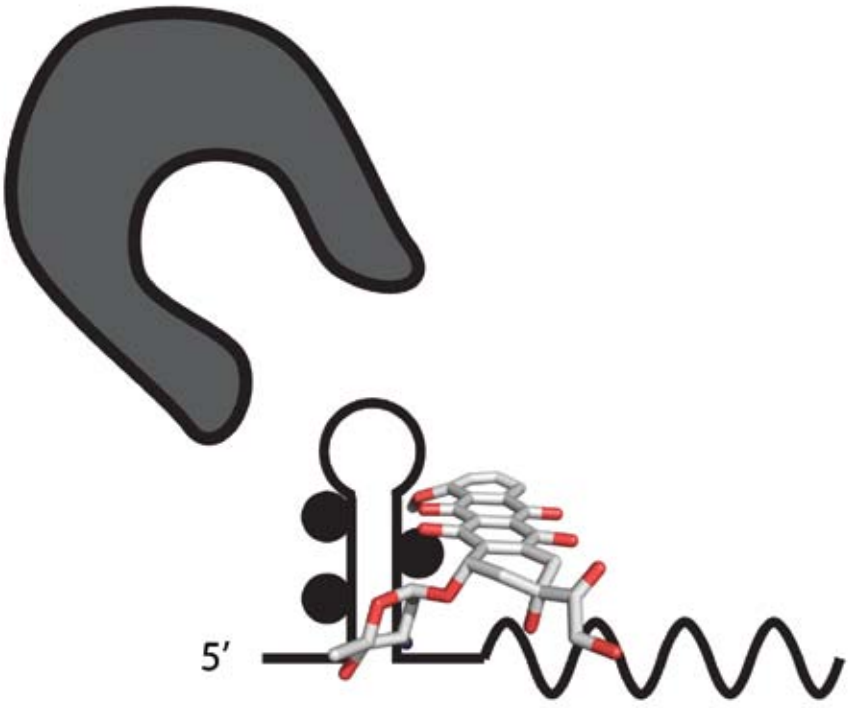
A widely utilized chemotherapy drug, doxorubicin, has recently been shown to bind to a mammalian 5' untranslated region Iron Responsive Element (IRE) RNA. In conjunction with the Iron Regulatory Protein (IRP), IRE RNA is involved in cellular iron homeostasis at the translational level. This tight RNA-protein complex prevents ribosomal assembly, hindering translation initiation of iron storage proteins, i.e. ferritin, under low cellular iron conditions. Conversely, iron overload is conducive to complex dissociation, allowing for up-regulation of the same proteins. However, this system is not entirely efficient. Some anemic patients receive adjuvant chelation therapies upon chronic blood transfusions to sequester excess labile iron. The use of doxorubicin to promote RNA-protein dissociation could potentially allow for downstream up-regulation of ferritin (see figure 1 below). In this work, we show how doxorubicin interacts specifically with IRE RNA using multidimensional nuclear magnetic resonance, fluorescence spectroscopy, and electrophoretic mobility shift assays. All three approaches converge on the observation that the IRE-IRP complex formation is disrupted by doxorubicin. Obtaining further data on the RNA-protein-drug interactions may lead to unveiling a validated RNA target as a complementary treatment of anemia.



531 A Defining a eukaryotic core mRNA interactome: the landscape of RNA-binding proteins in yeast and its conservation in mammals

Benedikt Beckmann[1], Alfredo Castello[1], Bernd Fischer[1], Rastislav Horos[1], Claudia Strein[1], Katrin Eichelbaum[1], Sophia Föhr[1], Thomas Preiss[2], Lars Steinmetz[1], Jeroen Krijgsveld[1], Matthias Hentze[1]

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We recently defined the mRNA interactome of proliferating human HeLa cells, identifying 860 mRNA-binding proteins (mRBPs) and implicating more than 300 previously unknown RBPs in RNA biology [1]. We here report the next step forward, work on the unicellular yeast *Saccharomyces cerevisiae*. Yeast is of particular interest for its availability of advanced genetic tools for studies in vivo, and -in conjunction with the mammalian interactomes- allows definition of conserved RBPs and their function across evolution.

We developed an adapted protocol for interactome capture [2] in yeast based on PAR-crosslinking (Photoactivatable-Ribonucleoside-Enhanced Crosslinking). Using quantitative proteomics, we identified 678 high confidence (m)RBPs (FDR<0.01), including many previously unknown ones. We confirm 101 out of the 120 recently reported RBPs [3], and newly identify 283 additional high confidence RBPs.

Cross referencing with human mRNA interactomes [1, 4] defines 259 members of an evolutionarily conserved "core" mRNA interactome including many RBPs involved in central processes of RNA metabolism (e.g. processing, transport, translation). One of the most unexpected aspects is the occurence of several members of the oxidoreductase family that was previously unlinked to RNA biology. Among others, these include GAPDH, thioredoxin 1 and 2, and alcohol dehydrogenase 1 (ADH1). Moreover, the emerging core interactome includes a defined set of enzymes from diverse pathways in intermediary metabolism, suggesting connections between cell metabolism and the regulation of gene expression [5]. Castello A, Fischer B et al. *Cell*. 2012; **149**(6)

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532 B Regulation of human telomerase by the helicase RHAU, a quadruplex resolvase.

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Telomere extension is mediated by telomerase, an essential eukaryotic reverse transcriptase, and human cells have developmental and tissue-specific strategies for telomerase repression to ensure a defined cellular lifespan[1]. Human telomerase is a ribonucleoprotein complex containing a protein component (hTERT) and an essential RNA component (hTR). The 5' region of hTR RNA contains several guanosine rich tracts that form four-stranded tetrad structures stabilized by hydrogen bonds (G-quadruplexes). Previous evidence suggests that a G-quadruplex within this hTR disrupts the formation of an important base-paired structure within hTR known as the P1 helix, a critical element in defining the template boundary for reverse transcription[2, 3]. RHAU, also known as DHX36, is an ATP-dependent RNA helicase that belongs to the DExH/D family of RNA modifying enzymes[4]. RHAU possesses the unique ability to preferentially bind and unwind G-quadruplexes[5]. Herein, we present the characterization of the RHAU-hTR quadruplex interaction using biophysical and structural approaches, confirming the importance of the RHAU-specific motif in the interaction with hTR. We demonstrate that the helicase activity of RHAU is sufficient to unwind the quadruplex and promote an interaction with 25 internal nucleotides to form a stable P1 helix, and we have investigated the functional implications of this interaction. Screening of the human transcriptome for novel RNA-quadruplex interaction partners of RHAU identified PITX1 mRNA as a hit, with the protein product being an established transcriptional repressor of hTERT[6]. We present data demonstrating that RNA quadruplexes in the 3'-untranslated region of PITX1 mRNA are RHAU binding sites, and we detail our investigations into the functional mechanisms whereby PITX1 regulation is achieved by RHAU and components of the RNAi machinery. Together, the data implicate the unwinding of RNA quadruplexes by RHAU in multiple facets of telomerase regulation.

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533 C 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, by measuring the binding dynamics of SR proteins on model RNAs. To this aim, we use FRAP (fluorescence recovery after photobleaching) on GFP-SR tagged proteins and GFP-RRM tagged domains. To measure binding on specific RNAs, we FRAP the transcription site of MS2-tagged RNAs that contain repetitions of a binding site for an SR protein. In the long term, we aim at translating the statistical binding data obtained by techniques such as CLIP into a residency time on RNA, and we would like to test the hypothesis that combinatorial binding and protein-protein interactions can stabilize individual binding events.

534 A GOLLD: a large, structured, noncoding RNA from bacteria and bacteriophages

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The Giant, Ornate, Lake- and Lactobacillales-Derived (GOLLD) RNAs belong to a class of large, structured non-coding RNAs found in many bacteria and phages. The function of these RNAs is unknown, but their size and conserved structure are reminiscent of large, sophisticated ribozymes such as ribosomes, self-splicing introns, and RNase P[1]. More than 600 of these RNAs have been found in both bacteria and phages, and they are frequently encoded next to tRNA genes. Although little is known about GOLLD RNAs, they are a candidate for a new class of ribozyme.

Commonly, large ribozymes perform their functions in complex with other proteins or RNAs. Because GOLLD is a ribozyme candidate, we sought to identify any interacting partners, which may help elucidate the RNA function. Using formaldehyde-crosslinking, we developed a bead-assisted pulldown procedure to identify interacting molecules. Preliminary data shows that much more rRNA was pulled down when GOLLD was expressed, compared with when GOLLD was not expressed. Therefore, most of the rRNA in the bead-bound fraction appears to have been cross-linked to GOLLD. In addition, reverse-phase chromatography shows that GOLLD RNAs co-elute with the 23S ribosomal RNA, which is consistent with previous results, suggesting that GOLLD RNAs may be performing functions related to ribosomes. However, more in-depth experiments are required to rule out any spurious interactions.

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535 B Dynamic transition upon protein-RNA complex formation: PTB RRM1 interaction with an IRES stem-loop

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We are examining the interaction of the N-terminal RNA recognition motif domain (RRM1) of the Polypyrimidine Tract Binding (PTB) protein with a stem-loop RNA that contains a UCUUU pentaloop present in the Internal Ribosomal Entry Site (IRES) of *Foot-and-Mouth disease virus* (FMDV), *Encyphalomyocarditis virus* (EMCV) and *Theiler's Murine Encephalomyelitis Virus (TMEV)*. Where as previous structure determinations of PTB-RRM1 bound to the single-stranded RNA CUCUCU showed that recognition is achieved through canonical RRM-RNA interactions via the β-sheet surface of RRM1 and the loops connecting the β-strands [1], our structure determination of PTB RRM1 bound to the stemloop RNA shows that the C-terminal tail of RRM1 which includes part of the extensive linker connecting RRM1 to RRM2 in PTB forms an additional α-helix which docks to β2 of the β-sheet. Interestingly the newly formed α-helix makes no contacts to the RNA raising the question of how the C-terminal part of RRM1 is able to sense the binding of a structured RNA to the β-sheet. NMR measurements and site directed mutagenesis at the interface of the C-terminal helix and the β-sheet indicate that RRM1 is in a dynamic equilibrium between conformations where the C-terminal helix is docked to the β-sheet and other conformations where it is not. NMR relaxation dispersion measurements reveal that a dynamic network connects the C-terminal tail with the adjacent β-sheet and remote structural elements which are involved in binding the stemloop. This network which couples binding of RRM1 to a stemloop RNA with formation of an additional secondary structure element shows how PTB can adapt to recognize an ordered RNA target in the context of IRES mediated translation.

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536 C AURA 2.0: empowering post-transcriptional regulatory networks discovery

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The increasingly recognized importance of post-transcriptional regulation (PTR) is leading to the collection of more data than ever before. However, this pieces of data remain dispersed throughout many isolated databases or even lying in the literature, posing multiple obstacles to data integration and eventually preventing the discovery of the regulatory networks underlying these processes.

In order to address this issue, we originally developed the Atlas of UTR Regulatory Activity (AURA), now at its second and vastly enhanced iteration. AURA is a meta-database focused on all aspects of post-transcriptional regulation as mediated by the untranslated regions of mRNA. On top of a rich UTRs annotation layer, AURA contains experimental data on RNA-binding proteins and noncoding-RNAs binding sites, cis-elements, phylogenetic conservation and much more. Collected data covers multiple species, namely human and model organisms such as mouse, yeast and zebrafish. To allow for seamless data integration workflows, AURA also offers several data mining features, with particular focus on network generation, functional enrichment and UTR sequence analysis.

Regularly updated with the newest data and additional features, AURA aims at becoming a valuable toolbox for the PTR researcher and to stimulate the formation of an active community of users and contributors, able to eventually benefit the whole research field. AURA (soon available in its 2.0 version) is freely accessible at http://aura.science.unitn.it .

537 A The QUA2 domain of GLD-1 recognizes an additional nucleotide and modulates RNA binding affinity

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The members of the STAR (signal transduction and activation of RNA) protein family are key players in post-transcriptional processing and are especially essential for developmental processes, including the germline and the vertebrate nervous system. Recent data hint to implications of these proteins in cancer [1] and neurological diseases like human inherited ataxia, multiple sclerosis or schizophrenia [2]. In order to gain more insights into the role of the STAR protein family in these diseases, it is inevitable to understand their molecular basis of RNA interaction.

All STAR proteins share a very high sequence similarity in their RNA-binding domain, an extended KH domain (KH-QUA2), and contain an N-terminal dimerization domain (QUA1), with the exception of SF1 [3] .The family member GLD-1 (Germline defective 1) is a germline specific translational repressor in *C. elegans* and was suggested to associate with 10% of all germline detected mRNA to date [4]. It is essential for germ cell development [5] and can therefore be seen as its key regulatory factor. GLD-1 (*Germline defective 1*) binds as dimer to a single, highly conserved TGE-repeat found within the 3' UTR of its mRNA targets. The importance of the RNA-binding domain for the function of GLD-1 is emphasized by critical mutations within the KH domain, leading to tumor formation or masculinization of the hermaphrodite germline [6]. While the mammalian homolog SF1 recognizes specifically a hexanucleotide RNA sequence, a recently conducted immunoprecipitation assay for GLD-1 proposed a consensus sequence spanning a heptanucleotide [7]. This emphasizes the need of structural data for the RNA binding domain of GLD-1, since the solution structure of SF1 is not sufficient to explain this difference in RNA recognition [8].

We determined the solution structure of the monomeric GLD-1 KH-QUA2 domain bound to its primary target site within the TGE repeat of the *tra-2* gene [9]. In contrast to SF1, the QUA2 domain adopts a slightly different orientation and is thus indeed able to specifically recognize an additional nucleotide with an increase in RNA-binding affinity. In addition, this structure provides a rational for previously published mutagenesis data and subsequently answers the remaining questions about RNA specificity of GLD-1. In conclusion, our data explains the differences to the homologous structure of SF1, provides thus a rational for previous studies on GLD-1 and forms an important basis to understand the impact of the QUA2 domain on RNA binding for the whole STAR protein family.

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538 B Control of Mammalian Germ Cell Differentiation by the RNA-Binding Protein DAZL

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Female and male gametes (egg and sperm) differentiate from embryonic germ cells. Translational control by RNA-binding proteins (RBP) is a central mechanism of germ cell biology and many of the germ cell RBPs are essential and conserved across a wide range of species. Despite this conservation, RBP interacting transcripts and the spatiotemporal regulation of these transcripts have remained largely elusive. Mammalian primordial germ cells (PGC) are specified in the proximal epiblast around embryonic day 7.5 and subsequently migrate to the somatic gonad. Upon entering the gonad, PGCs downregulate pluripotency markers and initiate the sex-specific differentiation programs. Female PGCs initiate meiosis before arresting in diplotene whereas male PGCs undergo cell cycle arrest and initiate spermatogenesis after birth. DAZL is a conserved RBP expressed in PGCs when they enter the gonad and its expression in germ cells is essential for both the downregulation of the pluripotency program and the sex-specific cellular differentiation events. *Dazl* gene inactivation in mice leads to embryonic germ cell death and sterility. Despite its central role in germ cell differentiation and survival we ignore the transcripts bound and regulated by DAZL. We have used iCLIP, an unbiased whole genome approach to identify and characterize DAZL-RNA interactions in mammalian germ cells. We show that DAZL-regulated transcripts are enriched for genes encoding cell cycle regulators of all phases of the cell cycle. We also show that DAZL binds preferentially to the 3' UTR of its target transcripts near the stop codon. Finally we have combined single molecule FISH and immunofluoresence to characterize the DAZL-RNA interactions in vivo. DAZL appears to regulate the cell cycle transitions occurring in germ cells by binding to the 3' UTR and promoting the translation of cell cycle regulators but the molecular mechanism regulating DAZL-RNA interactions remains to be addressed and will further our understanding of translational control of germ cell biology and fertility.

539 C Structural and Dynamic Investigation on ETR-3 RRMs and their Interaction with AU-rich RNAs

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The ELAV type RNA-binding protein 3 (ETR-3) is a RNA binding protein involved in many aspects of RNA metabolism. ETR-3 interacts with UG repeats in the context of splicing [1]. Sequence specificity for UG repeats and notably the UGUU motif has been confirmed by systematic evolution of ligands by exponential enrichment (SELEX) [2]. However, ETR-3 has also been shown to regulate the cyclooxygenase-2 (COX-2) mRNA stability and translation by binding to AU-rich sequences in the 3'UTR region [3].

ETR-3 belongs to the CUG-BP and ELAV like factor (CELF) family. The members have two RNA recognition motifs (RRM) separated from a third one by a divergent domain (approx. 200 amino acids). The RRMs are extremely conserved among the family; over 90% identity between ETR-3 and CUG-BP1, its closest homolog. The solution structure of CUG-BP1 RRM3 in complex with $(UG)_3$ has been solved by NMR spectroscopy[4]and X-ray crystallography structures of CUG-BP1 RRM1 and RRM2 bound to RNAs containing the UGUU motif have been published [5]. Although these structures provide very detailed insight in the binding mode to UG rich RNAs, it remains unclear how ETR-3 recognizes AU rich RNAs and how both RRM1 and RRM2 are oriented upon RNA binding.

We aim at solving the structure of ETR-3 RRM12 and RRM3 bound to AU rich RNA. This will allow us to explain the discrepancies between the natural targets and the sequences obtained by SELEX. We established that RRM1 and RRM2 are semi-independent in the free form and we could show that both RRM12 and RRM3 bind to 5'-AUUUAAUU-3' sequence found in COX-2 mRNA. Dynamics studies on RRM12 in complex with the octamer demonstrated that RRM12 tumbles slower in complex than the free protein suggesting a rigidification of RRM12. In addition, we have a preliminary structure of RRM3 in complex with 5'-UUUAA-3'. In cell experiments demonstrated that RRM12 is sufficient to regulate the translation and unlike splicing, the divergent domain is not necessary for COX-2 mRNA translation inhibition.

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540 A Interactions between RBFOX2 and pre-microRNA-20b terminal loop

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Several RNA binding proteins originally known for their role in pre-mRNA processing have recently been found to also operate during the biogenesis of microRNAs. RBFOX2, a known regulator of alternative splicing, specifically binds to GCAUG motifs in the pre-mRNAs to regulate inclusion or exclusion of flanking exons. We identified several chemically synthesized pre-miRNA hairpins that bound to RBFOX2 *in vitro*. One of these, hsa-miR-20b contained the characteristic GCAUG recognition motif in the terminal loop region. As determined by surface plasmon resonance (SPR), the recombinant RRM domain of RBFOX interacted with the syn-pre-miR-20b with moderate affinity (3.6 μM). Mutation of G5 residue, which is essential for RBFOX2 binding to GCAUA completely abrogated the binding. The overexpression of miR-20b by transfection of its chemically synthesized precursor or by a pri-miRNA plasmid suppressed RBFOX2 protein levels. Upon G to A mutation in the loop, RBFOX2 suppression activity was lost. These data reveal a novel connection of RBFOX2 to microRNAs, in addition to alternative splicing.

541 B RNA-binding protein EWSR1 regulates CCDC6

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Dysregulation in the interactions between RNA and RNA-binding proteins (RBPs) by mutations, translocations or over expression results in several diseases. Altered protein expression of the ubiquitously expressed FET family of proteins FUS, EWSR1 and TAF15 has been shown to cause neurological diseases as well as sarcomas and leukemias. PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Crosslinking & Immunoprecipitation), a technique to study the RNA interactome of any RBP, was applied to the FET family proteins as described in our previous study (Hoell et al, Nat Struct Mol Biol 2011) and global RNA targets of these proteins were defined. In the present study we focussed on EWSR1 which is fused to several DNA binding proteins (e.g. FLI-1, ERG, ETV-1) to form active transcription factors in Ewing Sarcoma.

Ewing Sarcoma is the second most common bone and soft tissue malignancy in adolescents and young adults. Research so far focused nearly exclusively on the above-mentioned fusion proteins and the lost RNA binding capabilities of the C-terminal portion of EWSR1 remained unexplored. In order to study the functional impact of the loss of one wild type allele of EWSR1 on sarcomagenesis, we tested several mRNA targets predicted by PAR-CLIP including MDM2, CCDC6, CBFB and FGF9. Our results showed that there was a clear reduction in the expression levels of all the above selected genes in the EWSR1 knock down samples compared to the controls. Among the selected targets we chose to further investigate CCDC6 (Coiled Coil Domain Containing 6) which showed the strongest regulation.

CCDC6 is important in cell cycle regulation and acts as a check point control for transition of cells from S to G2 phase. It is also predicted to be a tumor suppressor gene and is down regulated in adenocarcinoma of the lung, colorectal carcinoma, thyroid carcinoma and small cell lung cancer. Interplay between CCDC6 and EWSR1 was further explored using forward and reverse genetic approaches in HEK 293 T cells and Ewing Sarcoma cell line MHH-ES-1. Protein levels of CCDC6 were down regulated upon EWSR1 knock down and up regulated upon EWSR1 over expression. Furthermore, EWSR1 knockdown results indecreased proliferation rates and increased cell death compared to the controls hinting at a defective cell cycle progression. Defects in cell cycle and proliferation are often known to trigger carcinomagenesis. Taken together, our results confirm the regulation of CCDC6 by EWSR1on mRNA and protein level and the role they play in regulating cell cycle which further helps us to understand the underlying mechanisms in the development of Ewing Sarcoma.

542 C Dead End, a protein counteracting miRNA-mediated repression of tumour suppressor genes, contains non-canonical RNA binding domains

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The protein Dead End (Dnd1) is necessary for proper development of germ cells in zebrafish and mammals [1]. Truncations in the Dnd1 gene have been shown to promote the formation of germ-cell tumors [2]. Recently the molecular function of Dnd1 has been exposed as a negative modulator of miRNA activity, when it was shown that it can counteract repression of several tumour suppressor genes (p27, LATS2) by the miR-221 family by blocking the accessibility of the mRNAs targeted by these miRNAs [3,4,5]. It was suggested that Dnd1 blocks access of miRNAs to their targets by binding to conserved U-rich regions (URRs) in close proximity to the miRNA seed sequences in the mRNA target 3'UTRs (see Figure 1 below).

Dnd1 contains two RNA recognition motifs (RRMs). To understand how Dnd1 recognizes its targets and inhibits miRNA-based gene silencing at the molecular level we aim to solve the three-dimensional structure of the Dnd1 RRMs in complex with RNA derived from one of Dnd1's 3'UTR-targets. We have identified short RNA oligomers derived from the p27 tumour suppressor mRNA 3'UTR that bind to the Dnd1 RRM1 and double RRM12 as shown by NMR chemical shift perturbation mapping and ITC. In addition, ITC measurements have shown that both RRMs are necessary for tight binding, suggesting that the two RRMs are working cooperatively in recognizing their mRNA-targets.

The solution structure of RRM12 shows that Dnd1's both RRMs contain non-canonical helical and beta-hairpin extensions to the classical RRM fold. The RNA binding site includes these non-canonical elements in addition to the RRM1 canonical beta-sheet RNA interaction surface. NMR based structure determination of a RRM12-p27-3'UTR complex is underway.

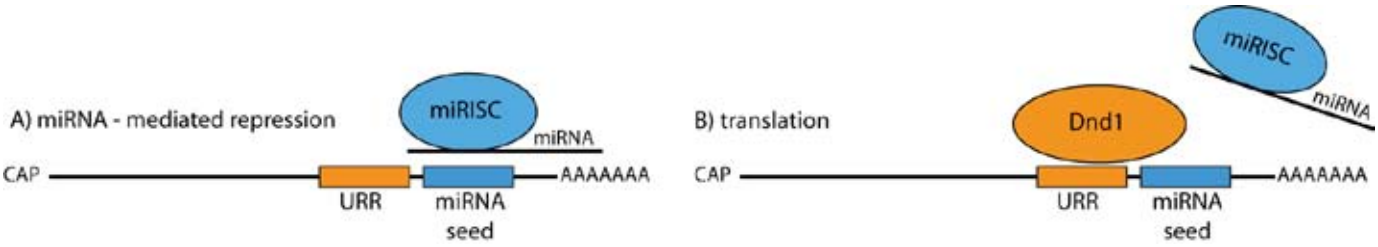

Figure 1:

A) miRISC binding through recognition of a complementary 'seed' sequence by the miRNA in the complex prevents translation of the mRNA

B) When Dnd1 binds to U-rich regions (URRs) next to the miRNA recognition sequence it blocks access of miRISC and the mRNA is translated

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543 A Potential substrates of the RNase MRP complex in cell cycle regulation

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The RNase MRP complex is composed of the RNA component of mitochondrial RNA processing endoribonuclease (*RMRP*) and five additional protein components. This RNAse MRP complex plays a role in the tailoring of the 5'end of the 5.8S rRNA.

RMRP is the first non-coding RNA which is associated with a human disease. Patients with cartilage-hair hypoplasia (CHH) have recessive mutations in *RMRP*; they are affected by skeletal dysplasia and a predisposition for various tumours. Recent studies have shown that *RMRP* also forms a complex with TERT (telomerase reverse transcriptase). This RNA-protein complex may have a RNA-dependent RNA polymerase activity. An isolated deficieny in TERT causes Diskeratosis congenital (DC). Intruigingly, the clinical manifestations of DC are very similar to CHH. In RNA-protein complex formation, the limiting factor is TERT. One consequence of impaired TERT function is progressive telomere shortening in proliferating cells. This results in cell cycle arrest and the induction of DNA repair mechanisms. Cell cycle genes such as p53 and p21 (also known as cyclin-dependent kinase inhibitor 1 or CDK-interacting protein 1) play an important role in these processes. Malfunction of these genes may lead to tumor formation and cellular senescence.

A negative correlation between expression of TERT and *RMRP* level has already been shown in some cell lines. The interaction of *RMRP*, TERT, and cell cycle genes could provide an explanation for the tumour susceptibility of CHH patients.

We will present preliminary data on the role of *RMRP* in cell cycle regulation and senescence.

544 B Post-transcriptional regulation of SMN2 expression by hnRNP G and LARP Family proteins

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Spinal Muscular Atrophy (SMA) is a recessive autosomal neurodegenerative disease caused by the loss of Survival Motor Neuron 1 gene (*SMN1*). Previous studies have implicated SMN in RNA metabolism, namely in the biosynthesis of small nuclear ribonucleoprotein complexes, and there is evidence that SMN is involved in axonal mRNA transport. Furthermore, in humans there is an almost identical genomic copy of *SMN1*, the *SMN2* gene. *SMN2* contains a silent mutation in exon 7 that greatly impairs splicing, leading to the predominant production of a truncated SMN protein that is thought to be rapidly degraded. Interestingly, SMA severity is inversely correlated to the number of *SMN2* copies present in the genome. Therefore, SMA would be a strong target for the development of new therapies regarding mRNA stability and translation control. It is widely accepted that mRNA stability and translation are greatly influenced by the action of RNA binding proteins and microRNAs that bind untranslated regions of mRNAs. Hence, identification of proteins binding to SMN2 mRNA would provide a greater insight on mechanisms underlying its expression and ultimately uncover novel targets that favor mRNA stabilization and/or increased translation. In this work we have used *in vitro* transcribed biotin-labeled probes covering the 3' UTR of SMN2 mRNA to perform pull-down assays to identify proteins binding its 3'UTR. Several proteins were identified by *peptide-mass fingerprinting*, among which were proteins described as having a role in mRNA metabolism, namely hnRNP G (hnG) and members of the La-Related Protein (LARP) family, including a novel LARP1 isoform. As LARP family members remain poorly studied, we characterized LARP isoform expression in the cell lines used in this work by RT-qPCR, confirming the existence of the novel isoform identified by mass-spec. Overexpression of hnG and LARPs resulted in increased expression of both a luciferase reporter containing the SMN 3'UTR sequence and of the endogenous SMN protein. Characterization of SMN mRNA levels identified different mechanisms of action for these proteins at the level of mRNA stability and translation. In order to further clarify the effect of hnG and LARPs on SMN2 expression, hnG and LARPs were co-transfected with various SMN2-3'UTR deletion mutants fused to *firely* luciferase. Our data supports a role for hnG and LARPs as positive modulators of SMN mRNA expression.

545 C Characterization of the potential role for RNA-binding protein FUS/TLS in DNA damage response: A quantitative proteomic approach

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FUS/TLS (fused in sarcoma/translocated in liposarcoma) is a ubiquitously expressed protein of the hnRNP family, that has been discovered as fused to transcription factors in several human sarcomas and found in protein aggregates in neurons of patients with an inherited form of Amyotrophic Lateral Sclerosis [Vance C. et al., 2009].

FUS is a 53 kDa nuclear protein that contains structural domains, such as a RNA Recognition Motif (RRM) and a zinc finger motif, that give to FUS the ability to bind to both RNA and DNA sequences. It has been implicated in a variety of cellular processes, such as pre-mRNA splicing, miRNA processing, gene expression control and transcriptional regulation [Fiesel FC. and Kahle PJ., 2011].

Moreover, some evidences link FUS to genome stability control and DNA damage response: mice lacking FUS are hypersensitive to ionizing radiation (IR) and show high levels of chromosome instability and, in response to double-strand breaks, FUS is phosphorylated by the protein kinase ATM [Kuroda M. et al., 2000; Hicks GG. et al., 2000; Gardiner M. et al., 2008].

Furthermore, preliminary results of mass spectrometric identification of FUS interacting proteins in HEK293 cells, expressing a recombinant flag-tagged FUS protein, highlighted the interactions with proteins involved in DNA damage response, such as DNA-PK, XRCC-5/-6, and ERCC-6, raising the possibilities that FUS is involved in this pathway, even though its role still needs to be clarified.

This study aims to investigate the biological roles of FUS in human cells and in particular the putative role in DNA damage response through the characterization of the proteomic profile of the neuroblastoma cell line SH-SY5Y upon FUS inducible depletion, by a quantitative proteomic approach. The SH-SY5Y cell line that will be used in this study expresses, in presence of tetracycline, a shRNA that targets FUS mRNA, leading to FUS protein depletion (SH-SY5Y FUS iKD cells). To quantify changes in proteins expression levels a SILAC strategy (*S*table *I*sotope *L*abeling by *A*mino acids in *C*ell culture) will be conducted on SH-SY5Y FUS iKD cells and a control SH-SY5Y cell line (that expresses a mock shRNA) and the relative changes in proteins levels will be evaluated after five and seven days upon FUS depletion, by nanoliquid chromatography coupled to tandem mass spectrometry (nLC-MS/MS) and bioinformatics analysis.

Preliminary experiments demonstrated that the SH-SY5Y FUS iKD cells, when subjected to genotoxic stress (high dose of IR), upon inducible depletion of FUS, showed a increased phosphorylation of *g*H2AX with respect to control cells, suggesting an higher activation of the DNA damage response.

546 A A novel PAR-CLIP based approach using RRM mutations reveals RNA recognition mechanisms of HuR

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HuR is a ubiquitous, multi-functional RBP that contains three RNA recognition motifs (RRMs) which allows it to recognize several closely related low complexity U-rich elements including the AU-rich element (ARE). However, it is unclear how each of the RRMs contributes to recognizing these elements and whether they have distinct preferences among the global set of targets. This is especially true for the third RRM which is known to bind U-rich RNA but has also been suggested to bind to poly(A) sequences; moreover, RRM3 is thought to be pivotal for *in vitro* multimerization of HuR. To address this we have combined a mutational approach with a modified version of PAR-CLIP, a technique for identifying global RNA-protein interactions. Our novel approach includes correcting for previously unaccounted for background binding and using a combination of deletion and point mutants designed from published RRM/RNA co-crystal structures. Our data agrees with previous findings that all three RRMs are not required for RNA recognition but that at least two RRMs are required for binding to most sites. Furthermore, our data also demonstrate that RRM3 can recognize sequences similar to RRMs 1 and 2 and that inactivation of RRM3 doesn't dramatically alter the sequence preference of HuR. Interestingly, while the three RRMs are at least partially redundant for recognizing similar sequences our data suggests that they have unique roles in determining where HuR binds globally. Specifically, inactivation of RRM3 resulted in fewer HuR binding sites in close proximity to other HuR binding sites, especially within intronic sequences. This suggests that RRM3 initiates and/or stabilizes multimerization of HuR *in vivo*, a mechanism previously proposed to explain how HuR competes with miRNAs. However, since our data indicate multimerization is predominately in introns this may relate to the ability of HuR to regulate splicing as well. This mechanism of RRM3-dependent multimerization potentially extends beyond HuR and may apply to the roughly 100 other 3 RRM-containing RBPs, nearly all of which share similar inter-domain spacing patterns.

547 B The Runt domain of AML1 (RUNX1) binds a sequence-conserved RNA motif that mimics a DNA element

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AML1 (RUNX1) is a key transcription factor for hematopoiesis that binds to the Runt-binding double-stranded DNA element (RDE) of target genes through its N-terminal Runt domain. Aberrations in the AML1 gene are frequently found in human leukemia. To better understand AML1 and its potential utility for diagnosis and therapy, we obtained RNA aptamers that bind specifically to the AML1 Runt domain. Enzymatic probing and NMR analyses revealed that Apt1-S, which is a truncated variant of one of the aptamers, has a CACG tetraloop and two stem regions separated by an internal loop. All the isolated aptamers were found to contain the conserved sequence motif 5′-NNCCAC-3′ and 5′-GCGMGN′N′-3′ (M:A or C; N and N′ form Watson–Crick base pairs). The motif contains one AC mismatch and one base bulged out. Mutational analysis of Apt1-S showed that three guanines of the motif are important for Runt binding as are the three guanines of RDE, which are directly recognized by three arginine residues of the Runt domain. Mutational analyses of the Runt domain revealed that the amino acid residues used for Apt1-S binding were similar to those used for RDE binding. Furthermore, the aptamer competed with RDE for binding to the Runt domain in vitro. These results demonstrated that the Runt domain of the AML1 protein binds to the motif of the aptamer that mimics DNA. Our findings should provide new insights into RNA function and utility in both basic and applied sciences.

548 C Predicting RNA-Protein Interactions: The Hunt for the Code of Recognition

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Predicting RNA-Protein Interactions: The Hunt for the Code of Recognition

Protein-RNA interactions are found to be pivotal in a wide variety of cellular processes ranging from gene regulation to host defenses against pathogens. In addition to their fundamental biological regulatory role, the impact of faulty protein-RNA based regulation on metabolic and immunological diseases emphasizes the motivation for understanding the principles behind protein–RNA recognition for medical research. In the presented study, covariant residue patterns between families of interacting protein and RNA were investigated. Co-crystal structures of RNA binding proteins and RNA targets were collected from the Protein Data Bank and filtered in order to only retain crystals of non-synthetic molecules. The protein and RNA sequences that were found to interact using a five Angstrom distance threshold were extracted from the co-crystal structures and subjected to structural alignment against the Pfam and Rfam databases. Covariance analysis of the alignments of protein-RNA pairs were conducted using mutual information and the recently published RIsearch method [1] for RNA-RNA interactions was adapted to infer protein-RNA specificity models. This approach was verified on examples with well-known specificity and by comparison to interactions found in the co-crystal structures.

The presented study constitutes a method which can predict novel protein-RNA interactions, aid in the mapping of regulatory networks, and contributes to deciphering the code of recognition between protein and RNA.

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549 A Testing Protein Sequestration Candidates for RNA-Mediated Disease

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RNA-mediated pathogenesis is a disease mechanism caused by the synthesis of mutant RNAs that have gained deleterious functions[1]. For microsatellite disorders where mutant expansions occur within non-coding regions, transcription leads to expansion RNAs (RNA^{exp}) that are toxic because they either sequester or trigger abnormal activities for RNA-binding proteins and possibly other types of cellular factors. For protein sequestration, this disease pathway is exemplified by the progressive neuromuscular disease myotonic dystrophy (*dystrophia myotonica*, DM), which is caused by C(C)TG expansions in either the untranslated or intronic regions of two different genes leading to the synthesis of $C(C)UG^{exp}$ RNAs that recruit and inhibit the alternative splicing activities of the muscleblind-like (MBNL) family of RNA-binding proteins[2]. Here, we use a crosslinking approach to confirm sequestration of MBNL2 to DMPK CUG and CNBP CCUG repeats in human autopsy brain tissues obtained from DM1 and DM2 patients, respectively. In parallel, we map MBNL2 binding sites to splicing target RNAs in normal and disease control samples to investigate how loss of MBNL2 function alters the central nervous system transcriptome. Finally, we use this assay to validate or refute the binding of proposed sequestered factors in human brains affected by other neurological diseases caused by microsatellite expansions. We propose this method as a validation assay to test the sequestration of specific factors by toxic RNAs in human disease tissues.

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550 B Computational study of interactions between amino acids and nucleobases in aqueous solvent

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The genetic code table provides a universally conserved link between the sequences of cognate mRNA and protein pairs. However, a question, which still remains unanswered, is why a particular nucleotide triplet codes for a particular amino acid? A potential connection between physicochemical properties of codons and cognate amino acids i.e. of mRNA and cognate protein sequences was recently explored[1]. Surprisingly, it was shown that mRNA coding-sequence pyrimidine content strongly correlates with the average propensity of protein sequences to be solubilized by pyrimidine mimetics (protein polar requirement), hinting at the possibility of complementary binding between these two biopolymers being not only responsible for defining the genetic code, but also playing an important role in present-day cells. To further examine potential interactions between proteins and their mRNAs on a microscopic level, molecular dynamics simulations were employed to derive scales of amino-acid solubility in nucleobase-water solutions. These were then used to obtain proteome-wide correlations of average sequence properties of mRNAs and cognate proteins and to compare them with previously observed ones using the polar requirement scale. Structural and energetic analysis of different amino acids in nucleobase-water solutions revealed that amino acids generally tend to interact more readily with nucleobases than with water molecules, with differing propensities depending on the exact system studied. Taken together, newly generated propensity scales provided proteome-wide correlations between compositional properties of mRNAs and cognate proteins that were comparable in strength to those obtained when the polar requirement scale was used, but differed in direction in several important cases. This allowed us to further explore the mRNA-protein complementarity hypothesis and probe its limits and overall validity.

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551 C Codon-usage effects and functional characterization of physicochemical complementarity between mRNA and cognate protein sequences

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We recently reported a persistent correlation between mRNA coding-sequence pyrimidine content and the propensity of cognate protein sequences to interact with pyrimidine mimetics as captured by the polar requirement amino-acid scale. Moreover, strong correlations were observed both on the whole proteome level and on the level of individual protein-mRNA sequence profiles. On the basis of these findings, we hypothesized that mRNA coding regions may in general be physically complementary to and therefore directly interact with cognate protein regions, especially if both polymers are unstructured. Here, we explore how codon usage affects these correlations by systematically varying the pyrimidine content of degenerate codons, while keeping the cognate protein polar requirement fixed. Next, we re-evaluate the correlations between the properties of two biopolymers, both on the level of sequence averages as well as individual profiles, and compare them against correlations observed for the native sets. We find that the levels of matching in present-day proteomes can be both significantly increased or decreased depending on particular codons, while still preserving the specific native codon usage bias for all proteomes tested (*M. jannaschii*, *E. coli*, *S. cerevisiae* and *H. sapiens*). Finally, we explore the relationship between the level of mRNA-protein complementary matching and biological function by analyzing the enrichment or depletion of functional Gene Ontology terms in different segments of the profile-matching distribution and find that certain molecular functions can easily be related to the mRNA-protein complementarity hypothesis.

552 A Establishment of a Fluorescence Cross Correlation Spectroscopy (FCCS) based assay to measure Argonaute – siRNA interaction

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Argonaute Proteins are the key players in the RNA interference mechanism (RNAi) by which double stranded RNA leads to the loss of an mRNA homologous sequence. Synthetic short interfering RNA duplexes (siRNAs) effectively can be used to downregulate certain genes of interest at the mRNA level. Therefore siRNAs targeting the same target RNA can exhibit different knockdown efficiencies or lead to unwanted off-target effects.

Here we report the measurement of the molecular interactions between siRNAs and Ago proteins via Fluorescence Cross Correlation Spectroscopy (FCCS). With this method information about the size, the rate of diffusion and concentration of each fluorescently labeled particle can be obtained. This is achieved by analyzing the time-dependent fluctuation of the fluorescence signal of Cyanine-5 (Cy5) labeled siRNAs and GFP fused Ago proteins through a confocal detection volume.

The aim of the project is to establish an FCCS based assay to measure direct interactions of Ago Proteins with siRNAs and to examine whether the affinity of a certain siRNA correlates with its knockdown potency *in vivo*. Furthermore this assay provides the possibility to identify potential regulatory sites of Argonaute proteins by comparing the binding kinetics of Ago mutants with the respective wildtype protein.

553 B Post-transcriptional control of macrophage activation by HuR.

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Post-transcriptional control of the expression of inflammatory molecules is emerging as an efficient and rapid way to regulate the development and the resolution of inflammation. It requires the assemblies of RNA-binding proteins (RBPs) and non-coding RNAs onto specific elements on their RNA targets in Ribonucleoprotein particles (RNPs) which control mRNA maturation, turnover and translation. One of the key players, with an established role in translation and turnover of inflammatory mediators is the RNA-binding protein HuR. HuR binds to AU-rich elements (AREs), and through this binding it has been suggested to act as a stabiliser of mRNAs, either by positively regulating their translation, or by inhibiting their decay. However, data have emerged suggesting its role as a negative regulator of pathologic inflammation: transgenic systems of inducible and macrophage specific HuR overexpression in mice suppress translation of inflammatory mRNAs (Katsanou et al 2005); mutant mice lacking HuR from the myeloid lineage (*LysCre^{+}Elavl1$^{fl/fl}$)* show exacerbations in the biosynthesis of inflammatory cytokines and chemokines (Yiakouvaki et al 2012). Therefore, HuR appears to be a pleiotropic regulator of the expression of inflammatory mRNAs, a role slightly more complicated than its original assignment as an mRNA stabilizer.

To further understand the role of this RBP in post-transcriptional control of inflammatory gene expression we applied PAR-CLIP on resting and activated bone marrow derived macrophages. Analysis of the RNA targets identified reveals HuR's differential contribution towards the development of macrophage activation.

554 C Repetitive RNA unwinding of a single RNA helicase A

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RNA helicase A (RHA) plays diverse roles in cellular machinery as a transcriptional co-activator, an essential cofactor for normal gastrulation during mammalian embryogenesis and a translational activator of complex cellular and retroviral mRNAs as like human immunodeficiency virus (HIV). Consisting of two double stranded RNA binding domains (dsRBDs) at the N-terminus, helicase core and a single stranded RNA (ssRNA)-binding RGG domain at the C-terminus, dsRBDs and RGG domain of RHA have been proposed to possess regulatory functions in its helicase activity. However, it has not been tackled yet how RHA plays a role as a transcriptional co-activator and how the subdomain as like dsRBDs controls the helicase activity of RHA owing to lack in the molecular detail of the RNA unwinding process by RHA. Here, we provided the detailed molecular mechanism of RNA unwinding by RHA, taking an advantage of a single-molecule technique, which unveiled the molecular details of the RNA unwinding process by RHA, including the characterization of every single step in the RNA unwinding process, the repetitive unwinding characteristic of RHA after its loading to dsRNA, and dsRBDs-controlled RHA loading or activation. Moreover, we directly showed that the repetitive RNA unwinding by RHA cleared the hydrogen bonds in dsRNA efficiently, making it easily access to the complementary ssRNA, which gives a hint how RHA acts as a transcriptional co-activator in a molecular point of view.

555 A The RNA-binding Protein Repertoire of Embryonic Stem Cells

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RNA-binding proteins (RBPs) play essential roles in RNA-mediated gene regulation, and yet the current annotation of RBP is largely limited to those with known RNA-binding domains. To systematically identify the RBPs of embryonic stem cells (ESCs), we here employ "interactome capture", which combines UV-crosslinking of RBPs to RNA in living cells, oligo(dT) capture, and mass spectrometry. From mouse ESCs, 555 proteins are defined here to constitute the mESC RNA-interactome, which includes 283 proteins not previously known to bind RNA. Interestingly, 39 novel RBP candidates are highly expressed in ESCs when compared to differentiated cells, suggesting that they may play important roles in stem cell physiology. Among them, two well-known E3 ubiquitin ligases (Trim25/Efp and Trim71/Lin41) are validated as novel RBPs, revealing a potential link between RNA biology and post-translational modification pathways. Our mESC RNA-interactome confirms RBPs recently found in HeLa and HEK293 studies and expands the atlas of RBPs with novel candidates, providing a valuable resource for the study of RNA-RBP networks in stem cells.

556 B Drosophila Gemin5 binds to UsnRNAs and another specific group of ncRNAs

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Pre-messenger RNA (pre-mRNA) splicing is an indispensable step in protein synthesis. Human RNA splicing is mediated by spliceosome, the molecular machinery that is comprised of various U small nuclear ribonucleoproteins (UsnRNPs). The UsnRNPs biogenesis is highly regulated by a protein complex called the Survival of Motor Neuron complex (SMN complex). The SMN complex is composed of the SMN protein, sm proteins and Gemin2-8. Gemin5 has been identified as the UsnRNAs-binding protein in SMN complex. *Rigor mortis* (Rig) is the gene homolog of human *Gemin5* in *Drosophila*. It is found highly expressed in the central nervous system of larvae and the ovaries of adult flies. We showed that Rig protein share the same function of human Gemin5 in binding UsnRNAs with high affinity and specificity. By means of RNA immunoprecipitation and next-generation sequencing, we further revealed that Rig binds to another group of non-coding RNAs, suggesting that Rig is involved in other pathways of RNA binding or RNA biogenesis.

557 C RBP atlas: an exploration of interactions between mRNA and proteins and their impact on cardiomyocyte biology

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RNA-binding proteins (RBPs) control all aspects of RNA fate and defects in their function underlie a broad spectrum of human pathologies. Employing a combination of UV-crosslinking of proteins to RNA in living cells with identification of those co-purifying with poly(A)+ RNA by mass spectrometry, we recently identified 860 proteins as the "mRNA interactome" of human HeLa cells.[1] Over 300 of these RBPs were not previously known to bind RNA and their identification sheds new light on RBPs in disease, RNA-binding enzymes of intermediary metabolism, RNA-binding kinases, and RNA-binding architectures. We have now adapted this approach to identify the mRNA interactome of murine HL-1 cardiomyocytes. The HL-1 cell line maintains the ability to contract and other differentiated cardiac morphological and functional properties in culture.[2] Our analyses reveal both, RBPs commonly detected in cells of different origin as well as more cardiomyocyte-specific RBPs. Ongoing work is focused on detecting changes in the cardiomyocyte mRNA interactome under conditions of pathophysiological stress and on identifying the RNA targets of selected cardiomyocyte RBPs using RBP pull-down followed by next generation sequencing of co-purifying RNA. Altogether, these investigations will map networks of post-transcriptional gene regulation in cardiomyocytes and might reveal their involvement in molecular processes commonly at play in heart disease.

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558 A Multifunctional interleukin-6 receptor aptamers

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An aptamer can form a defined tree dimensional structure which leads molecules to exhibit high affinities to inorganic or organic molecules. In our lab an RNA aptamer with high affinity (K_d = 20 nM) to the human interleukin 6 receptor (IL-6R) was selected. This aptamer triggers IL-6 receptor-mediated uptake without affecting IL-6R interactions with its natural ligands IL-6 and gp130. Furthermore, we were able to use the 19 nt long aptamer for cargo delivery into IL-6R presenting cells. The tree dimensional structure of the RNA aptamer was identified as an intrinsic all parallel quadruplex structure. The affinity of the aptamer to IL-6R was highly dependent on quadruplex structure formation and abolished by replacing one guanine being involved in quadruplex formation. Additionally, we could demonstrate that the IL-6R aptamer served as effective HIV *de novo* infection inhibitor by binding gp120 as primary target. Gp120 is presented on the surface of HIV particles and responsible for infection process. The presence of the aptamer during HIV *de novo* infection leads to prevention of infection due to blockade of gp120-CD4 complex formation. Structural similarities of IL-6R and gp120 could not be observed explaining the affinity of an aptamer to two different targets.

559 B Neurodegenerative diseases: quantitative predictions of protein-RNA interaction

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Although neurodegenerative diseases are traditionally described as protein disorders leading to amyloidosis (Rubinsztein 2006; Dobson 1999), very recent evidence indicates that protein-RNA associations are involved in a number of neuropathies. Using our computational method catRAPID (Bellucci et al. 2011) we analyzed interactions between protein and RNA molecules linked to neurodegeneration. In particular, we focused on: 1) *FMR1,* whose codon expansion (55-200 CGG repeats) in the 5' untranslated region of the gene is associated to Fragile X-associated Tremor/Ataxia Syndrome (FXTAS); 2) Iron regulatory protein 1 (IRP1) translational regulation of Alzheimer's and Parkinson's disease related genes through the recognition of IRE (Iron-Responsive Element).

According to our calculations, CGG repeats in the 5'UTR of *FMR1* gene, have a strong propensity to sequester MBNL1 and hnRNP-G and all the proteins (hnRNP-A1, hnRNP-A2/B1, hnRNP-C, hnRNP-D, hnRNP-E and hnRNP-C) that were found to colocalize with CGG repeats by Sellier et al. 2010. In agreement with experimental evidence we also find poor sequestration propensity for FMRP and CUGBP1, while we predict that PURa, which colocalizes with cytoplasmic CGG repeats in flies (Jin et al. 2007) but not in mammalian cells where it is strictly nuclear, interacts with CGG repeats. Interestingly, we predict that SAM68, essential for the recruitment of other proteins but not interacting with CGG repeats from experimental evidence, does not interact with CGG repeats. Nevertheless, among its annotated protein interactors we find that cold-inducible RNA-binding protein CIRBP and polypyrimidine tract-binding protein 2 PTBP2, have a propensity to be sequestered by CGG repeats which increases with its length, thus suggesting that they could mediate SAM68 sequestration.

With regards to Irp1, our method correctly predicts that the 5'-UTRs of ferritin and *APP* interact with IRP-1 in several regions along the protein sequence, which is consistent with experimental evidence (Rogers et al., 2002; Walden et al. 2006). In addition, in line with what reported by Cho et al., we discovered that 87% of the mutations in the CAGAGC motif strongly reduce IRP-1 binding ability. Interestingly, one RNA stem loop within the 5'-UTR of human *a*-synuclein transcript that has been predicted to be structurally related to the IRE element present in ferritin mRNA, has the highest propensity to bind to IRP-1 according to our calculations.

In conclusion, we propose catRAPID for predictions of protein-RNA associations, to flag putative interactions and select candidates for experimental studies. Our method allows processing of a large amount of protein-RNA pairs and can lead to finding previously unknown interactions.

560 C RNA helicase function in yeast ribosome biogenesis

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The synthesis of cytoplasmic ribosomes in Eukaryotes is best understood in the yeast *Saccharomyces cerevisiae*. Here, the pathway requires more than 200 non-ribosomal proteins, such as GTPases, nucleases and RNA helicases. In addition, 75 small nucleolar RNAs (snoRNAs) are involved and base-pair with pre-ribosomal RNA, and many are predicted to require helicase activities for their release. Since the knowledge on the 19 RNA helicases involved in ribosome biogenesis is still limited and molecular and regulatory functions of these enzymes seem to be diverse, the elucidation of their roles has remained a challenging task.

Using the UV cross-linking and analysis of cDNAs (CRAC) approach, Solexa deep sequencing and bioinformatics, we have identified RNA binding sites of helicases involved in ribosome biogenesis. One protein that we are studying is Rok1, a RNA helicase required for the maturation of the small ribosomal subunit. We have previously shown that Rok1 acts in the release of snR30, a box H/ACA snoRNA that is required for pre-rRNA processing. Interestingly, Rok1 crosslinks to a region in the eukaryotic expansion segment 6 of 18S rRNA at the snR30 basepairing site, which is in line with a direct involvement of Rok1 in the unwinding of snR30 from pre-ribosomal RNA.

We are currently analyzing CRAC data for several other RNA helicases, most of which are involved in the biogenesis of the large ribosomal subunit. These data will provide the basis for further functional analysis of the specific roles of RNA helicases in ribosome biogenesis.

561 A Knock-out mice and HITS-CLIP reveal that the SGs assembly factor G3BP preferentially binds intron-retaining transcripts and controls their stability in the brain

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Stress granules (SGs) are non-membranous cytoplasmic foci formed as a cellular protective response to environmental stress, such as elevated temperature, oxidative stress, hypoxia, osmotic shock, UV irradiation, glucose deprivation or viral infection. SGs share common properties with other granules like processing bodies or neuronal transport granules. G3BP (RasGAP-SH3-domain Binding Protein) is a key factor involved in SGs assembly. To assess the physiological function of G3BP, we generated viable *G3bp1*-knockout (KO) mice, which demonstrated behavioral defects linked to the central nervous system (CNS) associated with ataxia phenotype. Immunohistochemistry pinpointed high expression of G3BP in the cytoplasm of hippocampal neurons and Purkinje cells of the cerebellum of wild-type (WT) mice. Also, electrophysiological measurements revealed that the absence of G3BP1 leads to an enhancement of short-term potentiation (STP) and long-term depression (LTD) in the CA1 area of *G3bp1* KO mice compared to WT mice. Consistently, G3BP1-deficiency in neurons leads to an increase in intracellular calcium and calcium release in response to (S)-3,5-Dihydroxyphenylglycine (DHPG), a selective agonist of group I metabotropic glutamate receptors. HITS-CLIP (High-Throughput Sequencing after Cross-Linking and ImmunoPrecipitation) experiments were carried out on WT and KO mouse brain to identify G3BP-associated RNAs. Surprisingly, many G3BP's targets turn out to be non-coding RNA sequences, essentially snoRNAs and intronic sequences. Interestingly, transcripts with retained introns appear to be enriched in the cerebellum compared to the rest of the brain. G3BP1 depletion leads to a decrease in the expression of these intronic sequences in the cerebellum. In particular, G3BP1 is essential to repress mature *Grm5* (metabotropic glutamate receptor 5) expression in the cerebellum by stabilization of the intron in its pre-mRNA. This study suggests a new mechanism of gene regulation, important in the cerebellum, based on stabilization of silenced premature RNA transcripts, which might be converted to mature transcripts and translated or targeted for degradation upon G3BP depletion.

562 B A co-evolution network of binding contacts between protein L25 and 5S rRNA

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RNA-protein interactions are central to all critical processes in the living cells. With the growth in the number of structures of RNA-protein complexes and in sequence data, a systematic analysis and study of RNA-protein interactions could help us understanding recognition mechanisms of RNA-protein binding in order to predict them. Here we chose to study the complex structure of ribosomal protein L25 and 5S rRNA for which detailed structural interactions are available. We analyzed binding and co-evolution patterns between them both on structure and sequence. Mutual Information (MI), a co-evolution analysis, determines residue pairs that show a statistically significant correlations, which result from both direct couplings and multitudinous couplings. Direct-coupling analysis (DCA), can discriminate the two kinds of couplings and give direct information (DI) between residue pairs. We used DCA to characterize co-evolution between L25 and 5S rRNA.

First, we found a highly connected co-evolution network constituted by the RNA binding residues in L25. We applied DCA to L25 and found the result correlates with the structural contact map. Besides, the top co-evolving residue pairs were plotted as a network and clustered. The largest cluster is not only more connected but also includes most of the RNA binding residues. This implies RNA binding may result in co-evolution between RNA binding residues.

Secondly, we found RNA binding residues are separate in the sequence but close in structure. A score is assigned by sequence distance |i-j| divided by the contact distance between residue i and j. It is higher for RNA binding residues than average. RNA-binding was found to cluster on L25 surface and being more conserved than others. A main reason for this observation could be that the local secondary structure features prevent the sequentially close residues from spatial proximity while they are required to cluster in structure to bind to a RNA motif. Thus, L25 needs to separate the RNA binding sites in sequence and gather them in space.

Then, we applied DCA in 5s rRNA, which is the first time it is applied to RNA co-evolution analysis. The results not only show that DI offers less noise than MI but also maps well with RNA structure. The co-evolving nucleotide pairs correlate to most of the base pairs. It validates us DCA could be effective for RNA system and demonstrates its potential in RNA structure prediction.

Finally, DCA was applied between L25 and 5s rRNA. 1424 protein and RNA sequence pairs for different species were collected, aligned and measured by DCA. The top co-evolving residue nucleotide pairs correspond to the key binding sites: five of the top six direct coupling pairs are proximal in structure and three of them map to key hydrogen bonds. The same strategy was extended to L5-5S rRNA and L18-5S rRNA binding, and results were similar. This reveals the great potential of DCA in RNA-protein interaction prediction.

563 C Mechanism of action of the CCCH zinc finger protein TbZC3H11 upon heat shock

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Most organisms induce transcription of heat-shock genes in response to temperature upshifts. However, Kinetoplastids, including important pathogens of human, animals and plants, control their gene expression almost entirely at the post-transcriptional level. Thus, amounts of heat-shock proteins after heat shock are regulated by mRNA stability and translation efficiency.

We showed previously that mRNAs encoding chaperones and co-chaperones are selectively stabilized by the *Trypanosoma brucei* CCCH zinc finger protein ZC3H11. Many target transcripts that interact with ZC3H11 contain a non-classical AU-rich element (UAU repeats) in the 3'-untransalted region. It has been shown that the ZC3H11 CCCH zinc finger domain is important for the interaction, with little discrimination between $(UAU)_n$ repeats and the classical AU-rich element which is bound by Tis-11 family proteins, $(UAUU)_n$ repeats.

Members of mammalian Tis11 family, however, contain two tandem CCCH zinc finger domains through which they bind to AU-rich elements, but in contrast, ZC3H11 has only one such domain. Hence, ZC3H11 should dimerize to gain reasonable RNA-binding specificity. Indeed, multimerization was apparent in gel-shift assays, and co-immunoprecipitation of V5- and myc-tagged versions of protein also confirmed dimerization.

It was shown that ZC3H11 expression is significantly induced upon heat shock. So, currently we are investigating the mechanism by which amounts of ZC3H11 and its activity are regulated. Like TTP and BRF-1, ZC3H11 is phosphorylated, but precise sites of the modification are as yet unknown. We do, however, have preliminary evidence that levels of ZC3H11 are – like those of TTP and BRF-1 – regulated through proteasomal degradation. Indeed, pulse-chase/immunoprecipitation analysis revealed that half-life of ZC3H11 protein increases upon heat-shock. In addition, polysome profiling showed that ZC3H11 mRNA is shifted to polysome fraction upon heat shock, what might explain elevated amounts of protein in response to temperature upshifts.

We propose that ZC3H11 operates via interactions with MKT1 and PBP1, which result in recruitment of PABP to the 3'-UTR and consequent RNA stabilization by protection from deadenylation.

564 A Sequence specific code in RRM–RNA interactions

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RNA–recognition motif (RRM) is a well known RNA interacting protein domain. RRMs are commonly present in all kingdoms of life and play a role at every stage of RNA life cycle: processing, splicing, editing, export, degradation and regulation of translation. Despite the large body of data available on sequence-specific RRM-RNA interactions, including numerous structures of RRM–RNA complexes, the "recognition code" is still not fully understood, and we cannot predict the RRM ligand specificity from its protein sequence alone. In this project we are using a multidisciplinary approach towards better understanding of RRM–RNA recognition. By combining bioinformatics (including sequence analyses, artificial intelligence methods, protein structure prediction, and protein–RNA docking) and biochemical and biophysical methods, we aim to provide the general understanding of the recognition code of RRMs in the evolutionary context. Thus far, comparative analyses of the RRMs and RRM–like sequences were used to build a database, where RRMs are classified into families based on sequence similarity. *In silico* analysis of RRM–RNA complexes, for which structure information is available, has been used to infer global trends in the formation of specific protein–RNA contacts. This is the starting point to assign substrate specificity and/or modulating affinity within the various RRM's families. Computational predictions of new RRM–RNA specificities are first tested *in vitro* and will be validated in human cell lines and submitted to structural studies. We have already started characterizing substrate specificity of novel RRMs with unknown mode of action using *in vitro* selection (SELEX). This approach revealed new interactions that are currently being further characterized. We aim to obtain information about specificity and build structural models of RRM-RNA structure for at least one member of each RRM family present in our database.

565 B Regulation of mRNA metabolism by U2AF65 splicing factor - novel mechanisms for the coordination of gene expression?

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In eukaryotes, gene expression is a highly controlled process that requires a coordinated regulation at several levels. Post-transcriptional processes can quickly impact cell function through the modulation of mRNA transport, stability and translation. RNA-binding proteins (RBPs) were shown to determine the fate of several mRNA targets, thus affecting multiple cellular processes.

We have previously demonstrated that U2AF65 and PTB, two mammalian splicing factors that recognize pyrimidine tracts, associate with a discrete subset of cellular mRNAs. The functional classification of these interaction profiles revealed underlying mRNA populations encoding proteins involved in common cellular functions. This strongly suggests that their expression is coordinated through overlapping post-transcriptional networks defined by U2AF65 and PTB. In fact, the mRNA population associated with U2AF65 shows a significant enrichment for molecules that encode proteins involved in RNA processing and cell cycle regulation.

Here we characterize the function of U2AF65 as a modulator of the mRNA metabolism by taking advantage of the lambda N-based tethering system. The artificial tethering of U2AF65 downstream of the coding sequence (CDS) resulted in downregulation of the luciferase reporter. Furthermore, we demonstrate that overexpression of U2AF65 has an inhibitory effect on the expression of a reporter gene fusing the luciferase CDS with the 3'UTR of U2AF65, without affecting the reporter mRNA levels. The 3'UTR of U2AF65 presents several predicted U2AF65 binding motifs that bind to recombinant U2AF65. In fact, we show that overexpression of exogenous U2AF65 results in decreased endogenous U2AF65 protein levels, suggesting the existence of a feedback regulatory mechanism. These observations point to U2AF65 as a negative regulator of gene mRNA metabolism, in contrast with its known role as an enhancer of splicing and 3'end processing.

566 C Division of labor: separation of loading and unwinding units in an oligomer of the DEAD-box helicase Ded1p

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DEAD-box RNA helicases perform ATP-dependent RNA and RNP remodeling reactions, including unwinding of RNA duplexes. In several DEAD-box proteins, RNA, nucleotide, and protein binding depend on the oligomeric state of the protein, but the impact of oligomerization on the function of DEAD-box helicases has not been examined. Here we have investigated oligomerization by the DEAD-box protein Ded1p from *Saccharomyces cerevisiae*.

During duplex unwinding, Ded1p forms an oligomer with at least three units. Two units of Ded1p associate with single stranded RNA proximal to duplex regions, and generate nearly all of the ATP hydrolysis observed during an unwinding reaction. Replacing the single stranded RNA with DNA, eliminates the majority of ATPase activity while having no significant effect on substrate binding or unwinding activity. Immobilization of two units of Ded1p on the single stranded RNA with a non-hydrolyzable ATP analog inhibits ATPase activity while promoting unwinding. Together these data suggest that two units of Ded1p serve as a loading platform to direct a third unit of Ded1p to the duplex for unwinding. We further show that the loading and unwinding units of the oligomer are functionally distinct in both ATP utilization and RNA binding, and we find that the C-terminal domain of Ded1p is critical for oligomerization. Moreover, a physiological interaction partner of Ded1p, eIF4G, interacts through the C-terminus of Ded1p and prevents oligomerization. Collectively, our findings reveal that oligomerization plays an important and underappreciated role in the biochemical function of Ded1p.

567 A Manipulating endogenous RNAs with synthetic RNA-binding proteins

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Post-transcriptional regulation of gene expression is ubiquitous and fundamental for the control of cell growth, differentiation and the complex developmental programs of multicellular eukaryotes. Because of their modular structure, repeat domain proteins are particularly well suited for these processes and have been widely adopted throughout evolution. We have expanded the RNA recognition code of Pumilio and FBF homology (PUF) proteins, enabling the design of RNA-binding proteins with programmable specificities. Furthermore, in recent work we have created synthetic proteins from another family of RNA-binding repeat domain proteins: the pentatricopeptide repeat (PPR) proteins. These artificial proteins have revealed the code for RNA binding by natural PPR domains and provide unique tools for manipulating endogenous RNAs. We show that designer RNA-binding proteins can be used to selectively manipulate the expression of endogenous mRNAs in human cells. The design of proteins that can bind any RNA sequence of interest and modulate its function will be important to elucidate the mechanisms by which genes are controlled at the RNA level and may provide potential therapeutics in the future.

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568 B Imbalance of Zfp36l2-RNA-binding protein results in female infertility

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ZFP36L2 is a CCCH tandem zinc finger protein that can destabilize certain AU-rich element-containing transcripts in cell transfection studies. ZFP36L2 has been implicated in the physiological control of female fertility. In the C57/BL6 mouse, a mutation in *Zfp36l2* that results in the decreased expression of a form of ZFP36L2 in which the 29 amino terminal amino acids had been deleted, revealed that *DeltaN-Zfp36l2* eggs could be fertilized but did not progress beyond the two-cell stage of development. Also when *DeltaN-Zfp36l2* females were subjected to superovulation protocols they released 50% fewer eggs than the WT. This suggested a possible additional defect in ovulation and oocyte maturation despite evidence of normal ovarian histology.

To further investigate we introduced the *DeltaN-Zfp36l2* mutation into the SV129 mouse strain, which has been reported to respond well to superovulation protocols. *DeltaN-Zfp36l2* mutation in this strain also resulted in complete female infertility. Unexpectedly, these females failed to release oocytes using superovulation protocols, prompting us to investigate the oocyte maturation. Remarkably, only 20% of *DeltaN-Zfp36l2* oocytes matured *ex vivo,* while 80% of the WT oocytes maturated spontaneously. This suggests that *DeltaN-Zfp36l2* either inhibits processes involved in the release from, or favor those involved in the maintenance of, meiotic arrest.

The cAMP/PKA pathway is critical for maintaining meiotic arrest. Treatment of *DeltaN-Zfp36l2* oocytes with PKA inhibitors doubled the percentage of oocytes able to overcome the *DeltaN-Zfp36l2*-linked meiotic arrest. The investigation of the mechanism involved in this arrest, led us to discover that LHR mRNA is the first specific and physiological RNA target for Zfp36l2. Decreased expression levels of Zfp36l2 in the ovaries resulted in sustained high levels of LHR mRNA during ovulation. Therefore, lack of the normal down regulation of LHR mRNA levels in the ovaries results in anovulation and arrest of the oocytes. This is the first time that a imbalance of a specific mRNA *in vivo* is directly associated with Zfp36l2 function. Thus, the Zfp36l2-RNA-binding protein can be the basis of some cases of unexplained female infertility in humans.

569 C Structure-specific RNase footprinting in multiple cell types reveals protein-binding sites throughout the human transcriptome

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RNAs are continuously associated with RNA-binding proteins (RBPs), and these interactions are necessary for their function and regulation. RBPs bind to target RNAs through sequence and/or structure-specific interactions. Increasingly, *in vivo* binding sites and sequence motifs for RBPs are being determined through the use of Crosslinking and Immunoprecipitation (CLIP) experiments, and several variant protocols. While these approaches have proven useful in determining the binding locations of individual RBPs, no current method allows global assessment of the extent and diversity of RNA-RBP interactions in both unprocessed and mature mRNAs. We developed a structure-specific RNase-mediated protein footprint sequencing approach to reveal the protein-protected sites (PPSs) of RNAs throughout the transcriptome, and applied it to three human cell types. The structure-specific nature of this assay allows for empirical determination of RNA base-pairing probabilities at protein-interaction sites. From this analysis, we uncover general principles of RNA-protein interactions, including an increased propensity for protein binding in the 3' UTR near the stop codon. Furthermore, we identify known and putative RNA-protein interaction sites and RBP-bound sequence motifs, and using RNA-affinity chromatography combined with mass spectrometry, we identified proteins that bind to known and novel sequence and structural motifs. Finally, we find that disease-associated single-nucleotide polymorphisms (SNPs) often affect RNA-protein interactions, revealing a likely molecular mechanism for numerous SNPs. Overall, we provide a global view of RNA-protein interaction sites and protein-bound RNA secondary structure in multiple cell types and provide a framework for the study of these features on a global scale.

570 A Architecture of catalytic complexes essential for synthesis and co-translational insertion of selenocysteine in humans

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Fundamental and highly conserved biological processes such as RNA transcription, posttranscriptional RNA modification, mRNA splicing, and protein synthesis are governed by complexes formed between proteins and nucleic acids. Upon formation of a particular binary, ternary or a higher order nucleoprotein complex, a protein or RNA catalyzes a reaction yielding a product critical for another biological process. Surprisingly, structural studies on these complexes revealed that the major principles of protein-nucleic acid recognition are often not universal and unified. Perhaps the least understood are the interactions stabilizing catalytic complexes required for synthesis, decoding and co-translational insertion of the 21st amino acid, selenocysteine. These processes, which are essential for the structure and function of selenoproteins and a number of vital cellular functions, can be divided into three general steps. In the first step, which is conserved in all organisms, seryl tRNA synthetase 'erroneously' attaches serine to selenocysteine tRNA (tRNA^{Sec}). Subsequently, a specific kinase (PSTK) and synthase (SepSecS) convert the seryl group into the selenocysteinyl moiety while being attached to tRNA^{Sec}. In the final step, a specialized elongation factor, EFsec, selects and delivers to the ribosome selenocysteinyl-tRNA^{Sec} where it facilitates decoding of the selenocysteine UGA codon. Although the process outline was drawn over a decade ago, it is still not understood how these enzymes recognize tRNA^{Sec} among other, more abundant elongator tRNAs. Herein, we present results from biophysical studies on various complexes from the human selenocysteine pathway. To determine the architecture of the individual complexes and to understand how tRNA^{Sec} is specifically recognized, X-ray crystallography, small angle X-ray scattering, multi angle light scattering, and analytical ultracentrifugation methods were employed. Our results provide the first comprehensive view onto the organization of the selenocysteine synthetic machinery in humans.

571 B hnRNP A1 Interacts with Conserved RNA IRES Elements in Enterovirus 71

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Enterovirus 71 (EV71) is an emerging threat to public health, there are currently no approved vaccines or treatment. A key step in the early EV71 life cycle is the translation of viral non-structural proteins that initiate viral replication. Due to the lack of m^7G cap in the viral 5' UTR, EV71 utilizes its own highly structured six stem loop type I IRES to co-opt host proteins. A key IRES trans-acting factor involved in IRES regulation is hnRNP A1. Prior research has shown that hnRNP A1 relocates from the nucleus to the cytoplasm and interacts with Stem Loop II (SLII) of the EV71 IRES, making it likely that SLII is critical in viral IRES dependent activity. Despite its importance in modulating viral IRES mediated translation and thus downstream replication events, little is known about the mechanism of how hnRNP A1 and SLII interact. A series of RNA biophysical studies, bioinformatics, and viral assays were utilized to characterize the two-step binding nature of this interaction. We show that host hnRNP A1 interacts with SLII in a novel manner involving two distinct conserved motifs: (1) a lower five nucleotide bulge containing a canonical UAG hnRNP A1 nucleotide binding motif and (2) an apical six nucleotide hairpin loop containing a CCA hnRNP A1 binding motif. *In vivo* studies further demonstrate that mutation of the UAG motif in the lower bulge abrogates IRES activity and viral replication in host cells. Since it is likely that the structure of SLII modulates this novel binding mechanism, a preliminary high resolution model of SLII was determined. Knowledge of this unique IRES:hnRNP A1 molecular mechanism can pave the way for novel anti-EV71 therapies and treatment.

572 C Human DIS3L2 exonuclease is involved in the processing of tRNA-derived small RNAs

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Processing of noncoding RNAs in eukaryotes involves the 3' to 5' exoribonucleolytic activity of type II ribonucleases that are part of the exosome complex. While in yeast it is known that the catalytic activity resides in Rrp44/Dis3 subunit, the functions of the three human Dis3 homologs have not been well characterized. In this work we aimed to uncover the physiological RNA targets of DIS3L2, the DIS3 homolog whose mutation is at the origin of the Perlman syndrome. We show that DIS3L2 is an exosome-independent cytoplasmic exoribonuclease, and through crosslinking and immunoprecipitation followed by RNA sequencing, we demonstrate that DIS3L2 is involved in the formation of tRNA-derived small RNAs (tRFs). A fraction of DIS3L2 along with its tRFs associates with ribosomes and DIS3L2 overexpression alters the polysome/monosome ratio, suggesting that DIS3L2 is involved in translational regulation. Most importantly, we found that the DIS3L2-dependent tRNA-derived fragments associate with Argonaute2 (AGO2) pointing to a crosstalk between the DIS3L2-dependent processing of tRNAs and AGO2-dependent regulation of gene expression the human cells.

573 A HIV Nucleocapsid Protein Precursors are Effective Nucleic Acid Chaperone Proteins

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During HIV-1 maturation, Gag is processed by the viral protease at five cleavage sites. Three different forms of nucleocapsid protein, NCp15 (NCp9+p6), NCp9 and NCp7 appear successively in this process. Previous work has shown that the virus containing NCp15 shows greatly reduced infectivity, while the virus with NCp9 is still infectious.[1] These data suggest that NCp15 lacks one or more necessary functions during viral infection. Mature NCp7 is a nucleic acid (NA) binding and chaperone protein, involved in destabilizing and remodeling NAs during reverse transcription. We hypothesize that HIV-1 NCp15 may be a poor chaperone, which may explain why the virus containing NCp15 is not infectious. To test this idea, the chaperone activity of NCp7, NCp9 and NCp15 was compared. Gel-shift annealing assays show that NCp15 anneals tRNA to the primer-binding site at a similar rate as NCp7, whereas NCp9 is the most robust chaperone protein. Sedimentation assays to measure NA aggregation show a similar trend, i.e., NCp9>NCp15~NCp7. Mutating all 8 of the C-terminal acidic residues of NCp15 to Ala improves the annealing and aggregation activity of NCp15 to the level of NCp9. NMR chemical shift perturbation results suggest that in solution, the p6 domain of HIV-1 NCp15 folds back and interacts with the zinc fingers of NCp7 at residues F16, A25, K33 and K38. Our results suggest that this interaction slightly reduces chaperone function.

Taken together, our data show that HIV-1 NC precursors are effective chaperone proteins and differences in chaperone activity do not explain the requirement for NCp15 processing. We also determined that mutations in NCp15, designed to eliminate the interaction of the p6 domain with TSG101 or Vpr, failed to rescue viral infectivity. A recent study showed that HIV-1 virions containing NCp15 have irregular core structures,[2] which may affect reverse transcription and viral replication. It has also been reported that NCp15-NA complexes have altered morphology compared to NCp7-NA complexes,[3] which may explain the irregular cores and the requirement for NCp15 processing. This work has been funded by NCI, contract no.: HHSN261200800001E with SAIC-Frederick, Inc.

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574 B Tethered Domains and Flexible Regions in tRNase ZL, the Long Form of tRNase Z

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tRNase Z, a member of the metallo-β-lactamase family, endonucleolytically removes the pre-tRNA 3' trailer in a step central to tRNA maturation. The short form (tRNase Z^S) is the only one found in bacteria and archaebacteria and is also present in some eukaryotes. The homologous long form (tRNase Z^L), exclusively found in eukaryotes, consists of related amino- and carboxy-domains, suggesting that tRNase Z^L arose from a tandem duplication of tRNase Z^S followed by interdependent divergence of the domains. X-ray crystallographic structures of tRNase Z^S reveal a flexible arm (FA) extruded from the body of tRNase Z remote from the active site that binds tRNA far from the scissile bond. No tRNase Z^L structures have been solved; alternative biophysical studies are therefore needed to illuminate its functional characteristics. Structural analyses of tRNase Z^L performed by limited proteolysis, two dimensional gel electrophoresis and mass spectrometry establish stability of the amino and carboxy domains and flexibility of the FA and inter-domain tether, with implications for tRNase Z^L function.

575 C Poly(A) binding protein 1 and formation of processing bodies in human

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Poly(A) binding protein 1 (PABPC1) is an essential translation initiation factor. PABPC1 circularizes mRNA together with mRNA cap binding proteins, bridged by eIF4G. While PABPC1 is found at stress granules and absent from processing bodies in human, we find a mechanism how PABPC1 contributes to formation of processing bodies. This helps us understand the dynamics of RNA-protein granules.

576 A Crystal structure of the active conformation of the Shigella flexneri VapC toxin at 1.9 Å resolution

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Toxin-antitoxin (TA) loci have been found in all prokaryotic genomes that have been sequenced to date and typically encode two proteins, a toxin that inhibits cell growth and an antitoxin that upon binding its corresponding toxin, neutralizes its action [1]. The VapC toxins contain an N-terminal ribonuclease motif belonging to the PilT N-terminus (PIN) domain type, harbouring four highly conserved acidic residues known to be essential for metal ion coordination. The toxin VapC (MvpT) from the Gram-negative pathogen *Shigella flexneri* is capable of globally down-regulating translation by specifically cleaving initiator tRNAfMet in the anticodon region following release from a tight complex formed with the antitoxin, VapB [2]. We have previously determined the crystal structure of the VapC-VapB toxin-antitoxin complex from *Shigella flexneri* [3] and a structure of the isolated form of an archaeal VapC is also available [4].

Here, we present the first structure of the active form of VapC from a pathogenic bacterium. Recombinant VapC from *Shigella flexneri* harbouring an active site mutation was overexpressed in Escherichia coli, purified to homogeneity, crystallized and the structure was determined at 1.9 Å resolution by x-ray crystallography. Comparison with the antitoxin-inhibited structure from the VapC-VapB complex [3] reveals that VapC most likely is active as a dimer in which movements of two α-helices surrounding the active site create a more compact active site conformation upon release of the antitoxin.

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577 B Alanine scanning of the aa-tRNA binding interface of Escherishia coli EF-Tu

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The extensive interface formed between EF-Tu and aa-tRNA includes more than 20 amino acid residues that are highly conserved among bacteria. In order to evaluate the individual contributions of these residues to the thermodynamics of ternary complex formation and their function on the ribosome, 22 point mutations of *E. coli* EF-Tu were prepared that usually changed the native amino acid side chain to alanine. After expression and purification, each mutant protein was activated with GTP, bound to aa-tRNA, and the dissociation rate of the complex (k_{off}) determined by a ribonuclease protection assay (1). While nearly all the mutant proteins were able to form a ternary complex, some of the EF-Tu residues showed aa-tRNA dependent thermodynamic contributions and many of the residues showed decreased binding affinities. The R377A EF-Tu mutant, in which arginine side chain influences secondary structure elements around the tRNA interface, has a faster k_{off} for every aa-tRNA tested. In addition, R223A mutation increases tRNA affinity for aa-tRNAs, presumably by slowing down the conformational changes that lead to dissociation of the ternary complex. Although the effect on binding affinities were quite variable among the different mutations, it is striking that the data closely resembles a similar set of data determined for the ternary complex of *Thermus thermophilus* EF-Tu and Phe-tRNA^{Phe} (2). This indicates that the protein-tRNA interfaces from the two bacteria are not only similar in sequence, but are similar thermodynamically.

We also studied the performance of these mutant proteins in decoding using ribosomes purified from *E. coli* (3). Except for R223A, most of the mutant proteins are active GTPases. It is possible that removal of R223 side chain alters the ribosome binding affinity of the EF-Tu. The ability of each mutant protein to release from aa-tRNA on the ribosome after the GTP hydrolysis step was evaluated using the T1 mutant of Tyr-tRNA^{Tyr}. This tRNA^{Tyr} variant has an altered T-stem that hyperstabilizes binding to native *E. coli* EF-Tu sufficiently that the rate of peptide bond formation (k_{pep}) is extremely slow (4-5). Most of the EF-Tu residues that interact with the 3'-ACCA end and T-stem residues of T1 Tyr-tRNA^{Tyr} are able to stimulate k_{pep} with T1 Tyr-tRNA^{Tyr} compared to wild type EF-Tu. Thus, by weakening binding to aa-tRNA, the EF-Tu mutations are able to overcome the rate limiting block in release of the hyperstabilized aa-tRNA from the protein on the ribosome. This suggests that the energetics of the interface between EF-Tu and aa-tRNA on the ribosome is similar to that of the free ternary complex.

578 C Evidence of direct complementary binding between mRNAs and cognate proteins

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The ability to interact with mRNA has recently been reported for many known RNA binding proteins, but surprisingly also for different proteins without recognizable RNA binding domains including several transcription factors and metabolic enzymes. In particular, direct binding to cognate mRNAs has been detected for various proteins creating a strong impetus to search for functional significance and basic physico-chemical principles behind such interactions. Here, we bioinformatically compare pyrimidine content of natural mRNA coding sequences with the propensity of cognate protein sequences to interact with pyrimidines (1). The latter is captured by polar requirement, an experimental measure of amino-acid solubility in aqueous solutions of pyrimidine mimetics pyridines. By analyzing proteomes of 15 different species, we find that pyrimidine density profiles of individual mRNAs remarkably mirror polar-requirement profiles of cognate protein sequences. For example, 4953 human proteins exhibit a correlation between the two with a Pearson coefficient $|R|>0.8$ (illustrated in Figure 1 for hemoglobin alpha-subunit, ATF3 and p53). In other words, pyrimidine-rich regions in mRNAs quantitatively correspond to regions in cognate proteins containing amino acids that prefer to interact with pyrimidine mimetics and vice versa. Moreover, by studying randomized genetic codes, we show that the natural genetic code is highly optimized to preserve these correlations. Finally, we derive interaction preferences between amino acids and RNA bases by analyzing known 3D-structures of protein-RNA complexes. Using this tool we both confirm the above findings for pyrimidines and also demonstrate an analogous behavior for purines with some exceptions. Overall, our results redefine the stereo-chemical hypothesis concerning the origin of the genetic code and provide evidence of direct templating of proteins from mRNAs before the development of ribosomal decoding. Moreover, our findings support the possibility of direct complementary interactions between mRNAs and cognate proteins even in present-day cells, especially if both are unstructured, with potential implications extending to all facets of nucleic acid/protein biology.

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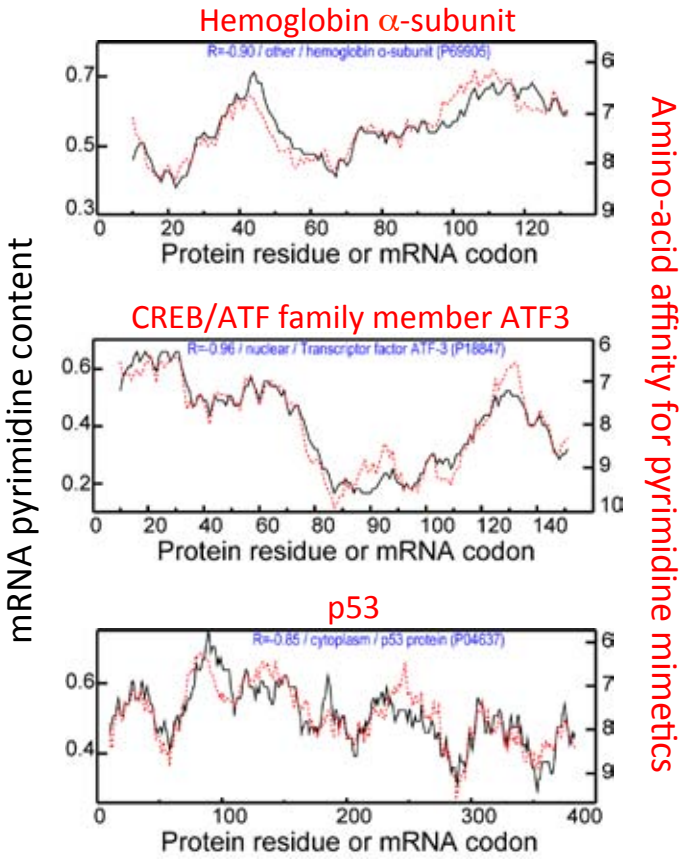

Figure 1

Examples of matching between mRNA coding-sequence pyrimidine-density profiles and cognate proteins' affinity profiles for pyrimidine mimetics. All profiles have been smoothed with a window of 21 codons/amino acids. Amino-acid affinity is defined in thermodynamic sense whereby low values indicate high affinity and vice versa.

579 A Responses of the mRNA interactome to genotoxic stress

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RNA-binding proteins (RBP) play critical roles in stress responses like DNA damage through interactions with elements in functionally-related RNAs, called regulons. One of the most cytotoxic forms of DNA damage are DNA double strand breaks (DSB), which can cause mutagenic events or even cell death. This project focuses on the plasticity of the global RBP network (mRNA interactome) and RNA-regulons of mouse fibroblasts (NIH-3T3 cells) in response to genotoxic stress. Using interactome capture [1; 2] and high-resolution proteomics, we will define the RBP´s that respond during genotoxic stress. By further employing deep sequencing and crosslink-IP approaches, we will identify genotoxic stress RBP targets and assay their influence on cell survival. Taken together, this project aims to address key questions of the DNA damage response and the implication of RNA-regulons.

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580 B A genome-wide RNAi screen identifies novel 40S ribosome synthesis factors in human cells

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Ribosome biogenesis is a complex, highly compartmentalized process that is assisted by more than 150 non-ribosomal proteins, known as trans-acting factors. Our current knowledge on ribosome biogenesis mostly derives from data in yeast, however little is known about this process in mammalian cells.

To shed light on the cellular repertoire of factors involved in ribosome synthesis in mammals, we have performed a genome-wide siRNA screen on 40S subunit biogenesis in HeLa cells using a visual assay relying on RPS2-YFP localization as read-out. Our approach allowed us to compile a list of approximately 400 factors that are required for biogenesis of the 40S subunit. Among the identified proteins are several expected factors, such as the ribosomal proteins themselves, human homologues of yeast ribosome biogenesis factors, as well as splicing factors and nucleoporins. In addition, we identify components of the ubiquitin-proteasome system, of various signaling pathways, metabolic enzymes and uncharacterized proteins.

Of our follow-up studies, we will present data on two different proteins that had not been previously linked to the maturation of ribosomes. One is an uncharacterized protein that we named CRBF1, for which we show that it localizes to nucleoli. Depletion of CRBF1 leads to defects in rRNA processing as demonstrated by FISH and Northern blotting experiments. Proteomic analysis identified CRBF1 as part of a pre-ribosomal particle, indicating a direct function for CRBF1 in ribosome synthesis. The second factor is an enzyme involved in amino acid metabolism that plays a central role in cellular energy homeostasis. Its requirement for ribosome synthesis might potentially explain some of the specific metabolic needs of cancer cell growth.

581 C Characterization and in vivo functional analysis of the Schizosaccharomyces pombe ICln gene

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Numerous studies indicate that assembly of the eukaryotic spliceosomal snRNPs is a multistep process following an ordered pathway. The methylosome and the SMN (Survival of Motor Neuron) complexes are essential players in early steps of this pathway. The methylosome, composed by pICln, WD45 and PRMT5 proteins, recruits Sm proteins via the pICln subunit and symmetrically dimethylates arginines within the C-tails of Sm proteins. The SMN complex further facilitates the loading of Sm proteins onto the snRNAs resulting in the formation of snRNP particles. In these early steps, pICln is an important regulator of snRNP assembly since it acts as an assembly chaperone while the SMN complex acts as a catalyst.

To expand our understanding of pICln and SMN functional relationships in vivo, we performed a genetic analysis of an uncharacterized S. pombe pICln homologue. Although not essential, the S. pombe ICln protein is important for optimal yeast cell growth. The human pICln gene complements the iclnΔ slow growth phenotype demonstrating that the identified SpICln sequence represents the bona fide human homolog. Consistent with the role inferred for human pICln using in vitro experiments, we found that the SpICln protein is required for optimal production of the spliceosomal snRNPs and for efficient splicing in vivo. Genetic interaction approaches demonstrate furthermore that modulation of ICln activity is unable to compensate for defects induced by SMN mutations, and reciprocally. Using a genome-wide approach and RT-PCR validation tests, we show also that splicing is altered differentially in iclnΔ cells. Our data are consistent with the emerging view that splice site selection and spliceosome kinetics are highly dependent on the concentration of core spliceosomal components.

582 A Dissecting RNA-binding protein complexes

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The cytoplasmic control of mRNAs is essentially facilitated via specific mRNPs expected to comprise transcript specific and dynamic subsets of RNA-binding proteins (RBPs). Some of these associate directly with the RNA via specific cis-determinants whereas others are expected to associate primarily via protein-association and form only transient and potentially unspecific contacts with targeted mRNAs.

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The RNA-binding protein IGF2BP1 (IGF2 mRNA binding protein) was demonstrated to stabilize the c-myc (MYC) mRNA, presumably by associating with a cis-determinant in the coding region of the MYC transcript , the so called ‘Coding Region instability Determinant (CRD)’. Moreover, the protein which is also termed ZBP1 (Zipcode Binding Protein 1) regulates the spatiotemporal control of b-actin mRNA (ACTB) translation and directs subcellular sorting of this transcript at least in primary cells. All these regulatory processes are presumed to be facilitated by a number of distinct mRNPs of unknown composition. To reveal how IGF2BP1-associated RBPs modulate the fate of ACTB and MYC mRNAs in tumor-derived cells we established an MS2-tethering approach using in vitro transcribed RNAs. This allows us to investigate which RBPs associate directly or indirectly with the analysed transcripts. If and how identified RBPs modulate the fate of MYC and/or ACTB transcripts is currently analysed by loss-of-function analyses (LOF) using shRNA-mediated knockdown.

583 B Reconstitution of the Uridine-rich snRNP Assembly Machinery

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Splicing U snRNPs are the major *trans*-acting factors of the pre-mRNA processing spliceosome. These factors contain a common RNP core composed of seven Sm proteins bound to an snRNA whose assembly is mediated by PRMT5 and SMN complexes. Assembly of snRNPs from RNA and protein is an essential pre-requisite for spliceosome formation. *In vivo*, this is facilitated in a highly complex biogenesis pathway. Initially, the snRNA to be assembled into an snRNP is exported from its site of transcription to the cytoplasm. Here, the assembly of the Sm proteins onto U snRNAs takes place. The snRNP is eventually transported to the nucleus and incorporated into the spliceosome. Several groups including ours have identified a unique machinery that assists the assembly of spliceosomal U snRNPs. This machinery consists of two cooperating units termed SMN-complex and PRMT5-complex, respectively. While the SMN-complex loads Sm proteins onto the snRNA and hence acts as an RNP-assembler, the PRMT5 complex functions upstream in this pathway. It acts as an assembly chaperone by forcing Sm proteins into a higher order structure (termed 6S complex) required for the subsequent transfer onto the SMN-complex. Here we present the total reconstitution of the U snRNP assembly machinery from recombinant sources, which allowed us mechanistic insight into its mode of action. The 6S snRNP assembly intermediate forms in a stepwise manner on the PRMT5 complex and is, upon completion, displaced by pICln or pICln-Sm complexes, thus allowing a new round of 6S formation. The Sm proteins pre-arranged in the 6S complex are then transferred en bloc onto the SMN complex. The SMN complex functions as a scaffold that allows a kinetic proofreading and the association of Sm proteins only with cognate RNA targets. Reconstituted SMN complex containing the SMN(E134K) missense mutation, which is linked to spinal muscular atrophy (SMA), interferes with the RNP-chaperone system and prevents productive U snRNP assembly. Our data reveal an elaborate interplay of U snRNP assembly factors and provide new insight into the cellular defects in SMA.

584 C Ribosomal protein clusters orchestrate the hierarchical construction of eukaryotic large ribosomal subunit structural domains in vivo

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Ribosomes, the protein factories of the cell, are fundamental players that link genotypes with phenotypes. These nanomachines are made up of two subunits, each characterized by ribosomal proteins interspersed over a complex lattice of the ribosomal RNA (rRNA) core. Biogenesis of ribosomes in eukaryotes begins in the nucleolus with the transcription of precursor ribosomal RNA (pre-rRNA). This primary transcript is then modified, processed, folded, and assembled with ribosomal proteins, as nascent subunits traffic to the cytoplasm. In yeast, these events require more than 200 *trans*-acting biogenesis factors, which are thought to facilitate alternating cascades of rRNA folding and protein binding.

Assembly of small subunits is better understood relative to large subunits, both *in vitro* in bacteria and *in vivo* in eukaryotes. Construction of small ribosomal subunits proceeds via the formation of two stable intermediates: the body domain is first formed, followed by the creation of the head domain. In eukaryotes, assembly of these structural domains is also correlated with early and late pre-rRNA processing steps, respectively. In contrast, description of assembly intermediates for eukaryotic 60S subunits has largely been limited to cataloguing their protein composition, and understanding very late cytoplasmic steps in subunit maturation. Information on the timing of construction of assembly neighborhoods in pre-60S particles, and how this is coupled to protein binding and pre-rRNA maturation remain fragmentary. In this study, we began to fill this gap by systematically analyzing the roles of r-proteins in 60S subunit assembly *in vivo*.

We show that assembly of eukaryotic large ribosomal subunits proceeds via the formation of three sequential pre-ribosomal intermediates that correspond to the proper folding and construction of specific neighborhoods in the large subunit. The solvent-exposed interface of the large subunit is first stably assembled, followed by the domain surrounding the rim of the polypeptide exit tunnel, then finally, the central protuberance and the subunit interface bearing the catalytic center. We also discuss how formation of these structural neighborhoods is coupled with catalysis of pre-rRNA processing steps and nucleocytoplasmic export, by stable association of critical *trans*-acting biogenesis factors required for these events. This significantly contributes to our understanding of how the large ribosomal subunit and similarly complex ribonucleoproteins are put together in living cells.

585 A 5′-cytosine methylation of C2278 in 25S rRNA stabilizes 60S ribosomal subunit in yeast

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During ribosome biogenesis the eukaryotic ribosomal RNAs are extensively modified at more than 100 sites by methylation and pseudouridylation. The direct role of most of these modifications for either the biogenesis, stability or activity of the ribosome remains unclear. Among these modification is a highly conserved 5'-methylation of the cytosine 2278, located at the base of the helix 71 in the domain IV of the yeast 25S rRNA. The equivalent site is methylated in both eukaryotes and prokaryotes, however, its function is unknown. We have identified Rcm1p as the long elusive RNA methyltransferase of C2278 in yeast. Deletion of *RCM1* gene and/or snR75, snoRNA guiding 2'-O-ribose methylation of an adjacent G2288, leads to destabilization of 60S ribosomal subunit. SILAC analyses confirm partial loss of a group of ribosomal proteins located in the vicinity of the modified residues. SHAPE analyses confirm changes in the 25S rRNA conformation of the helix 71 and the surrounding. Our results show that the two studied modifications are important for stability of the rRNA structure and interaction with ribosomal proteins.

586 B Identification of a chloroplast ribonucleoprotein complex containing trans-splicing factors, intron RNAs and novel components

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Chlamydomonas reinhardtii is widely used for analysis of nucleus-encoded factors that are thought to promote the maturation of chloroplast precursor RNAs. To elucidate the function and composition of ribonucleoprotein complexes that are presumably part of a transcript specific chloroplast spliceosome, we are studying the expression of the chloroplast encoded *psaA* gene [1]. The *psaA* gene is separated into three exons, which are widely distributed over the plastom and flanked by consensus sequences typical for group II introns. The exons are transcribed individually and the major transcript is then assembled in *trans*. Here, we present a novel *trans*-splicing mutant, which is affected in splicing of the first *psaA* intron. Genomic complementation led to the identification of the mutant gene encoding Raa4, a protein of 112.4 kDa, which shares no strong sequence identity with other known proteins [2]. The chloroplast localization of Raa4 was confirmed by confocal fluorescence microscopy, using a GFP-tagged fusion protein. RNA binding-studies showed that Raa4 binds specifically to domains D2 and D3, but not to other conserved domains of the tripartite group II intron. In addition, we used a combined experimental approach including yeast-two hybrid screening, tandem affinity purification (TAP) and mass spectrometry to identify putative interaction partners of Raa4.

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587 C The structural organization of the box C/D sRNP

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The post-transcriptional modifications of ribonucleotides occur during the biosynthesis of the RNA in functionally important regions and are essential for the structure, folding, stability and functions of the host RNAs [1].

Our research is focused on the most common RNA modification, which is ribose 2'-OH methylation. RNA methylation in eukaryots and archaea is mediated by box C/D s(no)RNP complex. The archaeal sRNP complex is asymmetric and constituted by three core proteins assembled around small non-coding guide RNA. The gRNA contains two similar conserved motifs: box C/D and box C'/D'. The C box (RUGAUAG, being R purine) is close to the 5' end and the D box (CUGA) is near the 3' end. The related boxes C' and D' are in the internal region. The gRNA in sRNP forms base pairs with complementary target RNAs and selects the 5th nucleotide upstream from box D for methylation [2].

The complex assembly is initiated by L7Ae interaction with the K-turn and K-loop motifs of the box C/D guide RNA. Nop5 binds to the preformed L7Ae-RNA core subcomplex and facilitates binding of the Fibrillarin, which is a SAM-dependent methyltransferase [3].

Recently, the box C/D sRNP has been crystallized using a symmetric guide sRNA consisting of two separate strands, base-paired with the corresponding targets [4]. The structure suggests that the catalytically active complex is constituted by two copies of each protein assembled around one molecule of gRNA (~200 kDa) and is consistent with the classical mono-RNP model.

Another study demonstrates that the full box C/D sRNP complex is constituted by four copies of each protein and two copies of gRNA (di-RNP, ~ 400 kDa). A cross-methylation mechanism, during which the movement of Fibrillarin to the substrate-guide double strand facilitates release of one copy of L7Ae, was proposed [5].

Therefore, the assembly architecture and the functional regulation of this important enzyme remain to date contradictory.

Here we embark in the structural study of the box C/D sRNP complex from *Pyrococcus furiosus* in solution with a combination of NMR, small angle nuclear scattering (SANS) and restrained molecular modeling. Our study reveals a surprising mode of assembly of the complex in solution, which supports the di-RNP hypothesis, while showing a substantially different mode of RNA and proteins assembly with respect to that previously published [5]. Our structure explains the selectivity of the methylation and turn-over and furthermore provides a structural understanding for the asymmetry of the complex in all organisms.

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588 A Budding yeast telomerase RNA: Zooming in for more definition of a large RNA.

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Budding yeast telomerase RNA: Zooming in for more definition of a large RNA.

The RNA components of telomerases appear to be subject to extremely rapid evolution. For example, RNA transcription and maturation pathways vary widely and the RNA cannot easily be grouped with others. We showed that the budding yeast telomerase RNA, called Tlc1, can be classified as a snRNA. Not only is the 5'-TMG cap and a passage through the nucleolus fitting that model, we determined that the 3'-termination pathway is dictated by the classical non-coding RNA termination factors Nrd1 and Nab3 at sites near the mature polyA- 3'-end of the RNA. In order to understand its biology better, we also began investigating the transcriptional control of the Tlc1 RNA. Guided by phylogenetic comparisons, we discovered canonical SCB boxes in the promoter and a series of heterologous gene expression experiments demonstrate that these short sequence elements can be functional for directing cell cycle controlled transcription. Targeted mutagenesis of the SCB has functional consequences such as reduced levels of the RNA and short telomeres. Finally, using a tagged version of Tlc1 as target, RNA mediated ChIP experiments show a clear induction of transcription at the end of G1 of the cell cycle. We therefore hypothesize that the telomerase RNA, just as the RNA encoding Est1p, is made specifically at the entry of S-phase, together with a number of S-phase specific genes.

I will also present our investigations on a sub-structure in Tlc1 that has as of yet unknown functions. This approach, again based on phylogenetic comparisons, uncovered a new conserved substructure that we call CS2a on the stem-loop (SL) IVc. It is near an area shown to be involved in the binding of Est1p and therefore could be intimately associated with telomerase-telomere interactions. Indeed, targeting this new CS2a element by mutagenesis yielded evidence for loss of telomerase function *in vivo*. This effect can be explained by a loss of Est1-binding and therefore is Est1p-dependent. Surprisingly however, specific mutations that should only affect the predicted distal structure of SL IVc, but not Est1p-association, strongly affected catalytic telomerase activity. Using strains that carry a deletion of the *EST1* gene, we were able to conclude that this new function of the distal area in SL IVc we call TeSS, for Telomerase Stimulating Structure, indeed is Est1p-independent. I will present our description of the molecular functions of TeSS and hypothesize that this substructure may be a new commonality between all telomerase RNAs.

589 B Characterisation of the human UTP-B complex and its role in ribosome biogenesis

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Ribosome biogenesis in eukaryotes requires a multitude of cofactors, many of which are recruited as pre-assembled modules. This has been analysed best for the assembly of early 90S pre-ribosomal intermediates in yeast, where several subcomplexes and RNPs assemble co-transcriptionally on the pre-rRNA. Among them is the UTP-B complex, which in yeast consists of six proteins, Pwp2, Dip2/Utp12, Utp6, Utp13, Utp18, and Utp21.

We are studying the roles of RNA helicases and other cofactors in ribosome biogenesis. Using affinity purification and mass spectrometry to analyse the composition of the human UTP-B complex, we identified homologues of yeast UTP-B proteins as well as candidates for human-specific components of the complex. Among them is the DEAD-box RNA helicase DDX21, for which we have identified putative binding sites on pre-rRNA using UV Crosslinking and Analysis of cDNA (CRAC) and deep sequencing. Interestingly, the potential interaction sites of DDX21 map to rRNA sequences of both the small and the large ribosomal subunit and some of the crosslinking sites cluster in the 3D structure of the ribosome. Preliminary results suggest a role of DDX21 in the regulation of snoRNP binding to pre-ribosomal complexes and it might have other roles in ribosome synthesis.

Taken together, we show that the human UTP-B complex has acquired additional components compared to its yeast counterpart, enabling it to perform more extensive functions in ribosome biogenesis.

590 C Messenger Ribonucleoprotein Assembly Requires the DEAD-box Protein Dbp2 and Enzymatic Modulation by Yra1

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Eukaryotic gene expression involves numerous biochemical steps that are dependent on RNA structure and ribonucleoprotein (RNP) complex formation. DEAD-box RNA helicases are one class of enzymes that play fundamental roles in RNA and RNP structure in all aspects of RNA metabolism. However, the precise biological roles for the vast majority of these enzymes are not fully understood.

In an effort to define the biological roles of DEAD-box proteins, our laboratory recently provided evidence that the DEAD-box protein Dbp2 functions in nuclear gene expression steps in *Saccharomyces cerevisiae*. Moreover, our studies showed that Dbp2 is an enzymatically active ATPase *in vitro* that is required for both repression of aberrant initiation and proper transcription termination. Furthermore, we found that Dbp2 associates with transcriptionally active chromatin, suggesting that this enzyme functions in co-transcriptional mRNP assembly. We now provide evidence that *DBP2* interacts genetically and physically with the mRNA export factor Yra1. In addition, we find that Dbp2 is required for *in vivo* assembly of mRNA-binding proteins Yra1, Nab2 and Mex67 onto poly(A)+ RNA. Strikingly, to uncover the biochemical mechanism, we find that Dbp2 displays strong annealing activity at levels higher than any other published DEAD-box proteins to date. Yra1 controls this activity by inhibiting duplex unwinding without decreasing ATP hydrolysis activity. Here, we will present evidence that Dbp2 functions as an mRNA chaperone, constituting a previously unrecognized mechanism for co-transcriptional assembly of mRNPs in the nucleus.

592 B Structural basis of Brr2-Prp8 interaction and its implications for Retinitis Pigmentosa disease type 13 and U5 snRNP biogenesis

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U5 small nuclear ribonucleoprotein particle (U5 snRNP) is one of the five canonical subunits of the spliceosome and three of its protein components, Brr2, Prp8 and Snu114, play a crucial role in the formation of the spliceosome's active site. Brr2 is a Ski2-like helicase (246 KDa in yeast), which disrupts the extensively basepaired U4/U6 snRNA duplex and allows U6 snRNA to engage in an intricate RNA interaction network that forms the active centre of the spliceosome. Here we present the structure of yeast Brr2 in complex with the Jab1/MPN domain of Prp8, which has been shown to stimulate Brr2 helicase activity [1]. The residues in the Jab1/MPN domain, whose mutations in human cause the degenerative eye disease Retinitis Pigmentosa type 13 (RP13), are found at or near the interface with Brr2, providing crucial insights into the molecular pathology of RP13. In the cytoplasm Prp8 forms a precursor complex with U5 snRNA, seven Sm proteins, Snu114 and Aar2 but after nuclear import Brr2 replaces Aar2 to form mature U5 snRNP [2]. Together with the recent structure of $Prp8^{885-2413}$-Aar2 complex [3], our structure provides important insight into U5 snRNP biogenesis.

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593 C Surveillance of spliceosomal snRNP assembly in the cell nucleus

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Assembly of spliceosomal snRNPs and their incorporation into the spliceosome has been well described. Here, we provide evidence about a surveillance pathway that detects snRNP assembly intermediates and sequesters them in a nuclear structure called the Cajal body. We inhibited different stages of tri-snRNP assembly and in all cases observed retention of immature snRNP complexes in Cajal bodies. Next, we show that the protein SART3 is essential for targeting and retention of non-assembled snRNPs in Cajal bodies. We provide evidence that SART3 associates with U4 and U6 snRNPs as well as with coilin, a building protein of Cajal bodies. SART3 thus represents the factor that interacts with non-assembled snRNPs and anchors them in Cajal bodies. In addition, depletion of SART3 sensitizes cells to inhibition of snRNP formation, which provides a functional significance for surveillance pathway. Finally we show that Cajal bodies are inducible structures and inhibition of snRNP assembly stimulates formation of Cajal bodies in primary fibroblasts that normally lack Cajal bodies. Surprisingly, induced Cajal bodies accumulate only those snRNPs whose assembly pathway was perturbed, which provides the first evidence that individual metabolic pathways that concentrate in Cajal bodies are independent.

594 A Implication of the SMN complex in the biogenesis and steady state level of the Signal Recognition Particle

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Spinal muscular atrophy is a severe motor neuron disease caused by reduced levels of the ubiquitous SMN protein. SMN is part of a complex that is essential for spliceosomal UsnRNP biogenesis. Signal Recognition Particle (SRP) is a ribonucleoprotein particle crucial for cotranslational targeting of secretory and membrane proteins to the endoplasmic reticulum. SRP biogenesis is a nucleo-cytoplasmic multistep process in which the protein components, except SRP54, assemble with 7S RNA in the nucleolus. Then, SRP54 is incorporated after export of the pre-particle into the cytoplasm. The assembly factors necessary for SRP biogenesis remain to be identified. Here, we show that 7S RNA binds to purified SMN complexes *in vitro* and that SMN complexes associate with SRP in cellular extracts, and we identified the RNA determinants required. Moreover, we report a specific reduction of 7S RNA levels in the spinal cord of SMN-deficient mice, and in a *S. pombe* strain carrying a temperature-degron allele of SMN. Additionally, micro-injected antibodies directed against SMN or Gemin2 interfere with the association of SRP54 with 7S RNA in X. *laevis* oocytes. Our data show that reduced levels of the SMN protein lead to defect in SRP steady-state level and describe the SMN complex as the first identified cellular factor required for SRP biogenesis.

595 B The rRNA methyltransferase Bud23 interacts with the DEAH-box RNA helicase Ecm16 to promote cleavage at A2

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The small ribosomal subunit assembles co-transcriptionally on the nascent primary transcript. Early processing events at A0, A1 and A2 require U3 snoRNA in the context of the SSU Processome. Cleavage at site A2 releases the pre-40S particle from the primary transcript. We previously identified Bud23 as a conserved eukaryotic methyltransferase responsible for the base modification G1575 in yeast (G1338 in bacteria) and more recently we have found that Bud23 is required for efficient cleavage at A2. Here, we report that Bud23 interacts with the DEAH-box RNA helicase Ecm16 (Dhr1). Ecm16 has previously been implicated in cleavages at A2 and, to a lesser degree, at A1. RNA helicases often require protein cofactors to provide substrate specificity and these interactions are typically through the N- or C-terminal domains of helicases that extend beyond their catalytic cores. We used yeast 2-hybrid analysis to map the binding site of Bud23 to the N-terminal extension of Ecm16. To characterize the particle that Ecm16 acts on, we used a catalytically inactive Ecm16 mutant. Whereas wild-type Ecm16 does not stably associate with preribosomal particles, this mutant does and sediments at approximately 40S. Immunoprecipitation of this mutant reveals a striking accumulation of 21S rRNA and contains U3 snoRNA, Mpp10 and Bud23 among others. 21S rRNA has undergone cleavage at A1 and A3 but not A2. As U3snoRNA is required for cleavages at A0, A1 and A2 these results show that the cleavage pathway has been arrested before A2 cleavage. The accumulation of Mpp10 and Bud23 in the arrested Ecm16 particle is reflected in the altered sedimentation of these proteins, now cosedimenting with Ecm16 at ~40S. These results imply that Bud23 enters the preribosomal particle while U3 is present and shortly before cleavage at A2. Although Bud23 binding is important for 40S biogenesis, its methyltransferase activity is not. We propose that Bud23 recognition of its binding site signals completion of folding of the major domains of the small subunit, coupling Ecm16 activity to assembly status of the subunit.

596 C The 5S RNP couples ribosome production to p53 regulation

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Ribosomopathies, including Diamond Blackfan anemia and Treacher Collins syndrome, are genetic diseases caused by defects in ribosome biogenesis. In several of these ribosomopathies, activation of the tumour suppressor p53 in response to impaired ribosome production plays an important pathogenic role. Paradoxically, many ribosomopathies are also associated with cancer predisposition. It is proposed that when ribosome biogenesis is blocked, two ribosomal proteins, RPL5 and RPL11, function to activate p53. RPL5 and RPL11, together with the 5S rRNA, form the 5S RNP, an essential sub-complex of the large ribosomal subunit (LSU). Both RPL5 and RPL11 bind to and inhibit HDM2, the E3 ubiquitin ligase that targets p53 for degradation. Conflicting reports suggest that RPL5 and RPL11 function either together or independently to repress HDM2. Furthermore, it is currently unclear whether the third component of the 5S RNP, the 5S rRNA, also plays a role in this process. Indeed, mechanistic insight into 5S RNP formation, its integration into the ribosome, and how this is coupled to p53 regulation is lacking in human cells.

Here we show that the 5S rRNA, as well as RPL5 and RPL11, is indeed required for p53 activation when ribosome biogenesis is blocked. In addition, the whole 5S RNP complex is required for p53 homeostasis in normal cells. We find that the levels of free, non-ribosomal 5S RNP in the nucleoplasm correlate with the amount of p53 in the cell. Indeed, our data indicate that factors that regulate 5S RNP formation, its nucleolar retention/localisation and its integration into the ribosome, determine the amount of free complex that can interact with and regulate HDM2 in the nucleoplasm. For example, knockdown of the human ribosome biogenesis factors RRS1 or BXDC1 inhibits 5S RNP nucleolar localisation and causes p53 activation. Furthermore, the known tumour suppressors, PICT1 and PAK1IP1, that both regulate p53, are in fact essential LSU biogenesis factors and PICT1 is needed for 5S RNP recruitment into the ribosome.

Following oncogene overexpression $p14^{ARF}$ inhibits ribosome biogenesis and activates p53 by repressing HDM2. We show that all three components of the 5S RNP are required for $p14^{ARF}$ mediated activation of p53. This provides evidence that blocking ribosome production is important for the cellular response to oncogene misregulation. Our data therefore reveal that the assembled 5S RNP complex, rather than its individual components, is a critical modulator of multiple signalling pathways coupling the regulation of cellular proliferation to ribosome biogenesis.

597 A Dissecting the splicing-dependent mRNA binding of ASAP complexes

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The exon-junction complex (EJC) is a multi-protein complex, which is deposited on the mRNA by the spliceosome. It binds in a sequence-independent manner 20-24 nucleotides upstream of an exon-exon junction. The EJC core consists of four different proteins: eIF4A3, MAGOH, Y14 and Barentsz (Btz). This core complex serves as a binding platform for many associated proteins referred to as peripheral EJC components. Within the peripheral EJC, the proteins RNPS1, Acinus and SAP18 form a stable trimeric sub-complex, the ASAP complex. The recently solved crystal structure of the ASAP complex reveals how RNPS1, Acinus and SAP18 interact in vitro. However, it remains elusive how the ASAP complex assembles in living cells and which factors mediate its interaction with spliced mRNA and the EJC core.

We studied ASAP complex assembly in cell extracts with a combination of co-IP experiments and in vitro splicing assays. Using deletion mutants, we defined domains of RNPS1 and Acinus that are required for ASAP complex formation and mRNA interaction. Furthermore, we identified single point mutations of RNPS1, Acinus and SAP18 that partially or completely disrupt the ASAP complex and analyzed their capability to bind mRNA. Interestingly, formation of the trimeric complex is a prerequisite for the interaction with spliced mRNA and its bound protein complexes. The molecular basis of the interaction between ASAP complex and EJC is currently further characterized.

Taken together, our data suggest that interactions of EJCs and ASAP complexes contribute to the assembly of splicing-dependent mRNPs.

598 B The Ribosome-OME I: Big Data & the Ribosome

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Ribosomes are abundant. Each contains one copy of each of 80 ribosomal proteins (RPs). Therefore, to a first approximation one might expect cells to live with equal numbers of the mRNAs for each of the RPs. The massive transcriptome data for human cells and tissues provides an opportunity to test this assumption. Yet, this expectation has not been satisfied, due in large part to the following problem: each authentic RP gene is diluted by an average of 20 pseudo-RP genes[1], nearly perfect cDNA copies presumably inserted into the genome during the abundant ribosome synthesis of oogenesis. Thus mapping the reads of an RNA-seq analysis against the genome leads to many ambiguous identities. While the various mapping programs deal with this problem in different ways, the result is often an unreliable measure of the actual value, sometimes suggesting 100-fold differences in the level of mRNA for the different RPs.

Modifying the usual mapping systems to provide more accurate assessment of the actual values of RP mRNAs led us to two observations: I) there is substantial variation of the abundance of any given RP mRNA among different cell types, and even among different determinations on the same cell type, e.g., from the ENCODE consortium. II) there is a reproducible difference between the mRNA levels for different RPs, but only over a ~five-fold range. Nevertheless, this result suggests either a substantial regulation of translational rate AND/OR an overexpression of many RPs, with rapid degradation of the excess, as has been recently suggested[2].

An interesting example is RPL41, that encodes a protein of 24 AA, 17 of which are R or K. It consistently has more mRNA than any other RP. Is this because the efficiency of translation on monosomes is low, or because of the concentration of basic amino acids leads to slow translation.[3]

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599 C Cytoplasmic RNA regulatory networks orchestrate male gametogenesis

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Tissue and organ formation requires the generation of complex protein expression patterns. Regulated protein synthesis, in the form of cytoplasmic post-transcriptional mRNA regulation, is a powerful mechanism to shape protein gradients across cells and tissues in space and time. Translational control is vital during metazoan germ cell development and early embryogenesis. Our past research was devoted to elucidating the molecular mechanisms of germ cell development in *Caenorhabditis elegans*. We primarily focused on the regulation of the tumor suppressor GLD-1, itself an RNA-binding protein and translational repressor. While GLD-1 protein expression is essential for female meiosis and *gld-1* mRNA regulation is a paradigm of translational control, loss of *gld-1* expression has no impact on male germ cell development. However, by studying the RNA regulatory network in males, we discovered essential and novel roles of highly conserved RNA-modifying enzymes and RNA-binding protein families.

A pivotal mechanism of translational control is anchored around the poly(A) tail, which influences mRNA stability and ribosomal engagement. Two opposing forces, deadenylases and non-canonical poly(A) polymerases, are envisioned to dynamically regulate poly(A) tail lengths in the cytoplasm, as a consequence of mRNA-associated factors, such as CPEBs. By focusing on male meiosis, we identified an intricate RNA regulatory web built of all four encoded CPEBs, which interface with two distinct cytoplasmic poly(A) polymerases, GLD-2 and GLD-4, and members of the PUF (Pumilio/FBF) protein family. Importantly, the process of meiotic chromosome segregation in most species is different between male and female germ cells. We find that these conserved sex-specific differences are reflected in a rewiring of the poly(A) tail control network in male vs. female germ cells to achieve gamete-specific gene expression. I will present our biochemical, genetic and cell biological data that reveal spatially and temporally resolved activities of all four CPEBs as master regulators of male germ cell development.

600 A PX1 regulates protoxylem cell fate via RNA processing

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Plant hormones cytokinin and auxin have been established as essential regulators of Arabidopsis vascular cell fate. Protein AHP6, a member of cytokinin transduction cascade, has been demonstrated to play a critical role during protoxylem development. In order to put AHP6 protein in a broader functional context, we made genetic screen, using *AHP6prom:GFP* as a marker of cytokinin activity in the protoxylem cells.

We isolated mutant *px1*, which shows a decreased *AHP6prom:GFP* activity, accompanied with aberrant protoxylem formation and altered hormonal responses. *PX1* codes for a weak allele of an embryonic lethal gene (EMB2016) which is, based on homology to known Drosophila proteins, involved in RNA processing, however with unclear function.

In order to elucidate the molecular role of EMB2016 complex, we made a tandem affinity purification (TAP) experiment. We identified homologs of EMB2016 interactors known from Drosophila, underlying the evolutionarily conserved character of the EMB2016 module, and mRNA adenine modifying enzyme, showing thus a novel functional link in this respect. An extensive phenotype analysis confirmed functional relevance of these interactions. In addition, transcriptional profiling revealed broad changes in auxin and cytokinin responsive genes expression in the *px1* genetic background.

In summary, we demonstrate the importance of adenine modifications in mRNA within the framework of hormonal regulations and developmental processes.

601 B Hepatitis B virus post-transcriptional element promotes mRNA export via the cellular mRNA export machinery TREX

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In eukaryotes, mRNA export factors are recruited to intron-containing mRNA during splicing, but recruited to intronless transcript probably via specific cis-elements. Hepatitis B viral RNAs are unspliced RNAs that are exported dependent on post-transcriptional element (PRE). However, the mechanism for PRE-mediated mRNA export is still unclear. We found that PRE drastically enhances cytoplasmic accumulation and expression of cDNA transcipts. Systematic deletion analysis indentified two ~100 nt minimum sub-elements in PRE. MS2-MBP affinity purification of proteins bound to these subelements identified TREX components as well as several TREX-associating RNA-binding proteins. Consistent with this result, TREX components efficiently and specifically associate with in vitro transcribed PRE, and this association is both cap- and ATP-dependent. Importantly, knockdown of TREX components and the mRNA export receptor TAP inhibited PRE-mediated mRNA export. Furthermore, by comparison of two functional sub-elements, we identified a 12 nt consensus motif which was sufficient to promote mRNA export when multimerized. Together, our results indicate that PRE enhances the export of intronless mRNA by recruiting TREX. Our study suggests that HBV RNAs competes with cellular mRNAs for cellular mRNA export proteins, facilitates viral RNA export and simultaneously inhibits cellular mRNA export.

602 C Insights into the nuclear mRNA export machinery of Trypanosoma brucei

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Trypanosoma brucei is a unicellular protist parasite causing devastating diseases such as human sleeping sickness and Nagana in sub-Saharan Africa. Trypanosomes diverged early in evolution and show significant differences in their gene expression compared to higher eukaryotes. For instance, due to a lack of individual gene promoters, large polycistronic pre-mRNA molecules are transcribed from which individual mRNAs mature by *trans*-splicing and polyadenylation. In the absence of transcriptional control, regulation of gene expression occurs on the post-transcriptional level mainly by control of transcript stability and translation. We are investigating the process of nuclear mRNA export as a potential additional way of post-transcriptional gene regulation in *T. brucei*. The active translocation of mature mRNA from the nucleus to the cytoplasm is well understood in higher eukaryotes ranging from yeast to human - model organisms belonging to the eukaryotic supergroup of Opisthokonta. Their export factors such as the TREX complex and the export receptor Mex67-Mtr2 are well described and appear to be highly conserved. Trypanosomes are members of the supergroup of Excavata and the high divergence between Opisthokonta and Excavata makes it difficult to identify orthologous export factors based on sequence similarity. Hence, biochemical approaches are needed to identify the export machinery of the parasites. One of the few conserved factors are the orthologs of yeast Mex67 and Sub2. Here, we report the functional characterization of TbMex67. RNAi mediated downregulation of TbMex67 affects cell growth and leads to a nuclear retention of bulk mRNA. In contrast to all known orthologs, Mex67 of kinetoplastid species including *T. brucei* and other important pathogens like *T. cruzi* and *Leishmania* spp. contains a N-terminal zinc finger motif. We show that this parasite-specific feature is indispensable for the function of TbMex67. Overexpression of mutated versions of TbMex67 causes a dominant negative effect indicating that essential interacting proteins are sequestered. We used tandem affinity purification of PTP-tagged TbMex67 to identify such proteins and isolated two candidates that are essential for mRNA export as well. TbMtr2 interacts with TbMex67 to form the stable export receptor Mex67-Mtr2. TbIMP1 in contrast, belongs to the family of importins and is required for shuttling of TbMex67. Our data show that the heterodimeric export receptor is conserved throughout the eukaryotic kingdom. However, the zinc finger motif is unique to kinetoplastids and may play a parasite specific role.

603 A Assembly of TREX complex components on mRNAs

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After their transcription in the nucleus, mRNAs are exported to the cytoplasm as ribonucleoprotein complexes (RNPs). Correctly processed mRNAs associate with a varying set of adaptor proteins that eventually direct the recruitment of the general export receptor NXF1. Although many studies have been carried out on export adaptors, the exact determinants for the interaction of the mammalian export machinery with export competent mRNAs remain unclear.

Here, we analyze the recruitment of the export factors UAP56, DDX39 and ALYREF using in vitro spliced mRNAs. Surprisingly, no preferential interaction of UAP56, DDX39 and ALYREF with spliced mRNAs was detected. While all three factors favor an association with the 5' end of spliced and non-spliced mRNAs, a contribution of exon junction complexes and 3' sequences to the RNA-binding of UAP56, DDX39 and particularly ALYREF could be detected. Hence, the cooperative interaction of export factors with exon junction complexes on mRNA with multiple introns may explain the preferential export of spliced mRNAs.

This study provides insights into the process of mammalian mRNA export and its dependence on splicing and the presence of exon junction complexes. Next, we aim to define the hierarchy of export adaptor recruitment leading to the efficient export of mammalian mRNAs.

604 B Growth cone local mRNA translation of nuclear proteins in the spatio-temporal regulation of neurite outgrowth

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Neuronal cells exploit local mRNA translation to regulate crucial processes such as neuronal survival, axon pathfinding and synapse formation. Local translation and retrograde transport of transcription factors emerges as a new paradigm to regulate nuclear gene expression in response to signaling events at distal ends of axons and dendrites. We identified a panel of mRNAs that encode nuclear proteins in growth cones of extending neurites (protrusions that are the precursors of axons and dendrites), suggesting that an analogous paradigm might also operate at the very initial phases of neuronal differentiation. We present results on a growth cone localized mRNA encoding a histone-interacting protein with a described role in transcriptional regulation. We show that the knock-down of this mRNA in neuronal cells induces transcriptional changes and impairs neurite outgrowth. Conversely, the overexpression of the encoded protein induces robust neurite outgrowth. Interestingly, the overexpression phenotype is strictly dependent on the presence of the mRNA 3'UTR, which we identified as a determinant that mediates growth cone mRNA localization. We are currently testing whether this protein is locally synthesized in growth cones and retrogradely transported to the cell nucleus. Our initial results suggest that local mRNA translation of a histone-interacting protein might serve as a mechanism to couple the dynamic neurite outgrowth process with transcriptional regulation in the nucleus.

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605 C Genome-wide identification of mRNAs associated with Survival of Motor Neuron proteins and whose axonal localization is decreased upon SMN deficiency

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Spinal Muscular Atrophy is a neuromuscular disease resulting from mutations in the *SMN1* gene which encodes the Survival Motor Neuron (SMN) protein**.** SMN is part of a large complex that is essential for the biogenesis of spliceosomal small nuclear RNPs. Using SMA animal models, it has been reported that SMN deficiency, similar to that occurring in severe SMA, alters the stoichiometry of snRNAs and causes widespread pre-mRNA splicing defects in numerous transcripts of diverse genes (1, 2). However, whether the specific degeneration of motor neurons is caused by one or more aberrantly spliced transcript or by the cumulative effect of numerous splicing alterations remains to be determined. SMN also colocalizes with mRNAs in granules that are actively transported in neuronal processes and it was recently shown that SMN knockdown results in a reduction of poly(A) mRNA levels in the axonal compartment of primary motor neurons (3), suggesting thereby that SMN-containing complexes might be involved in the axonal localization of a large number of mRNAs. However, the full repertoire of SMN-associated RNAs has not yet been characterized.

To address this question, we have used murine motor neuron-like NSC-34 cells and RNA Immuno-Precipitation experiments coupled to microarray analyses (RIP-Chip) to perform a genome-wide analysis of RNA species present in mRNP complexes containing either the full length SMN protein (fl-SMN) or a recently described truncated axonal form (a-SMN). This approach allowed us to identify distinct but overlapping subsets of mRNAs associated with fl-SMN and a-SMN. Combination of fluorescent *in situ* hybridization (FISH) and immuno-fluorescence (IF) experiments indicated that several mRNAs colocalize with the SMN proteins in neurites and axons of differentiated NSC-34 cells. Interestingly, the axonal localization of some of these mRNAs is affected in SMN-depleted cells, suggesting that SMN-deficiency could result in the mislocalization of numerous mRNPs required for axonogenesis.

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606 A Tetramolecular Fluorescence Complementation for Detection of Specific RNAs in Vitro

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Asymmetric localization of mRNA is a mechanism to regulate gene expression spatially as well as temporally and contributes to many important developmental processes. To study localization mechanisms various probes for RNA imaging have been established. These probes can be nucleic acid- or protein-based and give a characteristic fluorescent signal upon binding to target RNAs. Still, nucleic acid-based probes–*e. g.* molecular beacons or forced intercalation probes–cannot be produced inside cells, complicating *in vivo* imaging.[1] Protein-based probes fused to split fluorescent reporters can be expressed by the cellular machinery. Sequence-specific binding of proteins to their target RNA and subsequent reconstitution of the fused fluorescent reporter allow RNA detection. However, the traditional fluorescent reporters based on split-GFP are large and self-assemble spontaneously, causing significant background.[2]

To circumvent these limitations we used a three-body split-GFP and developed a reporter system for detection of specific RNA *in vitro*.[3] This system consists of four components: two Pumilio variants each fused to just one β-sheet of GFP, a GFP detector, and the target RNA, which triggers assembly of the whole complex (see figure 1 below). We used this system to differentiate between closely related RNAs after as little as 10 minutes with a background fluorescence of merely 1.4 %.[4] Since complex background of RNA and cell lysate did not prevent fluorescence complementation, detection of RNA with our system could become possible *in vivo*.

The ability to detect single stranded RNA sequence specifically might also be useful for detection of SNPs or discrimination of microRNAs. We currently extend our system to allow fast and easy detection of any given RNA of interest.

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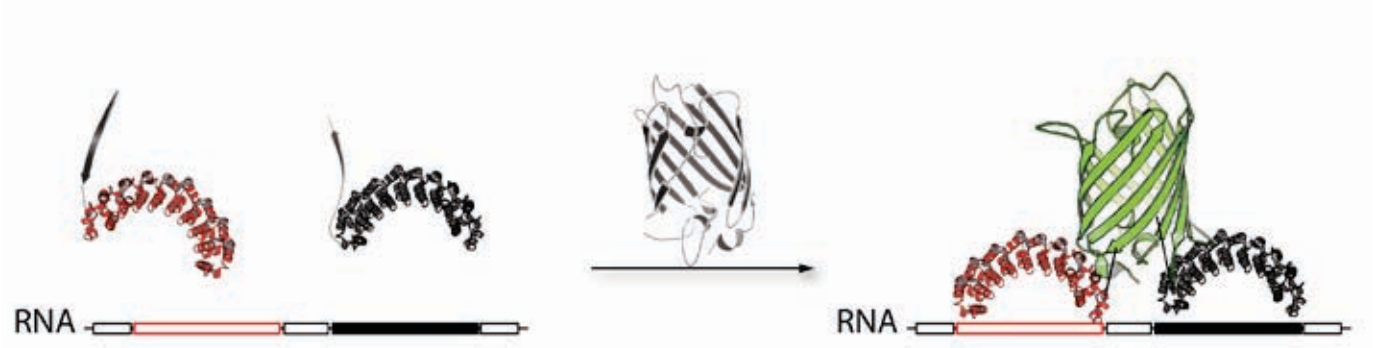

Figure 1: Tetramolecular Fluorescence Complementation (TetFC) for sequence-specific RNA detection. Binding of two Pumilio proteins to the target RNA at designated sites forms a complex that can recruit a GFP detector, thereby leading to fluorescence.

607 B Resolving conflicts between Transcription and Replication: a new potential role for the mRNA export factor Yra1, regulated by its post-translational modifications.

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The maintenance of genome integrity is one of the most challenging tasks eukaryotic cells have to face. One considerable source of endogenous damage is generated by defects in DNA replication that lead to replication forks stalling and collapse. Particularly during S-phase, cells have to resolve conflicts between replication and transcription, when DNA synthesis is impaired by the RNA polymerase and by factors involved in mRNA processing and export. The mechanism underling this stress response is not yet elucidated.

Intriguingly, one common factor of these processes is the essential and conserved protein Yra1/REF. Yra1 has a well-characterized role as mRNA export adaptor working in association with the mRNA export receptor Mex67/TAP and the poly(A) binding protein Nab2. We previously reported that Yra1 is a pluri-ubiquitinated protein and we showed that its ubiquitination by the E3 ubiquitin ligase Tom1 is involved in mRNA export regulation (Iglesias, Tutucci et al. 2010).In addition Yra1 is found at origins of replication, but its function in this context is unknown (Swaminathan, Kile et al. 2007).

Our recent work suggests a new role for the mRNA export factor Yra1 in the regulation of cell cycle progression, and shows that Yra1 ubiquitination and in turn Yra1 levels, are sensitive to replication stress caused by different DNA damaging factors. Moreover, we observed that Yra1 is a sumoylated protein and we identified the SUMO-dependent Slx5/Slx8 complex as the second E3 ubiquitin ligase complex involved in Yra1 post-translational modification. Interestingly this ubiquitin-ligase complex binds the DNA and deletion of either gene is paralleled by a general increase in genomic instability (Nagai, Dubrana et al. 2008).

With this work we propose Yra1 as a new factor involved in genome integrity maintenance and we suggest that Yra1 is a sensor of DNA damage and a player in the S-phase stress response. In addition, the fact that Yra1 is present both at the transcription sites and at origins of replication, suggests a role for this protein in resolving conflicts between transcription and replication during S-phase.

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608 C Ubiquitin and SUMO regulate Yra1: linking mRNA export factor to genome stability

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The nuclear periphery is a fundamental compartment to organize DNA domains and ensures proper transcription, replication and genome integrity.

The Nuclear Pore Complex has a well-established role in telomere tethering and subtelomeric repression at the nuclear periphery (Therizols, et al. 2006) and the loss of NPC subunits is synthetic lethal with components of the homologous recombination pathway (Pan X, et al. 2006). Intriguingly, the NPC physically interacts with the SUMO-dependent ubiquitin ligases Slx5-Slx8 that localize at irreparable DSBs (Nagai, et al. 2008). Moreover, the NPC-bound SUMO-protease Ulp1 prevents accumulation of DSBs (Palancade, et al. 2007) underlining the importance of sumoylation and ubiquitination in the DNA damage response.

Our earlier work (Iglesias, Tutucci et al. 2010) has shown that Yra1, an essential protein acting at the nuclear periphery and involved in transcription elongation, 3' processing, transcription termination and finally mRNA export, is released from the mRNP following ubiquitination by the E3 ligase Tom1 allowing proper mRNA export. Our recent studies identified Slx5-Slx8 as an additional E3 ligase involved in the regulation of Yra1 ubiquitination. Consistently, Yra1 is sumoylated and regulated by the protease Ulp1. Notably, the levels of Yra1 ubiquitination change in response to DNA damage. Our data highlight the importance of both sumoylation and ubiquitination in the regulation of Yra1 and suggest a new potential role of this mRNA export factor in the control of genome integrity.

609 A mRNA transport and translation regulate neuritogenesis

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Post-transcriptional regulation of mRNA processing is involved in neural development and disease. Transport of mRNA and local translation in neurites are important mechanisms for neuron to control their development and function including neuritogenesis, synapse formation and synaptic plasticity. We have been focusing on two factors in neuronal RNA granules, the RNA-binding protein hnRNP Q1 and DEAD-box RNA helicase DDX3, to explore how they may regulate mRNA transport and translation during neuritogenesis. Using siRNA-mediated knockdown, we found that hnRNP Q1 and DDX3 could regulate neuritogenesis likely by regulating mRNA transport and translation, respectively, in primary cortical neurons and N2A cells. Knockdown of hnRNP Q1 increased neurite complexity in both primary cortical neurons and N2A cells. A search for mRNA targets of hnRNP Q1 identified functionally coherent sets of mRNAs involved in Cdc42-mediated cytoskeleton remodeling. Knockdown of hnRNP Q1 indeed reduced the level of several mRNAs encoding the components of the Cdc42/N-WASP/Arp2/3 pathway in neurites. This result suggests that hnRNP Q1 may participate in localization of mRNAs encoding Cdc42 signaling factors in neurites, and thereby regulate actin dynamics and control neuronal morphogenesis (Mol. Cell. Biol. 32, 2224, 2012). Knockdown of DDX3 inhibits neurite outgrowth in both cortical neurons and N2A cells. A screening of potential mRNA targets of DDX3-regulated translation identified functionally coherent genes involved in the Rac1-mediated signaling pathway. We demonstrated by using immunoblotting, immunostaining and quantitative RT-PCR that DDX3 knockdown reduced the level of Rac1 protein but not mRNA. Moreover, RNA pulldown and *in vitro* translation showed that DDX3 associated with the 5' UTR of Rac1 mRNA and regulated Rac1 translation in N2A cells. Furthermore, overexpression of constitutively active Rac1 could partially rescue neurite outgrowth defects in N2A cells. Therefore, our and others' studies indicate that functionally coherent mRNAs can be regulated by single proteins as mRNA regulons at the mRNA transport and translation levels in neurons. Cdc42 and Rac1 are critical molecules in regulating cytoskeleton remodeling and cell polarity. Identification of mRNA regulons encoding Cdc42 and Rac1 signaling factors may provide us insights into how they regulate neuritogenesis as well as build polarity in neurons.

610 B S6K1 alternative splicing modulates its oncogenic activity and regulates mTORC1

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Ribosomal S6 Kinase 1 (S6K1) is a major mTOR downstream signaling molecule which regulates cell size and translation efficiency. Here we report that short isoforms of S6K1 are over-produced in breast cancer cell lines and tumors. Overexpression of S6K1 short isoforms induces transformation of human breast epithelial cells. The long S6K1 variant (Iso-1) induced opposite effects: It inhibits Ras-induced transformation and tumor formation, while its knockdown or knockout induced transformation, suggesting that Iso-1 has a tumor suppressor activity. We further found that S6K1 short isoforms bind and activate mTORC1, elevating 4E-BP1 phosphorylation, cap-dependent translation and Mcl-1 protein levels. Both a phosphorylation-defective 4E-BP1 mutant and the mTORC1 inhibitor rapamycin partially blocked the oncogenic effects of S6K1 short isoforms, suggesting that these are mediated by mTORC1 and 4E-BP1. Thus, alternative splicing of S6K1 acts as a molecular switch in breast cancer cells elevating oncogenic isoforms that activate mTORC1.

611 C Altered microRNA expression profile in ALS: Role in the regulation of NFL mRNA levels

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Amyotrophic lateral sclerosis (ALS) is a progressive, adult onset neurodegenerative disease of motor neurons (MN). The MN degeneration is associated with the formation of neurofilament aggregates and a selective suppression of low molecular weight neurofilament (NFL) mRNA. The preferential localization of NFL mRNA to P-bodies in ALS suggests that the suppression of its levels is related to an increase in RNA degradation. In addition, in vitro experiments in which human NFL (hNFL) mRNA was incubated with spinal cord (SC) homogenates pretreated with RNase showed that RNA species contribute to NFL mRNA destabilization in ALS. Considering that microRNAs (miRNAs) are key mRNA stability determinants and play a critical role in degeneration, they presented a likely candidate to explain our previous results. MiRNAs are small non-coding RNAs that participate in mRNA degradation mainly through base pairing interactions with the mRNA 3' untranslated region (UTR). In this study our objective was to characterize the miRNA expression profile in ventral lumbar SC tissue in sporadic ALS (sALS) and controls. We analyzed a large group of miRNAs and we found that the majority of dysregulated miRNAs are down-regulated in sALS. Ingenuity Pathway Analysis showed that these dysregulated miRNAs are linked with nervous system function and cell death. We used two prediction algorithms to develop a panel of miRNAs that have recognition elements within the hNFL 3'UTR. Finally, we investigated the functional relevance of these miRNAs using reporter gene assays and rqRT-PCR from HEK293T cells co-transfected with different hNFL mRNA 3'UTRs linked to a reporter gene and miRNAs predicted to interact with the 3'UTR. Our data suggest a potential role of several miRNAs in the selective decrease of NFL mRNA observed in ALS that could contribute to the etiology of neurofilamentous aggregates and the ALS pathology.

612 A Novel TAL1 targets beyond protein coding genes: identification of TAL1-regulated microRNA genes in T-cell acute lymphoblastic leukemia

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The transcription factor TAL1 is downregulated early in T-cell development and frequently overexpressed in T-cell acute lymphoblastic leukemia (T-ALL). To identify a TAL1-dependent microRNA gene expression profile, we ectopically expressed TAL1 in the TAL1-negative T-ALL cell line P12 and performed low density array analysis. Initially, we identified eight microRNA genes whose expression changed significantly upon TAL1 overexpression. We then validated these results by quantitative PCR analysis after enforcing or silencing the expression of TAL1 in TAL1-negative and TAL1-positive T-ALL cell lines, respectively. This approach confirmed that miR-135a, miR-223 and miR-330-3p are upregulated by TAL1, whereas miR-146b-5p and miR-545 are downregulated. To assess the possibility that these microRNAs are direct targets of TAL1, we searched publicly available TAL1 ChIP-seq data (GSE29181) for the presence of TAL1 binding peaks up to 10kb upstream of the transcription start site (TSS) of each microRNA gene. We identified one peak in a putative promoter region for miR-146b, suggesting that this gene may be a transcriptional target of TAL1. Furthermore, two peaks can be observed upstream of miR-223 TSS. Because miR-223 has already been shown to play a pro-tumorigenic role in T-ALL, although not in the context of TAL1, we performed TAL1 ChIP-qPCR in JURKAT and CCRF-CEM cells using primers designed for the genomic areas covered by the two peaks in the miR-223 locus. We confirmed that upon TAL1 IP there is more than 2-fold enrichment in the amplified area within 3.5kbs upstream of the miR-223 TSS. These results indicate that miR-223 is a direct target of TAL1 in T-ALL. Interestingly, analysis of microRNA gene expression profiles in different T-ALL subsets revealed that TAL/LMO primary samples display higher levels of miR-223 ($p=0.03$) and tend to express lower levels of miR-146b-5p ($p=0.09$) than other T-ALL cases. Moreover, miR-223 and miR-135a appear to follow the same pattern of expression along normal human thymocyte development as TAL1, with high levels in CD34+ T-cell precursors and sharp downregulation in more differentiated subsets. In contrast, miR-146b-5p, which is negatively regulated by TAL1, is mostly expressed in mature, single-positive thymic subsets. Overall, our studies identify several TAL1 downstream micro RNA target genes, of which miR-146b and miR-223 may be directly regulated, and suggest that they may be components of TAL1 downstream regulatory networks involved in normal hematopoietic development and in T-ALL. Their actual participation in this malignancy and/or in TAL1-mediated physiological effects in hematopoiesis requires investigation. We are currently exploring the functional consequences of these microRNAs modulation in the development of the malignancy. We Expect to present these results in this congress.

613 B Retinitis pigmentosa mutations of hBrr2 reduce splicing fidelity

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hBrr2 is a DExD/H-box RNA helicase crucial for pre-mRNA splicing and its mutations cause autosomal dominant retinal disorder retinitis pigmentosa. In this study, we prepared S1087L and R1090L mutations of human Brr2 using BAC recombineering and expressed them stably in human cell culture. Mutations in hBrr2 did not compromise snRNP assembly and both mutants were incorporated into the spliceosome as the wild-type protein. Surprisingly, cells expressing RP mutants exhibited increased splicing efficiency of the LDHA gene. Next, we found that depletion of endogenous hBrr2 enhanced usage of a cryptic splice site while splicing at the correct splice site was inhibited. Proper splicing of optimal and cryptic splice sites was restored in cells expressing wild-type hBrr2 but not in cells expressing the RP mutants. Taken together, our data suggest that hBrr2 is an important factor in splice site recognition and that the RP-linked mutations S1087L and R1090L affect this hBrr2 function, possibly leading to the increased usage of cryptic splice-sites.

614 C An Exon-Specific U1 approach to correct SMN protein deficiency in spinal muscular atrophy (SMA)

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A significant proportion of disease-causing mutations affect pre-mRNA splicing inducing skipping of the exon from the mature transcript. We recently showed that modified U1snRNAs targeting non-conserved intronic sequences downstream of the 5'ss (Exon Specific U1s) are able to correct different types of splicing mutations associated with defective exon definition (Fernandez et al HMG 2012). Using coagulation *F9*, *CFTR* and *SMN2* models, we identified an ExSpeU1 able to rescue exon skipping caused by mutations at the polypyrimidine tract, the 5'ss or the ESEs. To investigate their efficacy and potential applicability *in vivo* we used a cellular model of spinal muscular atrophy (SMA) where improvement of SMN2 exon 7 skipping due to a silent exonic substitution is a reliable therapeutic strategy. In SMA-derived primary fibroblasts, lentiviral-mediated transduction of ExSpe U1s rescued SMN2 exon 7 splicing pattern. The increase in the amount of the correct full length (FL) mRNA resulted in a corresponding improvement of the SMN protein to a level present in unaffected fibroblasts. Furthermore, using the Hek293 Flip-In cell system, we show that corresponding levels of SMN2 exon 7 splicing rescue can be obtained by expression of only one chromosome-integrated copy of the ExSpe U1 gene.To understand the ExSpeU1s splicing enhancing mechanism we investigate the U1 components and the possible direct interference with ISSs. All model systems showed an ISS downstream the 5'ss. However, the ISS deletions rescued with variable efficiency only some types of splicing mutations and U7 RNAs targeting the ISSs had no effect on splicing suggesting that ExSpeU1 are only partially acting through an antisense mechanism that target the ISS.. On the other hand, ExSpeU1 mutants previously reported to affect 70K and U1A binding when tested in vivo, only slightly reduce, or do not affect, the splicing enhancement. In the CFTR case, side-directed mutagenesis showed that an RNA secondary structure-dependent accessibility of the 5'ss regulates splicing. All together, these data suggest that multiple factors are involved in the ExSpeU1-dependent improvement of exon definition

615 A Ddx5/Ddx17 RNA helicases control multiple layers of gene expression during TGFß-induced Epithelial-to-Mesenchymal Transition (EMT)

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It is well known that Epithelial-to-Mesenchymal Transition (EMT) plays an important role in differentiation and is corrupted during tumorigenesis. TGFβ is a potent inducer of EMT, through the activation of Smads, leading to transcription of EMT master genes (Snail, Zeb, Twist) that regulate epithelial and mesenchymal gene expression. Evidence shows that EMT result from regulation of gene expression programs at the post-transcriptional level (alternative splicing and miRNAs). Ddx5 has been reported to favor EMT and is known to be a transcriptional coactivator of Smads. Ddx5 and its paralog Ddx17 are also implicated in alternative splicing.

To characterize the role of Ddx5/17 during TGFβ-induced EMT, we used the human mammary epithelial cell line MCF10A and showed that Ddx5/17 depletion by siRNAs inhibited the induction of EMT in response to TGFβ. Ddx5 and Ddx17 interact with smad3 and act as smad3 co-activator to induced expression of EMT transcription factor snail1&2. Consequently under ddx5/17 depletion, TGFβ no longer repressed the expression of E-cadherin and Ocludin, two proteins implicated in the cell-cell junction. Accordingly, cells are stilled attached and are not able to migrate. These results confirmed that Ddx5/17 are necessary for TGFβ -induced EMT.

Paradoxically, we observed that Ddx5/17 endogenous expression decreased after 48 hours of TGFβ treatment. We show that miR-181b is induced by TGFβ in a Ddx5/17 dependant maner and target Ddx5/17 in a feedback loop control. Our hypothesis is that Ddx5/17 decreased expression contributes to the development of cellular programs during EMT. Indeed, Ddx5/17 depletion increased: 1- the expression of Zeb1, a EMT transcription factor and others mesenchymal markers in response to TGFβ and 2- the formation of Actin stress fibers. Finally, using exon arrays, we show that Ddx5/17 participate to the regulation of alternative splicing events induced during EMT. Indeed Ddx5 and Ddx17 control alternative splicing of a large subset of genes including Enah, Stx16 and Fn1, genes relevant in the EMT process.

In conclusion, Ddx5 and ddx17 are necessary to initiate TGFβ signaling, but their decreased expression in a second phase could contribute to the completion of the cascade of events leading to TGFβ -induced EMT in particular at the splicing level. In this context Ddx5/17 control different steps of gene expression (transcription, miRNA and alternative splicing), to drive TGFβ -induced EMT.

616 B Overexpression of miR-29b and miR-122 in the invasive ductal carcinoma of the breast

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MicroRNAs (miRNAs) are key regulators of gene expression in eukaryotic cells. Current computational predictions suggest that miRNAs regulate the expression of 30% of human protein-coding genes. Several studies indicate that miRNAs regulate genes of signaling pathways involved in important cellular processes, including cell proliferation and apoptosis. The pro-apoptotic RNA-dependent protein kinase (PKR) signaling pathway is activated by PACT (protein activator of PKR). Recently, PACT was shown to be a target of miR-29b and miR-122 and the role played by these miRNAs in the breast cancer is still unknown. The aim of this study was to investigate whether the expression of miR-29b and miR-122 is deregulated in the invasive ductal carcinoma (IDC) of the breast using Formalin-Fixed, Paraffin-Embedded (FFPE) tissues. The expression of miRNAs and PACT was evaluated by real time PCR and immunohistochemistry, respectively. The normal breast FFPE samples were used as control. Our results showed an upregulation of miR-29b (8-fold; $p < 0.01$) and miR-122 (19-fold; $p < 0.005$) in the FFPE samples of IDC. We also observed that PACT expression was reduced by ~ 50% which is consistent with previous studies showing that miR-29b and miR-122 negatively regulate this PKR activator. Thus, the reduction of PACT expression may be accompanied by the decrease of the pro-apoptotic PKR pathway activation. Taken together, our results suggest that the overexpression of miR-29b and miR-122 in IDC is contributing for the inhibition of apoptosis and, therefore, these miRNAs may be involved in progression of the human breast cancer.

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617 C The RNA binding protein Quaking regulates monocyte adhesion and differentiation

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Circulating monocytes are actively recruited to sites of tissue injury. The adherence of these cells to the activated endothelium leads to their extravasation into the sub-endothelial space where they differentiate into macrophages. Despite the fact that the cell surface receptors that regulate this adhesion process, such as p-selectin glycoprotein ligand-1 and b-integrins, have been characterized, the factors that regulate monocyte activation, adhesion and differentiation are poorly understood.

Here, we show that the RNA-binding protein Quaking (QKI) critically regulates monocyte activation, adhesion and differentiation. First, we identified that QKI is lowly expressed in human peripheral blood monocytes, while FACS sorting of monocyte subsets from peripheral blood and subsequent RNA isolation revealed that QKI mRNA expression is potently induced in a specific, activated subset of human circulating monocytes ($CD14^+$ $CD16^+$). Interestingly, the activation and differentiation of monocytes into macrophages was coupled with a striking induction of QKI protein (n=6). This finding was supported by laser capture micro dissection and immunohistochemistry studies where we validated that QKI mRNA and protein is highly expressed in macrophages in human atherosclerotic lesions. To gain mechanistic insight we utilized lentiviral short-hairpin RNAs to specifically abrogate QKI protein levels in human THP-1 and U937 monocytes. A reduction of QKI resulted in decreased adhesion using a cell perfusion assay ($p<0,05$ n=6), together with perturbed migration and differentiation. Subsequently, our discovery that QKI colocalizes with RNA species within spreading initiation centers of adhering monocytes, indicates that QKI could play a central role in regulating the translocation of RNA species required for the development of focal adhesions. Finally, we demonstrate that the transplantation of bone marrow from mice with decreased levels of QKI (quaking viable), into LDLR-/- mice fed a high-fat diet, leads to significantly reduced macrophage content in atherosclerotic lesions ($p<0,05$ n=13).

Collectively, we have identified that QKI critically regulates monocyte activation, adhesion and macrophage differentiation. We therefore propose that the targeted reduction of QKI in monocytes could be an effective means of limiting the severity of the systemic or local inflammatory response in disease.

618 A Rho Guanine Nucleotide Exchange Factor: A Novel RNA Binding Protein Involved in the Pathology of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is an adult-onset progressive disorder characterized by degeneration of motor neurons. Although the cause of the disease remains elusive, protein aggregate formation in motor neurons, including neurofilamentous aggregates, is a neuropathological hallmark. Recent evidence supports the hypothesis that alterations in RNA metabolism in motor neurons can lead to the development of these aggregates. In mice, p190RhoGEF, a guanine nucleotide exchange factor, is involved in neurofilament protein aggregation in a RNA-triggered transgenic model of motor neuron disease. However, no information was known regarding the function of the human homologue of p190RhoGEF called Rho Guanine Nucleotide Exchange Factor (RGNEF) and its role in ALS. Here we studied the function of RGNEF and its involvement in the ALS pathology. We observed that RGNEF is an RNA binding protein that binds NFL mRNA. In addition, we demonstrated that RGNEF affects NFL mRNA stability via 3'UTR destabilization. The over-expression of RGNEF in a human cell line significantly decreased the level of endogenous NFL protein and its over-expression in yeast showed cytotoxicity comparable to TDP-43, another protein involved in ALS. When the pathology of RGNEF was analyzed we observed extensive cytoplasmic inclusions in ALS spinal motor neurons that co-localized with ubiquitin, p62/Sequestosome-1, TDP-43 and FUS/TLS (all of them known neurodegenerative markers). Finally, we investigated for genetic alterations performing sequencing of the gene that codifies for RGNEF (ARHGEF28). We found a new frameshift mutation and extensive regions of the gene with homozygosis in familial ALS cases. Our results provide further evidence that RNA metabolism pathways are integral to ALS pathology. This is also the first described link between ALS and a RNA binding protein with aggregate formation that is also a central cell signalling pathway molecule.

619 B Mechanisms of Post-Trascriptional Regulation of Gene Expression in Dementias

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MicroRNAs (miRNAs) are noncoding RNA of 18-25 nt, capable of regulating mRNA translation and gene expression at the post-trascriptional level. MiRNAs mis-expression is often associated with human diseases, such as cancers and neurodegenerative pathology conditions. The main objective of this study is an analysis of the post-transcriptional regulation by miRNAs of two important genes, MAPT and PGRN, involved in Frontotemporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17). This is one of the major degenerative dementia syndromes, characterized by atrophy of the prefrontal and anterior temporal lobes. Several studies identified 43 pathogenic mutations in MAPT, that encodes for microtubule associated tau protein. In the brain tau protein has important functions in microtubule (MT) assembly, stability and has a relevant role in neurogenesis, axonal maintenance and transport. The disruption of its function has devastating effects on neuronal integrity and in most neurodegenerative diseases forms neurofibrillary tangles (NFT) accumulation, that trigger neuronal loss. Recently, the discovery of 56 mutations in the progranulin gene (PGRN) explained the heterogeneity of patients affected by FTDP-17. PGRN encodes a secreted precursor protein called progranulin, that is expressed in neurons, microglia and represents an important growth factor involved in the regulation of multiple processes. MiRNAs may be a contributing factor in neurodegenerative diseases. This project focuses on the expression of selected miRNAs in cell lines and primary neurons and on the validation of the miRNAs targeting of PGRN and MAPT 3'UTR. Moreover, we aimed at understanding the influence of SNPs in PGRN 3'UTR on the effect of validated miRNAs and finding if there is a causal role of miRNA deregulation in FTD, through cell systems and mice brains.

620 C MicroRNAs as lung cancer biomarkers

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Lung cancer is the leading cause of cancer-related death worldwide, with a 5- year survival rate of only ~15%[1]. The cause of this high mortality is due to the poor prognosis caused by a late disease presentation, tumour heterogeneities and limited understanding of tumour biology. Histologically, lung cancer is classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is comprised of two most common subtypes, i.e., Squamous Cell Carcinoma (SQCC), Adenocarcinoma (ADC), and an additional type called Large Cell Carcinoma (LCC). With the emergence of targeted therapies directed against specific cellular alterations, an accurate classification became necessary[2]. In this context, the pattern of miRNAs expression could be useful in improving the classification of lung cancers and predicting their behavior. The relative quantification of miR-205 and miR-21 was reported by us and others to be a useful marker for differentiating subtypes of NSCLCs[3,4,5,6]. However, the majority of NSCLC patients have advanced disease stage at the time of diagnosis. It is therefore important to find diagnostic methods based on detection of early events. For this reason we compared the expression of miR-21 and miR-205 between cytological and histological samples to obtain a less invasive early method of diagnosis.

Besides ADC and SQCC, lung tumors include also a spectrum of neuroendocrine (NE) lesions with different histology, biology and dramatically different clinical behaviour, which include typical carcinoids (TC), atypical carcinoids (ATC), large cell neuroendocrine carcinomas (LCNEC) and small cell lung carcinomas (SCLC)[7]. In the present study we analyzed miR-375 and miR-192 expression to obtain a method to distinguish NE versus non-NE lung tumors and the different categories of NE lung tumors. By using a Support Vector Machine (SVM) algorithm we assessed whether, on the basis of miRNAs expression, it is possible to recognize different subtypes of lung cancers.

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621 A hnRNP A2/B1 regulated alternative splicing of key signal transduction components and is essential for breast cancer metastasis.

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Alternative splicing is a key control point in gene expression, and it is now becoming clear that it is also a process whose misregulation in cancer can contribute significantly to malignancy by regulating the expression of isoforms of various oncogenes and tumor suppressor genes. However, the role of alternative splicing regulators, or splicing factors, in cancer development and progression, is mostly unknown. Our recent studies indicate that some alternative splicing factors can be oncogenic, which partly involves the activation of known signaling pathways in novel ways.

Our results suggest that the splicing factor hnRNP A2/B1 is up-regulated in cancer and act as oncoproteins in several steps of cancer development and progression. Our aim is to use this factor as a model to reveal the functional roles of alternative splicing in cancer development, tumor maintenance and metastasis and the molecular mechanisms involved. Using a combination of both in vitro and in vivo cancer model systems in which we can manipulate the expression of hnRNP A2/B1 we discovered its role in cancer initiation and progression.

Since hnRNP A2/B1 is a broad regulator of alternative splicing, we hypothesize that its overexpression will change the splicing landscape of a large set of currently unknown target genes which might be novel determinants of survival, motility, invasiveness and other properties of tumor cells. In order to reveal the full spectrum of hnRNP A1/A2 splicing targets we performed RNA deep sequencing analysis of cells with hnRNP A2/B1 and discovered that it regulates several key pathways known to regulate invasion survival and metastasis.

This study discovered the biological functions of hnRNP A2/B1 in normal development and in cancer and identified the alternative splicing events it regulates. Furthermore, the therapeutic potential of our future discoveries is immense. Currently only a handful of drugable targets to treat cancer are available. The potential of identifying critical splicing events that are essential for cancer initiation or progression and can be modified by splice-site specific competitive oligonucleotides have great promise for future anti cancer therapy.

622 B The role of the simtron, miR-1225, and its host gene, PKD1, in autosomal dominant polycystic kidney disease

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the leading genetic cause of end stage renal disease and affects 1:1000 individuals worldwide. Currently, there is no cure for ADPKD. ADPKD has variable severity and heterogeneity, which emphasizes the need for the identification of disease modifiers and new therapeutic targets. The gene *PKD1* is responsible of 85% of ADPKD cases and contains the microRNA miR-1225 within highly conserved intron 45. However, the role of miR-1225 has not been examined in ADPKD pathogenesis. Here, we investigate the coexpression of miR-1225 and its host gene, *PKD1* and the potential functional role of miR-1225 in ADPKD. We find that miR-1225 expression is regulated in a tissue-specific manner in humans and is inversely related to *PKD1* expression during mouse kidney development. Also, we discovered that the SR protein SRSF5 is a regulator of miR-1225 biogenesis. In order to investigate the role of miR-1225 in ADPKD, we tested human pathogenic mutations in *PKD1* intron 45 and determined their affect on miR-1225 abundance and mRNA splicing. To further elucidate a possible role for miR-1225 in ADPKD *in vivo*, we designed antisense oligonucleotides (ASOs) that alter miR-1225 abundance without affecting *PKD1* pre-mRNA splicing and are testing them in mice. These data suggest a potential role for miR-1225 in disease pathogenesis along with its host gene, *PKD1*.

623 C Interplay of oncogenic transcription factor b-catenin with SR protein SRSF3 contributes to the alternative splicing of tumor-related genes

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b-Catenin regulates the expression of many genes critical for cell proliferation and fate determination during development and stem cell maintenance under the control of Wnt signaling pathway. Constitutively active b-catenin was found in epithelial cancers including colon cancer, which indicates it as an important regulator for cancer initiation and progression. Interestingly, transcription factor b-catenin could also binds RNA, so it could influence altered transcriptome by regulating the splicing step of gene expression. Here we demonstrate that mutant b-catenin found in colon cancer cells significantly alters the splicing patterns of tumor-related genes, including p53 tumor suppressor gene. b-Catenin recognizes and associates near the alternative splicing site and regulates the alternative splicing. Interestingly, SRSF3 protein occupied the same alternative exon of p53 and repressed the alternative splicing, which could be abrogated by b-catenin. Such a crosstalk of the oncogenic transcription factor and splicing regulator could contribute globally to multitudes of genes important for tumorigenesis. We will discuss on a novel mechanism for b-catenin to regulate alternative splicing, and propose the model how b-catenin enhance the oncogenic RNA expression profiles in cancer cells. It will explain partially why b-catenin have a greater impact on oncogenic RNA expression network. Finally, we hope to provide new concepts in the therapeutic intervention of cancer development, targeting aberrant RNA binding property of b-catenin protein in cancer cells.

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624 A Exploring the in vivo functions of the mammalian tRNA ligase

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Our laboratory has recently identified an elusive protein complex in human cells displaying tRNA ligase activity. The human tRNA ligase is a pentamer consisting of the catalytic subunit HSPC117, the DEAD box helicase Ddx1 and three proteins of unknown function - CGI-99, Fam98B and Ashwin. Only cells depleted of the essential subunit HSPC117 fail to complete tRNA splicing.

We envision additional roles for the mammalian tRNA ligase in RNA processing and RNA metabolic pathways, particularly given the more extensive functions of tRNA ligases in other organisms that include for example *hac1* mRNA splicing during the unfolded protein response as well as RNA repair. To elucidate the *in vivo* function of the mammalian tRNA ligase, we generated an HSPC117 knock-out mouse. Since the complete knock-out of HSPC117 leads to early embryonic lethality, we are currently focusing on the depletion of HSPC117 in the brain and cells of the immune system. Mammalian RNA ligation and RNA ligase enzymes are relatively unexplored. We hope that our mouse models will provide insights into the role of mammalian RNA ligation in diverse cellular functions, and potentially connect the failure of these functions to human disease.

625 B The FUS protein is required for cell proliferation

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FUS/TLS (fused in sarcoma/translocated in liposarcoma) protein, a ubiquitously expressed and highly conserved RNA binding protein, has been linked to a variety of cellular processes from mRNA processing to DNA repair. However, the precise function of FUS is not well understood. Recently, mutations in the FUS gene have been identified in familial and sporadic patients of Amyotrophic Lateral Sclerosis, a fatal neurodegenerative disorder characterized by dysfunction and death of motor neurons.

Based on the observation that some mutations in the FUS gene induce cytoplasmic accumulation of FUS aggregates, we decided to explore a loss-of-function situation (i.e. inhibition of FUS' nuclear function) to unravel the role of this protein. To this purpose, we have generated a SH-SY5Y human neuroblastoma cell line which expresses a doxycycline induced shRNA targeting FUS that efficiently depletes the protein. In order to characterize this cell line, we have characterized the poly(A) fraction by RNA deep sequencing. Preliminary results show that FUS depletion affects both mRNA expression and alternative splicing. Upon FUS depletion 330 genes are downregulated and 81 are upregulated. We also found that 395 splicing isoforms were downregulated, while 426 were upregulated. Currently, we are focusing our attention on the pathways which are mostly affected by FUS depletion. In addition, we are currently characterizing how FUS depletion affects cell proliferation and survival. We find that the lack of FUS impairs cell proliferation but does not induce apoptosis.

Finally, since MEFs and B-lymphocytes derived from FUS knockdown mice display major sensitivity to ionizing radiation and chromosomal aberrations [1,2], we are exploring the effects of DNA damage in FUS-depleted cells by monitoring important components of DNA Damage Response (DDR). Taken together, these studies may contribute to our knowledge of the role of FUS in these cellular processes and will allow us to draw a clearer picture of mechanisms of neurodegenerative diseases.

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626 C The Involvement of miRNA Dysregulation in Amyotrophic Lateral Sclerosis

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ALS is a neurodegenerative disease that specifically affects upper and lower motor neurons leading to progressive paralysis and death. There is currently no effective treatment. Thus, identification of the signaling pathways and cellular mediators of ALS remains a major challenge in the search for novel therapeutic approaches. Recent studies have shown that non-coding RNAs have a significant impact on normal CNS development and onset and progression of neurological disorders. Based on this evidence we specifically test the hypothesis that misregulation of miRNA expression is a common feature in familiar ALS. Hence, we are exploiting human neuroblastoma cell lines either expressing the SOD1(G93A) mutation or depleted from *Fused in Sarcoma* (FUS) as tools to investigate the role of miRNAs in familiar ALS. To this end we performed a genome-wide scale miRNA expression on these cells, using whole-genome small RNA deep-sequencing followed by quantitative real time validation (qPCR). This strategy allowed us to find a group of dysregulated miRNAs, which are predicted to play a role in the motorneurons physiology and pathology. We verified our data on cDNA derived from SOD1-ALS mice models at early stage of the disease and on cDNA derived from lymphocytes from a small group of ALS patients. In the future, we plan to define the mechanisms responsible for the miRNA dysregulation, by silencing or stimulating the signal transduction pathways putatively involved in miRNA expression and regulation.

627 A Differential LMNA splicing leads to metabolic disorders

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In humans, A-type lamins arise from *LMNA* gene by alternative RNA processing. The two main A-type lamins are lamin A and lamin C. Progerin is a truncated version of lamin A protein, involved in Hutchinson-Gilford progeria syndrome (HGPS), a premature aging disease. Most of the times, Progerin expression is due to a single splicing mutation in the exon 11 of *LMNA* gene (c.1824C>T; p.G608G).

Mutant mice carrying the equivalent HGPS splicing mutation in the *Lmna* gene (c.1827C>T; p.G609G) also accumulate progerin and phenocopy the main cellular alterations and clinical defects of HGPS patients. We demonstrate that changes in the splicing ratio between lamin A and progerin are key factors for lifespan since the $Lmna^{G609G/G609G}$ mice lived no longer than 5 months, whereas $Lmna^{G609G/+}$ mice lived up to one year. Strikingly, mice expressing only the lamin C isoform, due to targeted inactivation of lamin A (herein called $Lmna^{LCS/LCS}$ mice), lived longer than wild type mice. The initial characterization of $Lmna^{G609G/G609G}$ and $Lmna^{LCS/LCS}$ mice indicates that lamin isoforms expression influences adipose tissue homeostasis. We observed that progeria mice are lean compared to control mice whereas LCS mice become fat under chow diet. These modifications of body weight are correlated with an alteration of adipose tissue size without changes in food intake. Moreover oxymax analysis showed an increase in O2 consumption in progeria mice compared to the control mice, whereas LCS/LCS mice have a lower O2 consumption. Finally, microarray analysis of white adipose tissue revealed that a set of genes varies in opposite way between progeria and LCS mice. Interestingly, many of these genes are involved in energy metabolism. All these data showed that alterations in LMNA alternative splicing lead to a strong metabolic phenotype, characterized by a dysregulation of adipose tissue homeostasis.

628 B Uncovering the role of microRNAs in SMA

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Spinal muscular atrophy (SMA) is caused by low level of the survival of motor neuron protein (SMN). As a consequence, the α-motor neurons of the spinal cord die causing muscle weakness and paralysis. SMN is ubiquitously expressed and involved in the assembly and maturation of small nuclear ribonucleoproteins (snRNPs) and is thus essential for survival of all cells. The question why low levels of SMN cause a motor neuron-specific disease such as SMA still needs to be answered. An attractive hypothesis is that SMN may help to regulate the transport, translation or stability of mRNAs that are localised to the axon and that a defect in this function may be the primary cause of SMA. In neurons, translationally repressed mRNAs are transported along the dendrites as part of ribonucleoproteins particles (mRNPs). These mRNAs are kept in a translationally repressed state by several factors, including miRNAs. Upon synaptic activation, the miRNAs dissociate from their targets, and local protein synthesis takes place. My aim is to see whether SMN could interplay with miRNAs and regulate the activity of moto-axonal mRNPs. To investigate these aspects, an inducible human neuroblastoma SMN knock down cell line was created and micro RNA levels of certain miRNA candidates (based on a literature search) are being monitored by qPCR under control and knock down conditions. Preliminary results showed only little changes of the candidate micro RNAs in the cell line model. In a next step, motor neurons will be extracted by laser micro dissection from the severe mouse model and deep sequencing analysis will be performed to analyse the miRNA repertoire in these cells. This should help to define the repertoire of miRNAs affected by the loss of SMN in the severe SMA mouse. The localization of these candidate miRNAs in the cells will then additionally be monitored by miRNA in situ hybridisation in the cell line model and in slice cultures.

629 C Anti-inflammatory function of miR-146a in human primary keratinocytes and atopic dermatitis

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Previously, miR-146a has been shown to regulate immune responses in different cell types. We carried out miRNA expression profiling and determined elevated expression of miR-146a in keratinocytes and skin from atopic dermatitis (AD) patients. Our results show that miRNA-146a is up-regulated by proinflammatory cytokines, such as TNF-alpha and IL1-beta, but not by IFN-gamma in primary keratinocytes. To study miR-146a functions further, we transfected miR-146a precursors or inhibitors into primary keratinocytes treated with IFN-gamma, TNF-alpha or IL1-beta. These experiments reveal that miR-146a hinders the capacity of primary keratinocytes to produce several inflammation-related cytokines and chemokines, such as CCL5, IL-8 and IL-6 both in mRNA and protein level. Over-expression of miR-146a suppresses proliferation and cytokine induced apoptosis of primary keratinocytes. mRNA array and pathway analysis of miR-146a-influenced genes demonstrates that miR-146a down-regulates mRNAs encoding proteins from NF-kappaB pathway as well as proteins involved in regulation of apoptosis and proliferation. siRNA inhibition of two miR-146a targets from NF-kappaB pathway, CARD10 and IRAK1 show that both these proteins are needed for production of IL-8, however, only CARD10 influences the expression of CCL5. We also demonstrate that miR-146a-deficient mice acquire similar Th2 type skin inflammation as wild type mice in mouse AD model. Concordant with the *in vitro* studies, elevated expression of IFN-gamma and CCL5 was detected in inflamed skin from miR-146a-/- mice, which indicates that anti-inflammatory function of miR-146a becomes more important in the chronic phase of skin inflammation. Together, our data show that miR-146a has a strong anti-inflammatory effect in human keratinocytes and its expression level may influence the course and intensity of AD-related skin inflammation.

630 A Mutation of a Zinc Finger Polyadenosine RNA Binding Protein Causes Autosomal Recessive Intellectual Disability

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Intellectual disability affects between 1-3% of people across the world. Patients with intellectual disability suffer from significantly subaverage intellectual function (IQ ≤70), which impinges on quality of life. In a collaborative effort, we have recently identified the first gene encoding a polyadenosine RNA binding protein, ZC3H14 (Zinc finger CysCysCysHis domain-containing protein 14), which is mutated in inherited nonsyndromic autosomal recessive intellectual disability. This finding uncovers the molecular basis for disease in these patients 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 RNA binding proteins. Studies of ZC3H14 orthologs in budding yeast and *Drosophila* provide insight into the role of this protein in post-transcriptional regulation of gene expression, specifically in the proper control of 3'-end polyadenylation of mRNA. Despite studies in yeast and *Drosophila*, functional characterization of ZC3H14 in vertebrates is crucial for understanding the role of this protein in brain function and the molecular mechanism underlying intellectual disability in these patients. Therefore, we have developed a conditional *ZC3H14* knockout mouse utilizing the Cre/loxP system to extend our studies to vertebrate ZC3H14 and address our hypothesis that ZC3H14 is required for proper expression of target mRNAs that are critical for neuronal function. We utilized the EIIa-Cre transgenic line, in which Cre-recombinase is expressed ubiquitously, to knockout ZC3H14 expression. EIIa-Cre +, ZC3H14 -/- mice are viable, suggesting that *ZC3H14* is not essential. These *ZC3H14* knockout mice provide us with an optimal model to study the requirement for ZC3H14 in higher order brain function. We are currently performing preliminary analyses to define molecular and functional effects of the loss of ZC3H14 by examining aspects of neuronal morphology, overall brain architecture, and cognitive behavior. Additionally, we will extend our observations from yeast and *Drosophila* and specifically investigate the role of ZC3H14 in 3'-end polyadenylation of mRNA for the first time in the mouse model organism. By understanding how ZC3H14 regulates target mRNAs—and thus their expression—in mice, we can begin to define the role of ZC3H14 in for normal brain function. Our long-term goal is to understand how dysregulation of post-transcriptional control of mRNA in neurons leads to neuronal dysfunction and consequently impaired brain function.

631 B A novel function for MiR-10a in the pathogenesis of atopic dermatitis

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MiR-10 family genes, including miR-10a, are located in the Hox gene clusters and are known to be the regulators for Hox genes, suggesting they might have a role in fetal development. miR-10a is almost nonexistent in embryonic stem cell lines hES1 and hES2 and embryonic carcinoma Ep2102 line. miR-10a expression starts to increase during endodermal differentiation and is quite high in both embryonic and normal adult liver as well as several other inner organs. Additionally, miR-10a has been reported to be expressed in the skin, in conventional and induced regulatory T cells, where it is induced by retinoic acid and TGF-β. Besides other functions, retinoic acid and TGF-β control cell proliferation and promote regulatory T cell differentiation.

Our results demonstrate that miR-10a is upregulated in keratinocytes and skin biopsies from atopic dermatitis (AD) patients compared to healthy individuals. This finding led us to investigate further, whether miR-10a would be an important player in the pathogenesis of AD. First, we stimulated primary keratinocytes and peripheral blood mononuclear cells (PBMCs) with various pro-inflammatory cytokines, TGF-β, retinoic acid, histamine and their combinations. Histamine occurs naturally in mast cells and basophils, and is released upon allergen cross-linking of IgE antibodies and then triggers an inflammatory response. Our experiments revealed miR-10a to be upregulated by histamine in primary keratinocytes, but not by retinoic acid, TGF-β or pro-inflammatory cytokines. In PBMCs, retinoic acid stimulation induced notable upregulation of miR-10a, which was further enhanced by costimulation with TGF-β, whereas histamine treatment had no significant effect. To study the function of miR-10a in inflammatory response of KCs, we overexpressed miR-10a in KCs and observed downregulation of IRAK1 and IL-8 mRNAs. IRAK1 and IL-8 are both mediators of innate immune response involved in regulation of NF-kB pathway, but are not predicted as direct targets of miR-10a. Computational target prediction with Targetscan revealed transcription factor cAMP responsive element binding protein 1, also known as CREB1, and several MAP Kinase pathway genes as relevant and evolutionally conserved targets of miR-10a. Currently, we are studying the function of miR-10a in keratinocytes and verifying the the effect of miR-10a on putative targets. In conclusion, our data suggest that miR-10a has anti-inflammatory function in keratinocytes and migth influence the development of AD.

632 C Splice variant specific blood biomarkers of Parkinson's disease

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Diagnosis of Parkinson's disease (PD) currently relies on assessment of motor symptoms. Distinguishing between PD and atypical parkinsonian disorders (APD) such as progressive supranuclear palsy and multiple system atrophy is sometime difficult based solely on clinical symptoms. Identification of sensitive minimally invasive risk markers would be beneficial so that proper therapeutic intervention may be initiated as early as possible. We used splice variant-specific microarrays to identify mRNAs whose expression is altered in peripheral blood of early-stage PD patients compared to healthy and neurodegenerative disease controls participating in the Diagnostic and Prognostic Biomarkers in Parkinson Disease study. Thirteen splice variants were identified that can be used to distinguish samples from PD patients and controls with 90% sensitivity and 94% specificity[1]. Six splice variants were identified that may be used to classify APD patients with 95% sensitivity and 94% specificity[1]. Preliminary results indicate that 9 of the risk markers may also be used to distinguish PD patients from healthy controls who participated in the Harvard NeuroDiscovery Biomarker study.

Network analysis of splice variant and transcription/RNA stability PD risk markers and genes associated with PD identified the transcription factor HNF4alpha (HNF4a) as a potential therapeutic target for PD. HNF4a plays a role in regulating genes involved in gluconeogenesis, lipid metabolism, and fatty acid metabolism. *Hnf4a* gene expression is up-regulated in the substantia nigra of PD patients[2] and the striatum of Parkinsonian mice[3].

[1]Potashkin et al., PLoS one 7: e43595

[2]Zhang et al., PloS one 5: e11464

[3]Kurz et a;., PloS one 5: e11464

633 A Hepatitis C virus induced up-regulation of miR-27 expression promotes hepatic triglyceride accumulation

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MicroRNAs (miRNAs) are small RNAs that post-transcriptionally regulate gene expression. Their aberrant expression is commonly linked with diseased states, including hepatitis C virus (HCV) infection. To-date, there is a limited understanding of the biological relevance of HCV-induced changes in hepatic miRNA expression. Herein, we demonstrate that HCV replication induces the expression of miR-27 in cell culture and *in vivo* HCV infectious models. Furthermore, we establish that miR-27 overexpression in hepatocytes results in intracellular triglyceride accumulation and larger lipid droplets, as observed by triglyceride assays and coherent anti-Stokes Raman scattering (CARS) microscopy. This triglyceride accumulation coincides with miR-27 mediated down-regulation of host factors with known roles in triglyceride homeostasis, as measured by qRT-PCR. These repressed genes include PPAR-α, a transcription factor regulating the expression of genes associated with fatty acid catabolism, and ANGPTL3, an inhibitor of fatty acid uptake. We further demonstrate that treatment with a PPAR-α agonist, bezafibrate, is able to reverse the miR-27 induced lipid accumulation in Huh7 cells. This miR-27 mediated repression of PPAR-α signaling represents a novel potential mechanism of HCV-induced hepatic steatosis. This link was further demonstrated *in vivo* through the correlation between miR-27 expression levels and hepatic lipid accumulation in HCV infected SCID-beige/Alb-uPa mice. Lastly, overexpression of miR-27 was found to inhibit viral replication in hepatoma cells stably expressing full-length genomic HCV replicon. *Conclusion:* Collectively, our results highlight HCV's induction of miR-27 expression as a novel mechanism of steatosis, as well as the HCV therapeutic potential of miR-27.

634 B Knockdown of human Dyskerin, the protein linked to Dyskeratosis congenita, blocks large ribosomal subunit production and activates p53 via RPL5 and RPL11

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Dyskerin (Cbf5), NOP10, NHP2 and GAR1 are core components of H/ACA snoRNPs and as such, are important for ribosome biogenesis. In yeast, most H/ACA snoRNPs catalyse rRNA pseudouridylation, but two complexes, snR30 and snR10, are instead required for 18S rRNA processing. Utp23 is an snR30-specific protein that is also essential for just small subunit biogenesis. In humans, H/ACA snoRNPs are important for pseudouridylation although little is known about their role in pre-rRNA processing. The human H/ACA snoRNP proteins are also associated with scaRNAs (snRNA modification) and the telomerase complex (telomere maintenance). Mutations in Dyskerin, NOP10 and NHP2 result in a genetic disease, Dyskeratosis congenita (DKC). DKC patients have shorter telomeres, consistent with a telomerase defect. There is, however, evidence that this disease may also arise due to a defect in ribosome biogenesis.

The interaction between the essential yeast 18S rRNA processing factor, Utp23 and snR30 (U17 snoRNA in humans) is conserved in human cells. We now show that human UTP23 is also required for 18S, but not 28S and 5.8S, rRNA processing. In contrast, knockdown of the human H/ACA snoRNP proteins surprisingly lead to a block in the production of the large ribosomal subunit (LSU) RNAs, 5.8S and 28S, but not 18S rRNA processing. Northern blot analysis revealed that depletion of Dyskerin, NOP10, NHP2 or GAR1 blocks the primary cleavage in ITS1 at site 2. Similar defects are seen after the knockdown of LSU biogenesis factors, such as BOP1 or RBM28. Our data therefore suggest that the function of human UTP23 is separate from that of the H/ACA snoRNPs.

Several genetic diseases, such as Diamond Blackfan anemia, are due to defects in ribosome biogenesis. These defects are proposed to result in the accumulation of free ribosomal proteins, RPL5 and RPL11, which bind and suppress the activity of HDM2, a suppressor of p53. We show that knockdown of Dyskerin in MCF7 cells leads p53 activation in an RPL5- and RPL11-dependent manner. Taken together, our data indicate that Dyskerin, and the H/ACA snoRNPs are essential for LSU biogenesis in humans. This is in contrast to yeast where they are essential for SSU production. Furthermore, we also speculate that DKC patients are likely to have defects in ribosome biogenesis which result in increased p53 levels.

635 C Exonic splicing mutations in Mendelian disorders are more prevalent than currently estimated: the example of Lynch syndrome-associated MLH1 exon 10 variants

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The identification of a causal mutation is essential for molecular diagnosis and clinical management of Mendelian diseases. Even if new-generation exome sequencing has greatly improved the detection of exonic changes, the biological interpretation of most exonic variants remains challenging. More often than not, the impact of exonic variants is evaluated by assuming a direct effect on protein sequence. However, it is currently known that exonic variants can also affect RNA maturation, stability and/or translation.

To evaluate the prevalence of RNA splicing mutations among disease-associated exonic variants we use *MLH1*, a gene implicated in hereditary colorectal cancer (also called Lynch Syndrome), as a model system. Here, we analyzed the effect on splicing of all single-substitutions reported on the Leiden Open Variation Database in the exon 10 of *MLH1* (n=22), including 15 missense, 3 nonsense and 4 synonymous mutations. *Ex vivo* splicing assays with representative minigenes revealed that 17 out of the 22 mutations had an impact on splicing. We detected the following alterations: (i) creation of an internal splice site, (ii) increased exon 10 inclusion, and (iii) increased exon 10 skipping. Remarkably, a significant number of variants located away from splice sites increased/decreased exon 10 inclusion (n=3/n=7), an effect that could not be predicted by commonly used bioinformatics approaches. These variants were further analyzed by using a completely heterologous minigene particularly sensitive to the presence of splicing regulatory sequences. Our results indicate that indeed these mutations alter exonic splicing regulatory elements. This observation led us to test a newly developed in silico tool aiming at detecting exonic splicing regulatory elements. With very few exceptions, we found the predictions produced by this new tool to be concordant with our minigene-derived data. When possible, results were also compared with those obtained by analyzing RNA extracted from patients' blood cells, and found to be concordant.

Overall, this study shows that the exon 10 of *MLH1* is very sensitive to splicing mutations, and indicates that the number of exonic splicing mutations in Lynch Syndrome, and very probably in many other Mendelian disorders, may be currently underestimated. Moreover, our results highlight the predictive potential of a newly developed in silico tool in pinpointing exonic variants that affect RNA splicing.

636 A RNase MRP is involved in chondrogenic differentiation

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The ribonuclease mitochondrial RNA processing (RMRP) gene encodes the RNA component of a multi-protein-RNA complex called RNase MRP. This small nucleolar ribonucleoprotein particle is implicated in various cellular processes, including ribosomal biogenesis, mitochondrial RNA cleavage, cell cycle regulation and has been linked to telomerase. Mutations in the RMRP gene cause Cartilage Hair Hypoplasia (CHH). The phenotypic hallmark of CHH is dwarfism. We therefore hypothesized that RNase MRP is involved in chondrogenic differentiation of the growth plate during skeletal development.

To investigate the expression of RNase MRP during growth plate development RNase MRP protein subunits Rpp25, Rpp38 and Rpp40 were immunohistochemically detected in growth plates of 6 week old mice. Identical distribution patterns were observed: resting zone chondrocytes expressed RNase MRP proteins. Weak expression levels were observed in proliferative chondrocytes. Expression of RNase MRP proteins was clearly detectable in hypertrophic chondrocytes. This temporospatial expression was confirmed using *in vitro* culture models for chondrogenic differentiation. In chondrogenic ATDC5 and human bone marrow stem cells RMRP, Rpp25, Rpp30 and Rpp40 expression was upregulated from day 14 in differentiation onward, simultaneously with markers for chondrocyte hypertrophy. Chondrocyte proliferation and differentiation has shown to be tightly regulated by parathyroid hormone-related peptide (PTHrP), by preventing terminal differentiation of chondrocytes. We therefore investigated the effect of PTHrP on RMRP expression during chondrocyte differentiation in ATDC5 cells. PTHrP-mediated inhibition of chondrogenic differentiation displayed a dose dependent decrease in RMRP levels, indicating a relation between PTHrP, chondrogenic differentiation and RMRP expression. Interfering with RMRP expression using RMRP-specific siRNAs resulted in impaired chondrogenic marker expression. In addition, RMRP knockdown resulted in increased expression of early chondrogenic transcriptional regulator Bapx1/Nkx3.2, suggesting a regulatory role for RMRP in determining chondrocyte differentiation. Moreover, bone morphogenic protein-2, a known stimulator of hypertrophy, increases RMRP expression during chondrogenic differentiation of ATDC5 cells. Finally, transdifferentiation of CHH patient-derived dermal fibroblasts into chondrocyte-like cells indicated a more than 50% reduced Col10a1 expression in patient-derived fibroblasts as compared to control cultures.

In conclusion, our data indicate that RNase MRP is involved in chondrogenic development of the growth plate and appears to be predominantly associated with terminal hypertrophic differentiation. In addition, our data provide novel insight into the underlying molecular mechanism causing the CHH-associated skeletal phenotype.

637 B TDRD3 recruits FMRP and the topoisomerase TOP3ß to spliced mRNAs and provides a molecular link between schizophrenia and fragile X syndrome

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The fragile X mental retardation protein (FMRP) affected in fragile X syndrome is a key regulator of eukaryotic mRNA translation. However, how it is recruited into target mRNA-protein complexes (mRNPs) remains enigmatic. We have previously identified an interactor of FMRP, the Tudor domain containing protein TDRD3, whose binding is disrupted by the highly pathogenic I304N missense mutation of FMRP. TDRD3 also associates with the exon junction complex (EJC) and binds asymmetrically dimethylated arginines (aDMA) in histones and the C-terminal domain of RNA-polymerase II. Therefore, we reasoned that TDRD3 might function in the recruitment of FMRP to mRNPs. Here we report that FMRP and TDRD3 form a complex with a third component, the DNA topoisomerase TOP3β. Interestingly, deletion of the gene encoding TOP3β is associated with schizophrenia and intellectual disability. We provide evidence that this enzyme, previously implicated only in the unwinding of DNA, is a cytoplasmic RNA binding protein (RBP). Thus, the etiologies of schizophrenia and FXS might intersect at the post-transcriptional regulation of mRNPs containing TOP3β and FMRP. Interestingly, formation of the TOP3β-TDRD3-FMRP complex and its ability to recognize aDMA are essential for the concomitant integration of TOP3β and FMRP into mRNPs. The biochemical composition of these mRNPs and their association with polyribosomes further suggests that they are engaged in the pioneer round of translation. Hence, our data uncover a novel mechanism for the recruitment of FMRP into mRNPs that is independent of RNA cis-elements and provide a molecular link between two neuropsychiatric disorders.

638 C Spliceosome Integrity is Defective in the Motor Neuron Diseases ALS and SMA

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Two motor neuron diseases, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), are caused by distinct genes involved in RNA metabolism, TDP-43 and FUS/TLS, and SMN, respectively. However, whether there is a shared defective mechanism in RNA metabolism common to these two diseases remains unclear. Here, we show that TDP-43 and FUS/TLS localize in nuclear Gems through an association with SMN, and that all three proteins function in spliceosome maintenance. We also show that in ALS, Gems are lost, U snRNA levels are up-regulated and spliceosomal U snRNPs abnormally and extensively accumulate in motor neuron nuclei, but not in the temporal lobe of FTLD with TDP-43 pathology. This aberrant accumulation of U snRNAs in ALS motor neurons is in direct contrast to SMA motor neurons, which show reduced amounts of U snRNAs, while both have defects in the spliceosome. These findings indicate that a profound loss of spliceosome integrity is a critical mechanism common to neurodegeneration in ALS and SMA, and may explain cell-type specific vulnerability of motor neurons (*Tsuiji et al., EMBO Molecular Medicine, in press*). We also found that TDP-43 lacking its Gly-rich region can regulate splicing of one of its target *Sortilin1*, but does not localize in Gem, does not associate with snRNPs, and shows reduced activity to down-regulate the expression of its own mRNA. These results indicate that the C-terminal Gly-rich region of TDP-43, in which most ALS-mutations reside, is important for full activity of TDP-43 and may play crucial role in neurodegeneration. To verify our hypothesis, we generated knock-in mice with TDP-43 lacking the Gly-rich region, and the expression of TDP-43 lacking the Gly-rich region in brain and spinal cord was confirmed. Further progress will be discussed.

639 A The RNA-binding protein Quaking critically regulates vascular smooth muscle cell phenotype

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Rationale: RNA-binding proteins are critical regulators of post-transcriptional RNAs, and can influence pre-mRNA splicing, mRNA localization, and stability. The RNA-binding protein Quaking (QKI) is essential for embryonic blood vessel development. However, the role of QKI in the adult vasculature, and in particular in vascular smooth muscle cells (VSMC), is currently unknown.

Objective: We sought to determine the role of the RNA-binding protein Quaking (QKI) in regulating adult VSMC function and plasticity.

Methods and Results: We identified that the RNA-binding protein Quaking (QKI) is highly expressed by neointimal VSMCs of human coronary restenotic lesions, but not in healthy vessels. In a mouse model of vascular injury, we observed reduced neointima hyperplasia in Qk^{v} mice, which have decreased QKI expression. Concordantly, abrogation of QKI attenuated fibroproliferative properties of VSMCs, while potently inducing contractile apparatus protein expression, rendering non-contractile VSMCs with the capacity to contract. We identified that QKI localizes to the spliceosome, where it interacts with the myocardin pre-mRNA and impacts the myocd_v3 / myocd_v1 balance. This shift in the transcriptional coactivation activity of Myocardin following arterial damage is tightly coupled with QKI expression levels.

Conclusions: We propose that QKI is a central regulator of VSMC phenotypic plasticity and that intervention in QKI activity can ameliorate pathogenic, fibroproliferative responses to vascular injury.

640 B Investigating the role and regulation of microRNA-10a in Acute Myeloid Leukaemia bearing the Nucleophosmin1 mutation

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An increasing body of evidence has demonstrated the role of microRNAs in cancers. Acute Myeloid Leukemia (AML) associated with *Nucleophosmin1* gene mutation (*NPM1c+*) accounts for a third of AML cases (1). Our previous work has reported a unique microRNA signature in AML-*NPM1c+* samples which is dominated by 19.6-fold increase in hsa-miR-10a expression in the *NPM1c+* AML compared to wild type (wt) samples (2). We have demonstrated the role of miR-10a in apoptosis using human OCI-AML3 cell line, which is the only cell line known to harbour *NPM1c+*. The knock down of miR-10a results in an increase in cell death and a decrease in colony forming potential. These effects thought to be mediated through miR-10a targeting of *ARNT, GTFH1, ID4, KLF4, MAPRE1, NR4A3, RB1CC1* and *TFAP2C* (confirmed by luciferase assay), all of which are associated with neoplastic transformation. This suggests that miR-10a may act as a pro-survival factor *in vitro*. We aim to expand our study on miR-10a's leukaemic role in AML by knockdown of miR-10a in AML clinical samples. MiR-10a has been previously reported to be negatively regulated by methylation (3). To explore the mechanism that leads to the dysregulation of miR-10a in *NPMc+*AML, we assess the role of methylation of the CpG islands, which is located within 2kb upstream of the miR-10a promoter. Mutations in *DNA methyltransferase, DNMT3A* has been frequently found in *NPMc+*AML (4), leading to our investigation if *DNMT3A* mutations are associated with differential methylation patterns in *NPM1c+*AML, explaining the specificity of miR-10a overexpression in this AML subtype.

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641 C RNA binding protein FUS acts to mediate nuclear-mitochondrial communication

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Genetic mutations in & dysregulation of the gene encoding Fused in Sarcoma (FUS) have been associated with FUS proteinopathies, neurodegenerative disorders including ALS and frontotemporal lobar degeneration with FUS pathology (FTLD-FUS). However, cellular defects underlying FUS proteinopathies are unclear. Our recent work show sthat FUS expression leads to increased fragmentation of mitochondria and production of reactive oxygen species (ROS). Immunoelectron microscopy (IEM) and biochemical fractionation experiments reveal that FUS is detected not only in the nucleus but also in association with mitochondria. Increased FUS protein levels accompanied by marked mitochondrial damage have been detected in brain samples of FUS proteinopathy. Screening genetic modifiers of FUS-induced neurodegeneration using transgenic flies expressing the human FUS led to identification of mitochondrial protein HSP60 whose downregulation partially rescued FUS-induced neurodegeneration. Our study uncovers a previous unknown role of FUS in mediating nuclear-mitochondrial communication. Our data provide evidence for mitochondrial damage induced by FUS expression and suggest that mitochondrial defects may be an early event in FUS proteinopathies. Slowing down or reversing mitochondrial damage may provide therapeutic benefits in treating these devastating diseases. Mechanisms underlying FUS-induced mitochondrial impairment and implications of our findings will be discussed.

642 A FUS mutations strongly promote FUS-SMN and FUS-RNAP II interactions

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The motor neuron disease ALS has no known treatment and disease mechanisms are not understood. Previously, we characterized the interactome of the ALS-causative protein FUS and discovered that FUS associates abundantly with U1 snRNP and SMN, the protein deficient in the motor neuron disease spinal muscular atrophy (SMA). These and other data indicate that ALS and SMA share a molecular pathway (Yamazaki et al., Cell Reports 2012, 2:799). To gain insights into the function of FUS and its role in ALS, we have now carried out extensive analysis of the FUS interactome using FUS antibodies recognizing different epitopes. We found that FUS associates with RNAP II as well as several transcription-related proteins. To gain insights into the potential roles of these factors in ALS pathogenesis, we analyzed their interactions with FUS deletion mutants. As reported previously (Wang et al Nature 2008, 454:126), our data indicate that FUS forms an intramolecular interaction in which its N and C terminal regions bind to one another. We find a striking increase in the association of both SMN and RNAP II with FUS when it contains deletions that disrupt this intramolecular interaction. U1 snRNP binds with similar efficiency to full length FUS and the FUS mutants. Moreover, we find that the N-terminal prion-like domain and the C-terminal RGG-rich region show increased levels of binding to a number of proteins in nuclear extract. Thus, ALS-causing mutations in these regions of FUS could be pathogenic due to the increased interaction with SMN, RNAPII, and/or the proteins that we are in the process of identifying.

643 B Endogenous shRNA Induces the Large-scale Trans-determination of Mesenchymal Stem Cells into Hematopoietic Stem Cells with High Purity.

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Our preliminary studies in 2008 discovered 2564 new endogenous short hairpin RNAs (shRNAs) in human by bioinformatics and solexa sequencing, one (termed as shR-EID1 or shR-337) of which has been found to induce conversion of MSCs into HSCs by inhibiting the expression of EIA-like inhibitor of differentiation 1(EID1). EID1 interacts with CREB binding protein (CBP) and p300, which have at least 400 interacting protein partners (25), and these proteins play a crucial role during hematopoiesis. Mesenchymal stem cells (MSCs) can differentiate into cells of bone, endothelium, adipose tissue, cartilage, muscle, and brain. However, whether they can transdeterminate into hematopoietic stem cells (HSCs) remains unsolved.

We report here that human MSCs with CD44+,CD29+, CD105+, CD166+,CD133-,and CD34- could differentiate into hematopoietic stem cells (CD150+/CD49f+/CD133+/CD34+) and their descending blood cells in vitro, when transfected with a new endogenous shRNA. The sRNA was high-effectively delivered into MSCs by a novel peptide means. These induced MSC-HSCs could form different types of hematopoietic colonies as nature-occurring HSCs did. Upon transplantation into sublethally irradiated NOD/SCID mice, these MSC-HSCs engrafted and differentiated into all hematopoietic lineages such as erythrocytes, lymphocytes, myelocytes and thrombocyte. Furthermore, we demonstrated the first evidence that the transdetermination of MSCs was induced by acetylation of histone proteins and activation of many transcriptional factors. These transfected MSCs could be converted into other types of stem cells in the presence of appropriate cytokines. Together, our findings identify the endogenous shRNA that dictates a directed differentiation of MSCs toward HSCs and open up a new source for HSCs used for the treatment of blood diseases and artificial stem cell-made blood.

644 C Deep-sequencing of the Peach Latent Mosaic Viroid Reveals New Aspects of Population Heterogeneity

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Viroids are small circular single-stranded infectious RNAs that are characterized by a relatively high mutation level. Knowledge of their sequence heterogeneity remains largely elusive and previous studies, using Sanger sequencing, were based on a limited number of sequences. In an attempt to address sequence heterogeneity from a population dynamics perspective, a GF305-indicator peach tree was infected with a single variant of the *Avsunviroidae* family member *Peach latent mosaic viroid* (PLMVd). Six months post-inoculation, full-length circular conformers of PLMVd were isolated and deep-sequenced. We devised an original approach to the bioinformatics refinement of our sequence libraries involving important phenotypic data, based on the systematic analysis of hammerhead self-cleavage activity. Two distinct libraries yielded a total of 2,186 different PLMVd variants. Sequence variants exhibiting up to ~20% of mutations relative to the inoculated viroid were retrieved, clearly illustrating the high level of divergence dynamics within a unique population. Moreover, we show that ~50% of positions remained perfectly conserved, including several small streches as well as a small motif reminiscent of a GNRA tetraloop which are the result of various selective pressures. Using a novel hierarchical clustering algorithm, the different variants harvested were subdivided into either 7 or 8 clusters depending upon the library analyzed. We found that most sequences contained an average of 4.6 to 6.3 mutations compared to the variant used to initially inoculate the plant. This study provides a reliable pipeline for the treatment of viroid deep-sequencing. It also sheds new light on the extent of sequence variation a viroid population can sustain, and which may give rise to a quasi-species.

645 A Reduced HBsAg expression in occult HBV infection: alteration of a post-transcriptional regulatory mechanism?

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Background and aims. Occult hepatitis B virus (HBV) infection (OBI) is defined as low plasma level of HBV DNA with undetectable HBV surface antigen outside the pre-seroconversion window period. Previous work suggested that some OBI might be associated with various mutations in genomic regulatory elements negatively affecting viral replication. Recent studies suggested that Pre-S2/S mRNA splicing might be essential for S protein expression. On the other hand, such splicing must be strongly controlled to prevent the accumulation of nonfunctional spliced S mRNA. The functional relationship between S mRNA splicing and S protein expression and the potential negative effect of specific mutations was investigated in OBI carriers.

Methods. The Pre-S2/S mRNA 5' splice donor site sequence of 176 OBI and 381 HBsAg+ control strains (genotype A-E) were analyzed. The influence of mutations on local RNA folding was evaluated using MFOLD program. The effect of selected mutations on splicing and S protein expression was tested by transient transfection experiments in Huh7 cells with cloned S gene with or without the distant HBV posttranscriptional regulatory element (PRE) of OBIs and controls, as well as mutated whole genome.

Results. In vitro S mRNA splicing was confirmed irrespective of HBV genotype and was effective whether an autologous or heterologous promoter controlled S gene transcription. Splicing positions seemed to be mainly conserved across strains but splicing variants were also found. Irrespective of HBV genotype, 51% of OBI sequences presented substitutions adjacent to the 5' splicing donor site compared to 31% of controls ($P<0.05$). These substitutions were predicted to disrupt a putative stem-loop structure in 54% of OBI variants. OBI strains appeared to be competent in S protein expression. However, the amount of HBsAg in culture supernatant was significantly lower in the majority of cells transfected with the S sequences from OBI strains when compared to HBsAg+ strains. Mutating the 5' donor site prevented splicing and resulted in significant reduction of HBsAg production. Similarly, the double mutation A453G/G463A observed in two OBI strains interfered with splicing and HBsAg production.

Conclusions. These preliminary data indicate that the sequence and context surrounding a 5' splicing site within the S gene is important for efficient HBV S mRNA expression. This region within the S gene appears to be a new post-transcriptional regulatory element in addition to PRE essential for the efficient expression of unspliced S mRNA among HBV strains.

646 B Mechanistic insights into EMCV-IRES translation initiation

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Most of eukaryotic mRNAs are translated by the cap-dependent pathway which relies on the recognition of the cap structure. This is mediated by the eIF4F complex which contains the eIF4A helicase and the eIF4G platform which coordinates 40S and mRNA association. Viruses have developed many ways to hijack their host translation machinery, among which, internal entry of the ribosome which allows a direct recruitment of the initiation complex near or at the AUG codon, without requiring a 5'-cap. This mechanism relies on RNA structures called IRESes (Internal Ribosome Entry Sites), generally present in the 5'-UTR. Although numerous viral IRESes have been identified, they differ from one another, leading to the hypothesis that many different mechanisms exist. Initiation of translation on type II IRESes such as those present in the Encephalomyocarditis Virus (EMCV) and in the Foot and Mouth Disease Virus (FMDV) recruit eIF4G/A which in the presence of ATP restructures the border of the IRES. These conformational changes are thought to allow the recruitment of 43S pre-initiation complexes *via* the eI4G/eIF3 interaction. However, eIF4G mutants lacking the ability to contact eIF3 efficiently promote initiation on EMCV-IRES. This prompted us to evaluate a direct binding of the 40S subunit to EMCV-IRES. By using a combination of approaches we were able to demonstrate that EMCV-IRES directly binds the 40S subunit leading to a stable binary complex. Deletion mutants show that the region between the H and L domains is necessary for optimal 40S binding which is in agreement with their requirement for optimal IRES activity. This was further confirmed by footprinting analysis that identifies the H domain as part of the 40S binding site. In addition, as previous results on EMCV translation indicated that the nature of the downstream gene interferes with translation efficiency, we analyzed 40S binding from constructs presenting different open reading frames. Toe-print analysis of these RNAs in the presence of 40S subunits unravels a specific structure in the open reading frame that influences positioning of the mRNA in the 40S decoding groove. Taken together, these results allow us to refine the model for EMCV-IRES translation initiation.

647 C Modulation of HIV-1 gene expression by binding of UHM-containing splicing factors to a ULM motif in the Rev protein

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Human immunodeficiency virus type 1 (HIV-1) is a lentivirus encoding the Gag, Pol and Env proteins common to all retroviruses and several specific regulatory proteins. The HIV-1 regulatory protein Rev is essential for virus replication and ensures the expression of partially spliced and unspliced transcripts. Rev binds to the Rev-responsive element (RRE) in viral mRNAs in the nucleus and recruits factors required for the export of these viral mRNAs to the cytoplasm. Numerous additional functions have been shown for Rev and Rev has also been implicated in regulating the splicing of viral transcripts.

We identified a ULM-like motif in the RRE-binding region of the Rev protein. ULMs (UHM ligand motifs) mediate protein-protein interactions and modulate spliceosome assembly through their binding to UHMs (U2AF homology motifs). We therefore investigated whether the Rev ULM can interact with UHMs present in host splicing factors. Isothermal titration calorimetry and NMR titration experiments showed that Rev ULM binds to the UHMs of SPF45 and U2AF65. The crystal structure of the SPF45-UHM bound to the Rev ULM revealed that the Rev ULM adopts an extended conformation upon binding to SFP45. Structural analysis and biochemical experiments demonstrate that the highly conserved W45 in the Rev ULM is crucial for Rev-UHM interactions. Moreover, W45 was shown to ensure the ability of SPF45 to displace single Rev subunits from Rev-RRE SLIIB oligomeric complexes. Finally, we show, that W45 is important for proper processing of HIV transcripts in human cells. We propose that W45 and thus Rev-ULM interactions with UHM splicing factors contribute to the regulation of HIV-1 splicing and gene expression.

648 A HMGA1 interaction with HIV-1 TAR modulates basal and Tat-dependent HIV transcription.

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During transcription of the HIV genome the transactivating response element (TAR) located in the nascent viral transcript is playing a key role as a binding platform for host cellular co-factors as well as the viral transactivator of transcription (Tat). The Tat/TAR interaction is involved in the activation and recruitment of the host cellular positive transcription elongation factor b (P-TEFb), which subsequently releases the transcriptional block of RNA polymerase II in order to activate efficient viral transcription elongation.

We have recently identified the chromatin master regulator HMGA1 to interact with 7SK non-coding RNA in a regulatory fashion [1,2]. Here we show a highly specific interaction between HMGA1 and HIV-1 TAR, which leads to a reduction of HIV-1 promoter activity both in the absence and in the presence of Tat. HMGA1 competes with Tat for TAR binding and 7SK RNA competes with HIV-1 TAR for HMGA1 binding. These results support a model, in which HMGA1 blocks the binding of transcription activating factors --- such as Tat --- to TAR [3].

Thus, the interaction of HMGA1 with HIV-1 TAR might contribute to viral latency, which is the main reason for the inability of HAART to cure HIV infection.

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2. Eilebrecht, S., Becavin, C., Leger, H., Benecke, B.J. and Benecke, A. (2011) HMGA1-dependent and independent 7SK RNA gene regulatory activity. *RNA Biol*, **8**.
3. Eilebrecht S., Wilhelm E., Benecke B.-J., Bell B. and Benecke A. (2013) HMGA1 directly interacts with TAR to modulate basal and Tat-dependent HIV transcription. *RNA Biol*, **in press**.

649 B Thermodynamics of HIV-1 Reverse Transcriptase in action elucidates the mechanism of action of non-nucleoside inhibitors

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HIV-1 reverse transcriptase (RT) is a heterodimeric enzyme that converts the genomic viral RNA into proviral DNA. Despite intensive biochemical and structural studies, direct thermodynamic data regarding RT interactions with its substrates are still lacking. Here we addressed the mechanism of action of RT and of non-nucleoside RT inhibitors (NNRTIs) by isothermal titration calorimetry (ITC). Using a new incremental-ITC approach, a step-by-step thermodynamic dissection of the RT polymerization activity showed that most of the driving force for DNA synthesis is provided by initial dNTP binding. Significant differences are observed between nucleotide incorporation in elongation (RT bound to a DNA duplex) and in initiation (RT bound to the HIV-1 Primer Binding Site RNA/tRNALys(3) duplex) of reverse-transcription. Surprisingly, thermodynamic and kinetic data led to a complete re-interpretation of the mechanism of inhibition of NNRTIs. Binding of NNRTIs to preformed RT/DNA or RNA complexes is hindered by a kinetic barrier and NNRTIs mostly interact with free RT. Once formed, RT/NNRTI complexes bind NA either in a seemingly polymerase-competent orientation, or form high-affinity dead-end complexes, both RT/NNRTI/DNA or / RNA complexes being unable to bind the incoming nucleotide substrate.

650 C Domain interactions in adenovirus virus-associated RNA I mediate high-affinity PKR binding

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Adenovirus virus-associated RNA I (VA I) is a short, non-coding transcript that functions to inhibit the activity of protein kinase R (PKR), a component of the innate immunity pathway. PKR recognizes dsRNA and other RNAs containing duplex regions and is activated via dimerization and autophosphorylation. VA I contains three domains: an apical stem-loop, a highly-structured central domain, and a terminal stem. Although all three domains contain duplex regions that are of appropriate length for PKR recognition, previous work suggests that PKR binding is localized to the apical stem and central domain regions. We have characterized the stiochiometry and affinity of PKR binding to VA I and several deletion constructs using analytical ultracentrifugation. PKR binds to wild-type VA I with a 1:1 stoichiometry and a surprisingly high affinity (Kd = 334nM). Although PKR is capable of binding the isolated terminal stem, deletion of this domain does not affect PKR binding affinity or inhibition of autophorylation. Consistent with this observation, chemical probing data show that the secondary and tertiary structure of the apical stem and central domain do not change upon removal of the terminal stem. PKR is capable of binding to the isolated apical stem, albeit with greatly-reduced affinity, but does not bind to the isolated central domain. These results indicate that interactions between the apical stem and the central domain are necessary to form a high-affinity PKR binding site. Our data support a model whereby VA I functions as a PKR inhibitor because it binds a monomer tightly but does not permit PKR dimerization.

651 A Phosphorylation of hepatitis C virus RNA polymerase Ser29 and Ser42 by PRK2 regulates HCV replication

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Post-translational phosphorylation has important roles in regulating the structure and function of proteins and modulating protein-protein interactions for the rapid regulation of phosphosignaling pathways. In virus-infected cells, the function, stability, and subcellular localization of virus-encoded proteins can be altered by host kinase-mediated phosphorylation. Indeed, growing numbers of virus-encoded phosphoproteins have been reported recently and they are implicated in viral pathogenesis, virion assembly, and genome replication. For plus-strand RNA viruses, the viral RNA genome is replicated by virus-encoded RNA-dependent RNA polymerases (RdRps). RdRps of several plus-strand RNA viruses, including the hepatitis C virus (HCV), are phosphoproteins, and the phosphorylation of some viral RdRps has been shown to play an important role in viral replication.

The nonstructural protein (NS) 5B protein of HCV is the viral RdRp essential for viral RNA genome replication. We have previously shown that HCV viral genome replication is functionally linked to NS5B phosphorylation status regulated by the protein kinase C-related kinase 2 (PRK2). Here, we use a combination of biochemical studies and reverse genetics to show that the phosphorylation of HCV NS5B is required for viral RNA replication. Phosphoamino acid analysis of both *in vitro* and metabolically-labeled NS5B showed that PRK2 phosphorylates NS5B exclusively at the serine residues. By *in vitro* kinase assays using a series of deletions and site-specific mutants of NS5B and mass spectrometry analysis, we identified two PRK2 phosphorylation sites, Ser29 and Ser42, on the Δ1 finger loop region of NS5B that extensively interacts with the thumb subdomain of NS5B. Colony-forming assays using drug-selectable HCV subgenomic RNA replicons revealed that the prevention of phosphorylation by Ala substitution at either Ser29 or Ser42 impairs HCV RNA replication. Furthermore, reverse genetics studies using HCV infectious clones with mutations at Ser29 or Ser42 showed that preventing phosphorylation resulted in the suppression of viral replication. Molecular modeling revealed that phosphorylation of NS5B stabilizes the interactions between the Δ1 loop and the thumb subdomain, which are important for the formation of the closed conformation of NS5B known to be important for *de novo* RNA synthesis. Collectively, our results provide evidence that HCV NS5B phosphorylation has a regulatory role during the HCV RNA replication process.

652 B Structural rearrangements of HIV-2 RNA during dimerization

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Human retroviruses HIV-1 and HIV-2 are characterized by similar genome organization, virion structure and replication cycle. Like other retroviruses, they possess dimeric RNA genome assembled from two identical sense strands, interacting near their 5′-ends. The full-length HIV-2 RNA serve both as genomic RNA which are packaged into nascent virions and as the template for translation of Gag and Gag-Pol precursor polyproteins. HIV-2 dimerization and encapsidation signals are linked and located in the leader RNA region (5' UTR). Dimerization is a regulated process that involves several RNA structural changes governed by the Gag and NC proteins.

In order to gain a deeper understanding of HIV-2 RNA dimerization and packaging, we applied chemoenzymatic probing to the in vitro assembled loose dimer. Our studies indicated that the dimeric structure is held by the direct interactions of the palindromic sequences within SL1 and TAR domains. Our combined structural and functional analysis allowed us to characterize previously unknown structural rearrangements and extensive tertiary interactions crucial for the mature dimer formation. It is presumed that in the case of HIV-2, co-tranlational capture of the full-length RNA by nucleocapsid domain of Gag ensures encapsidation specificity Our results on nucleocapsid protein binding provide new insight into mechanism of HIV-2 genomic RNA differentiation from other *gag* mRNA species.

653 C RNA Packaging NTPase is Needed for Transcription in Double-stranded RNA Bacteriophage phi6

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Bacteriophage phi6 has three segments of genomic double-stranded RNA [S (2948bp), M (4063bp) and L (6374bp)] which are enclosed into a polymerase complex (PC), a particle displaying icosahedral symmetry. The PC of phi6 is a delicate enzymatic machinery which can selectively recognize and package the plus-sense single-stranded RNA (ssRNA) genomic precursors, preform minus-stranded synthesis to form dsRNA genomes (replication) and apply dsRNA as a template for plus-stranded synthesis (transcription). phi6 PC is composed of the main structural proteins P1, the RNA-dependent RNA polymerase P2, the packaging nucleoside triphosphatase (NTPase) P4 and the assembly cofactor P7. The hexameric packaging NTPase P4 resides on 5-fold symmetric vertex of the outer surface of PC. It is essential for the PC nucleation during self-assembly and required for phi6 ssRNA packaging and transcription. The particles with ~90% reduced level of P4, referred to P4-deficient particles, can only preform ssRNA packaging and replication but do not display the transcription activity. In this study, we applied the purified P4 on P4-deficient particles for assembly studies. The reconstituted PC products had high occupancy of P4 with a higher velocity in rate-zonal centrifugation than the P4-deficient particles. Further morphological analyses indicated that the P4 reconstitution induced conformation change of the shell from an expanded form to a compact form, which suggested that P4 occupancy level contributed to the dodecahedral symmetry of the empty PC. Our result also indicated that the transcription reaction was dependent on high occupancy of P4 hexamers on PC, which might be attributed to the access provided for export of nascent plus-sense ssRNAs.

654 A DNA habitat and RNA inhabitants: Relevant questions of a qualitative RNA sociology

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Background: Most molecular biological concepts result out of physical chemical assumptions on the genetic code that are basically more then 40 years old. Recent empirical data on genetic code compositions and rearrangements by mobile genetic elements and non-coding RNAs together with results of virus research and their role in evolution does not really fit into these concepts.

Results: If we look at the abundance of regulatory RNAs and persistent viruses in host genomes, the key players that edit genetic code of host genomes are consortia of interacting RNA agents and viruses that drive evolutionary novelty, integration into cellular DNA and regulation of cellular processes in all steps of development. There is increasing evidence that all cellular life is colonized by exogenous and/or endogenous viruses in a non-lytic but persistent lifestyle. They prefer cellular genomes as habitat and determine genetic host (group) identity.

Conclusions: If we take a step away from the mainstream molecular biology (including the physical chemical properties of quasispecies and mutant spectra) and systems biology (non-reductionistic physicalism) and move towards a more consortial thinking of cooperative ensembles of RNA stem loops, viruses, and subviral remnants of former infection events, we should move towards a kind of RNA sociology. Qualitative RNA-sociology emphasizes understanding of social interactions through analysis of active RNA sequences according contextual needs, i.e. the primacy of interactional motifs, that means functions prior to sequence syntax. In this respect the exaptations of former (infection derived) inventions to more appropriate cellular needs fit ideally into these investigations, that means the highly dynamic modular perspective on these interacting agents.

This talk will present some relevant questions of a qualitative RNA sociology that may complete molecular biological terms and methods.

655 B Distinct PPR proteins are responsible for coupling of mRNA editing, polyadenylation and translation in mitochondria of trypanosomes

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The majority of trypanosomal mitochondrial pre-mRNAs undergo massive uridine insertion/ deletion editing which creates open reading frames. However, our recent findings indicated that pre- and post-editing processing steps are also required to produce translation-competent mRNAs. Pre-editing addition of short 3′ A-tails exerts no influence on unedited pre-mRNA stability, but stabilizes transcripts that are edited beyond few initial sites. The post-editing extension of A-tails into A/U heteropolymers by KPAP1 poly(A) polymerase and RET1 TUTase commits fully-edited mRNAs to translation. To identify factors responsible for coupling of editing, polyadenylation and translation we built a comprehensive protein interactions network of respective machineries. The ensuing RNAi screen distinguished several pentatricopeptide repeat-containing (PPR) RNA binding proteins acting to: 1) stabilize mRNA prior to polyadenylation (PPR15); 2) block premature mRNA uridylation (PPR14) and 3) induce transcript-specific adenylation/ uridylation (PPR26 and PPR28). PPR15 is similar to the kinetoplast polyadenylation/ uridylation factor 1 (KPAF1) and is an integral subunit of the polyadenylation complex. PPR14 associates transiently with both KPAP1 and RET1, and is likely to be a membrane-anchored protein. Unexpectedly, PPR26 and PPR28 are stably associated with small ribosomal subunit (SSU) and the polyadenylation complex. Investigation of these PPR proteins suggested that pre-mRNA is initially stabilized by PPR15 binding which may also recruit poly(A) polymerase resulting in A-tail addition. Initiation of RNA editing at the 3′ region displaces PPR15 leaving A-tail as the main cis-acting stability element. We propose that SSU-associated PPR proteins, such as PPR26 and PPR28, recognize specific RNA sequences and stimulate transcript-selective A/U-tailing thereby committing mRNA for translation. Finally, PPR14 is likely to be actively disengaged from fully-edited mRNA to allow for A/U-tail addition.

656 C Identification of RNA targets for the nuclear multidomain cyclophilin atCyp59 and their effect on PPIase activity

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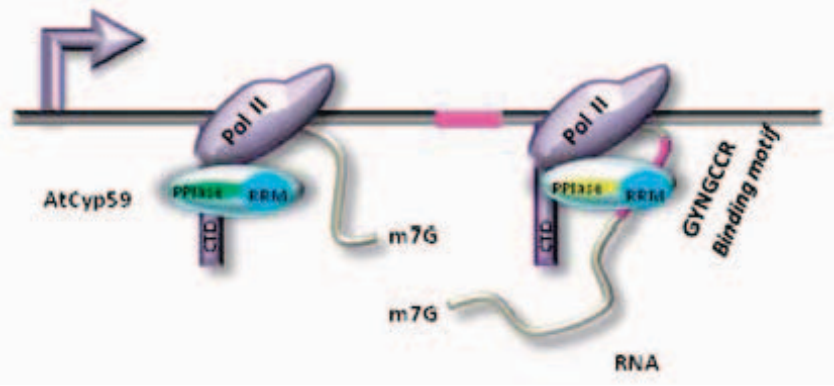
AtCyp59 is a multidomain cyclophilin containing a peptidyl-prolyl cis/trans isomerase (PPIase) domain and an evolutionarily highly conserved RRM domain. It has been shown to bind to the C-terminal repeat domain (CTD) of RNA polymerase II and to influence transcription significantly. To isolate RNA targets of AtCyp59 we employed a genomic SELEX method which is an unbiased approach to select potential RNA binding partners. Analysis of the selected RNAs revealed an RNA binding motif whose binding was verified by gel shift assays *in vitro* and by RNA immunopreciptation assays of AtCyp59 *in vivo.* Interestingly, genome-wide analysis showed that the consensus motif was present in at least 70 % of the annotated transcript and we show that this RNA motif is evolutionarily highly conserved. Most importantly, we show that binding also occurs on unprocessed transcripts *in vivo* and that binding of specific RNAs inhibits the PPIase activity of AtCyp59 *in vitro*. Taken together, the available data suggest that this type of cyclophilins have an important function in transcription regulation. Figure 1 presents a model where in the course of transcription RNA-dependent inhibition of the PPIase activity of AtCyp59 influences RNA polymerase II activity.



657 A Impact of specific RNA Pol II CTD phosphorylation patterns

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The human RNA polymerase II (Pol II) largest subunit contains a C-terminal domain (CTD) with 52 Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 (21 consensus and 31 non-consensus) repeats. Tyr1, Ser2, Thr4, Ser5 and Ser7 residues undergo dynamic phosphorylation and dephosphorylation throughout the transcription cycle in a gene specific manner. Whereas Serine modifications are known to orchestrate the binding of transcription and RNA processing factors to the transcription machinery, the impact of distinct phosphorylation combinations in guiding RNA-processing factors to specific genes remain to be investigated, as well as the role of consensus versus non-consensus heptapeptides.

To address these open questions, we perform in vitro phosphorylation studies and generate CTDs with defined modification patterns, either on a full-length 52 repeat or a shortened construct which lacks the non-consensus queue. Binding targets are identified in mass spectrometry approaches and localized by chromatin immunoprecipitation. Further in vivo studies will help understanding the distinct roles of specific CTD modifications.

658 B SC35 and Promoter-Associated Nascent RNA Function like HIV Tat and TAR to Regulate Transcription Pause Release

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Paused RNAP II at gene promoters produces short nascent RNA of unknown function, and the transition to productive elongation requires active recruitment of the transcription elongation factor P-TEFb. On the HIV-1 promoter, the virus-encoded protein Tat activates transcription elongation by relocating P-TEFb from the inhibitory 7SK complex to promoter proximally paused RNAP II via binding the TAR element in nascent viral RNA, but whether cellular genes use an analogous mechanism to activate transcription is unclear. We present evidence that SC35 (also known as SRSF2, a prototypical SR family splicing factor) has functions analogous to Tat, and that SC35 binding sites in nascent transcripts play roles equivalent to TAR in overcoming RNAP II pausing near the transcription start. These findings reveal an unanticipated SR protein function, a role for promoter-proximal nascent RNA at a discrete step in gene expression, and a unifying mechanism for transcription activation of viral and cellular genes.

659 C Not5 of the CCR4-NOT complex contributes to assembly of the SAGA coactivator complex.

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CCR4-NOT is a conserved eukaryotic multi-subunit complex that plays a role at every step of gene regulation. A variety of evidences in our laboratory indicate that a unique function of the CCR4-NOT complex might be to contribute to the assembly of other multi-protein complexes. One such complex is the histone acetyltransferase and deubiquitinase SAGA complex. Former data have revealed that global histone acetylation is reduced in mutants of SAGA and CCR4-NOT, in particular in cells lacking the Not5 subunit. Our current results show that there is both physical and genetical interaction between the two complexes. In addition we find severe alterations in SAGA complex integrity in cells lacking Not5, which can explain the acetylation defect previously observed in cells, and which also correlates with several modifications of the core subunits of the SAGA complex. Further characterization of SAGA in wild-type and *not5* mutant cells has indicated that Not5 might connect a GAPDH family member, Tdh3, also known as a moonlighting protein, to SAGA, and thereby contribute to proper SAGA assembly. Taken together, our results suggest a key role for Not5 in both SAGA integrity and function.

660 A Paraspeckle formation during NEAT1 lncRNA biogenesis is integrated by the SWI/SNF chromatin remodeling complex

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The paraspeckle is a unique subnuclear structure formed around the specific long noncoding RNA (lncRNA), NEAT1. NEAT1 lncRNA are comprised of two alternatively processed isoform transcripts, NEAT1_1 and NEAT1_2, in which the longer NEAT1_2 isoform is indispensible for paraspeckle formation (Sasaki et al., PNAS 2009). We recently identified 35 novel paraspeckle-localized proteins (PSPs) in which seven PSPs act as essential factors for paraspeckle formation through facilitating the steps required for the NEAT1 lncRNA biogenesis (Naganuma et al., EMBO J 2012). Here, we identified the SWI/SNF chromatin remodeling complexes as additional paraspeckle-localized factors that interacted with multiple essential PSPs. RNAi knockdown revealed that the intact SWI/SNF complex is required for the paraspeckle formation. This was supported by another observation that paraspeckle was undetectable in the SWI/SNF-deficient adrenal carcinoma cell line. What is the molecular mechanism underlying the function of the SWI/SNF complex in the parasepckle construction? Knockdown of the SWI/SNF components did not affect expression level of the essential NEAT1_2 isoform. Interestingly, the EU-pulse labeling experiment revealed that the transcript level of the newly synthesized nascent NEAT1_1 isoform was markedly increased in the SWI/SNF-depleted cells, although NEAT1_1 per se is dispensable for paraspeckle formation. We found that the association of the elongating form of RNA polymerase II (RNAPII) with phosphoserine 2 in the C-terminal domain was markedly increased in NEAT1_1 region and the most prominently increased at the NEAT1_1 terminator in the SWI/SNF-depleted cells. These data suggest that the SWI/SNF complex attenuates the elongation of RNA polymerase II (RNAPII) in the NEAT1_1 region. It raised an intriguing possibility that the optimized RNAPII elongation gives the proper environment for the assembly of various PSPs onto the nascent NEAT1 lncRNA which is required for the subsequent formation of the intact parasepeckle structure. We found that the RNA-protein and the protein-protein interactions between NEAT1 lncRNA and three essential PSPs were substantially weaken in the absence of SWI/SNF complex. We confirmed that the SWI/SNF complex interacted with RNAPII, the essential PSPs and NEAT1 lcnRNA. Taken together we argue that the SWI/SNF complex integrates the co-transcriptional ribonucleoprotein assembly onto the nascent NEAT1 lncRNA, which is required for the subsequent construction of the intact parasepckle, through controlling RNAPII elongation. Elucidation of the detail mechanism of the co-transcriptional assembly of ribonucleoprotein complexes onto NEAT1 lncRNA will tell us about the molecular basis underlying the fate determination of nuclear-retained lncRNAs distinct from that of mRNAs.

661 B The RNA exosome promotes a transcription termination pathway coupled to RNA decay

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The transcription cycle is composed of three essential steps that consist of initiation, elongation, and termination. Termination is probably the least understood of these three steps in eukaryotic cells. For protein-coding genes, termination is normally initiated by the endonucleolytic cleavage of the nascent mRNA, which generates an entry point for a 5'-3' exonuclease-dependent termination pathway known as the torpedo model. In contrast to this 5'-3' termination pathway, we provide evidence for a new mechanism of transcription termination that depends on the 3'-5' exonuclease activity of the RNA exosome. Accordingly, transcriptome-wide analyses of fission yeast cells depleted for exosome subunits reveal widespread accumulation of 3'-extended transcripts from coding and noncoding genes, whereas mature RNA levels are not reduced. Importantly, the detection of read-through RNAs in exosome-deficient cells strongly correlates with a genome-wide increase in RNA polymerase II density at the 3' end of genes, consistent with transcription termination defects in the absence of a functional exosome. We show that RNA exosome subunits are present along transcribed genes and are recruited in a transcription-dependent manner, supporting a direct role for the exosome in promoting transcription termination. Our results also indicate that the exonucleolytic activity, but not the endonucleolytic function of the Dis3 catalytic subunit is required for exosome-dependent termination. These findings support a reverse torpedo model in which the 3'-5' exonucleolytic activity of the RNA exosome promotes the release of RNAPII and the concomitant degradation of the nascent transcript.

662 C Widespread regulation of mRNA steady-state levels through alternative splicing-dependent mechanisms

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Differentiated cells acquire unique structural and functional traits through coordinated expression of lineage-specific genes. An extensive battery of genes encoding components of the synaptic transmission machinery and specialized cytoskeletal proteins is activated during neuronal differentiation, whereas genes required for neural stem cell (NSC) proliferation are turned off. We have recently shown that in non-neuronal cells, polypyrimidine tract-binding protein (Ptbp1/PTB/hnRNP I) represses splicing of 3'-terminal introns in pre-mRNAs encoding critical presynaptic proteins. This inhibits export of the incompletely spliced mRNAs to the cytoplasm and triggers their nuclear degradation. Clearance of these intron-containing transcripts occurs independently of nonsense-mediated decay (NMD) and requires components of the nuclear RNA quality control machinery. When Ptbp1 expression decreases during neuronal differentiation, the regulated introns are spliced out thus allowing translation-competent mRNAs to accumulate in the cytoplasm. Our unpublished work suggests that Ptbp1 additionally promotes expression of a large subset of non-neuronal genes by suppressing splicing patterns that lead to NMD. We show that this mechanism dampens the expression of these genes during neuronal differentiation. Thus, differentiation-induced changes in cellular mRNA steady-state levels appear to be frequently controlled by alternative splicing-mediated mechanisms.

663 A Alternative splice variant of chromatin regulators drive specific transcription

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Both alternative splicing and epigenetic regulations are deregulated in pathogenesis. These two processes are connected and recent studies describe how chromatin-born information can participate in the regulation of the alternative splicing. However, the question of how the alternative splicing can influence the chromatin regulation is not well evaluated. Here, we present evidences describing the functions of splicing variants coding for well-characterized chromatin regulators: the histone methyl-transferases EHMT2 and Suv39H2, the Polycomb subunit EED, and the NURF subunit BPTF. We showed that, while these chromatin regulators are expressed in all the tissues we tested, the expression levels of the different isoforms is cell-type specific. In particular, the relative proportion of the different EHMT2 isoforms is specifically correlated with the epithelial-mesenchymal transition, suggesting that alternative splicing affects the activity of these proteins on cell differentiation.

In order to further investigate the function of the different isoforms generated by alternative splicing, we have followed their activity on the transcriptional regulation of known target genes. For this purpose, we have developed a new strategy of siRNAs design where the sequence of the siRNAs we use, base-pair with splice junctions and so, target only spliced process mRNA which is expected to reduce off-target effects. This allows us to deplete the cells from either all the species of mRNA or just designated splice variants. Inversely, we have also generated isoform-carried lentiviruses, allowing us to rescue the expression of some alternative spliced variants.

Our study reveals how alternative splicing regulation affects the function of chromatin regulator, which in turn, modulate the transcription of target genes.

664 B Traf3 alternative splicing during T cell activation: functional consequences and regulatory insights

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The non-canonical NFkB (ncNFkB) pathway regulates the expression of chemokines required for secondary lymphoid organ formation and maintenance and thus plays a pivotal role in adaptive immunity. ncNFkB activity is controlled by the kinase NIK, which itself is negatively regulated by the full-length isoform of Traf3. Here we show that T cell specific and activation-dependent alternative splicing generates a Traf3 isoform lacking exon 8 (Traf3DE8) that, in contrast to the full-length protein, activates ncNFkB signaling. Traf3DE8 disrupts the NIK-Traf3-Traf2 complex and allows accumulation of NIK to initiate ncNFkB signaling in activated T cells. ncNFkB activity results in the expression of several chemokines, among them CxCL13, both in a model T cell line and in primary human CD4+ T cells. As CxCL13 plays an important role in B cell migration and activation, our data suggest an involvement and provide a mechanistic basis for Traf3 alternative splicing and ncNFkB activation in regulating T cell dependent adaptive immunity.

To start investigating the mechanism of activation-induced Traf3 alternative splicing we have used minigene analysis and defined an intronic cis-regulatory element. RNA-protein interaction studies and an siRNA screen have yielded candidate trans-acting factors that we are now testing for their involvement in regulating Traf3 exon 8 exclusion in activated T cells.

665 C The Bre5-Ubp3 complex links RNA surveillance to RNA Polymerase II regulation by ubiquitylation

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To identify novel nuclear RNA surveillance factors, yeast genome-wide screens were preformed for synthetic lethal (sl) interactions with loss of exosome cofactors Rrp47/Lrp1, Air1 or Air2. Each screen identified both Bre5 and Ubp3, which form a complex with protein de-ubiquitinase activity, suggesting links between RNA surveillance and ubiquitylation. Bre5 has a potential RRM and we confirmed RNA binding *in vitro* and *in vivo*. The CRAC UV crosslinking approach identified many Bre5 targets, transcriptome-wide. On intron containing genes, Bre5 hits were enriched over exon 2. Splicing-dependent transcription pausing over exon 2 has been reported, and a genetic screen using an allele of the splicing factor Prp45 that exacerbates this pausing also identified sl interactions with Bre5 and Ubp3. Ubp3 was previously shown to deubiquitylate RNAPII *in vitro* and levels of ubiquitylated RNAPII were elevated in *ubp3*Δ strains following DNA damage. CRAC was used to identify RNAPII interaction sites, transcriptome-wide; this provided data similar to ChIP, but strand specific and with nucleotide resolution. In addition, we specifically mapped the location of ubiquitylated RNAPII. This confirmed that levels of RNAPII ubiquitylation are elevated over Bre5 target genes. High-resolution, kinetic analysis of transcription and splicing of a reporter transcript revealed that the absence of Bre5 causes a delay in the release of RNAPII paused over exon II. We propose that splicing-induced, exon 2 pausing of RNAPII, results in its ubiquitylation to prevent further elongation while co-transcriptional splicing occurs. Recognition of the nascent transcript by Bre5 triggers de-ubiquitylation of RNAPII by Ubp3, allowing the polymerase to resume transcription following splicing. The Bre5-Ubp3 complex therefore provides a link between RNA surveillance and regulation of RNAPII activity by ubiquitylation.

666 A Double-stranded RNA-expressing plasmids selectively inhibit translation of exogenous mRNAs

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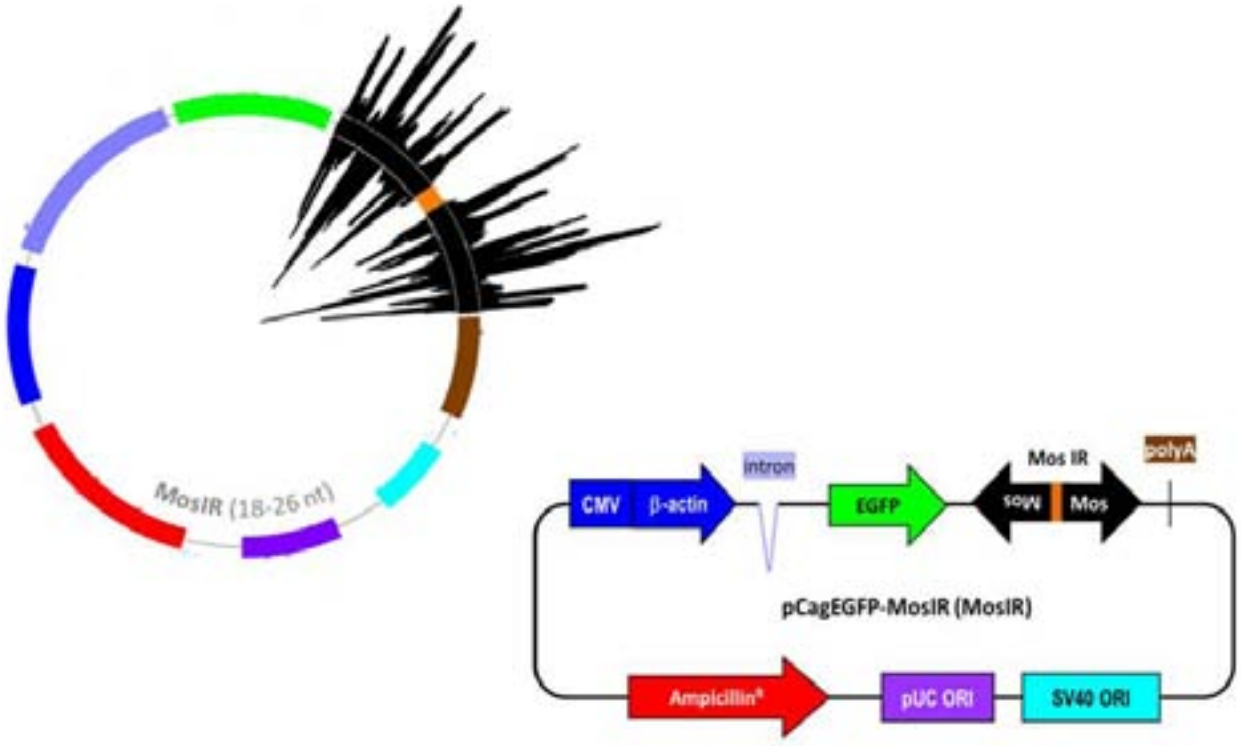
Double-stranded RNA (dsRNA) is linked to different effects in mammalian cells, including sequence-specific RNA interference, sequence-independent interferon response, and editing by adenosine deaminases. We have previously shown that long hairpin dsRNA expression in cultured cells does not necessarily activate the interferon response, it is poorly processed into siRNAs, and it is partially edited. Here, we demonstrate that dsRNA-expressing plasmids inhibit expression of transiently cotransfected reporter plasmids but they have a minimal impact on expression of endogenous genes or reporters stably integrated in the genome. The inhibition is concentration-dependent and independent of cell type, transfection method, or hairpin sequence. Deep sequencing data show low but detectable processing of hairpin RNA into small RNAs and preferential A-to-I editing of the dsRNA region (see figure 1 below). The inhibition likely occurs at the level of translation initiation and is mediated by the local and transient activation of protein kinase R. In conclusion, we demonstrate that expression from plasmids can be repressed if one of co-transfected plasmids generates dsRNA. We showed previously that the potential to generate dsRNA cannot be efficiently predicted and involves also commonly used plasmids. Moreover, our results indicate that cells can distinguish between endogenous and exogenous mRNAs and selectively inhibit the translation of foreign mRNAs in response to dsRNA.

Figure 1: Adenosine-to-inosine editing of small RNAs is specific to double-stranded RNA-forming region



667 B Molecular mechanisms of RNA Polymerase II termination mediated by contacts with Rat1 and CF IA

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Proper RNA Polymerase II (Pol II) transcription termination is needed to generate stable transcripts, prevent transcriptional interference with downstream loci, and allow recycling of Pol II back to the promoter. Termination is tightly coordinated with 3' end RNA processing and is intricately regulated by a variety of different *cis*- and *trans*-acting factors. Although many eukaryotic termination factors have been identified, the precise molecular mechanisms governing this essential process remain to be elucidated. A major limitation in our understanding of termination has been the lack of a defined, easily manipulated in vitro system. We have thus developed and characterized an *in vitro* transcription system for the assay of factor-driven Pol II termination. In this system, we generate promoter-driven stalled elongation complexes from yeast whole cell extract and challenge these complexes with purified termination factors. We show for the first time that the exonuclease Rat1, in complex with its stabilizing partner Rai1, can elicit release of stalled Pol II *in vitro* and can do so in the absence of other termination factors. We also find that Rtt103, which interacts with the Pol II C-terminal domain (CTD) and with Rat1, can rescue termination activity of an exonucleolytic-deficient Rat1 mutant. In light of our findings, we posit a model whereby functional nucleolytic activity is not the feature of Rat1 that ultimately promotes termination. Degradation of the nascent transcript allows Rat1 to pursue Pol II in a guided fashion and to arrive at the site of RNA exit from Pol II. Upon this arrival, however, our model suggests that it is perhaps the specific and direct contact between Rat1 and Pol II that transmits the signal to terminate transcription.

To further explore the role of direct interactions between Pol II and termination factors, we have characterized a Pol II variant with a deletion of the flap domain, an evolutionarily conserved, surface-exposed region within the vicinity of the RNA exit channel. This Pol II variant is not defective for transcription elongation but exhibits defective termination of short RNAs *in vitro* and of snoRNAs *in vivo*. Additionally, the mutation significantly alters the *in vitro* and *in vivo* interactions between Pol II and Cleavage Factor IA (CF IA), a complex important for both 3' end processing and termination. These findings suggest that contacts of proteins with the body of Pol II, in addition to those formed with the CTD, are important for efficient termination.

668 C IMAGEtags for imaging Pol II activity in real time with RNA reporters

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RNA based molecular imaging provides a new means of understanding of cellular events such as transcription and splicing. However the current techniques to visualize gene expression with RNA reporters are limited by the use of fluorescent proteins or by the need for highly abundant RNAs. We have developed an RNA reporter system known as IMAGEtags (Intracellular MultiAptamer GEnetic tags) for imaging promoter activity in real time in individual living cells by a FRET signal. The RNA reporter uses strings of RNA aptamers that can be expressed from an inducible or constitutive promoter. The tobramycin and PDC RNA aptamers were expressed in yeast from the GAL1, ADH1 or ACT1 promoters of a plasmid vector, which was transformed into the yeast cells. The cells are incubated with their ligands that are separately conjugated with the FRET pairs, Cy3 and Cy5. Following incubation with the dyes the cells are imaged. The constitutive ACT1 and ADH1 showed higher FRET signals compared to the control. FRET signals from the GAL1 promoter were seen to increase as a function of time after adding the galactose inducer and in parallel with the reporter RNA that was measured on a population basis. Cellular heterogeneity in FRET signals was also observed, which are consistent with the concept of stochasticity of transcriptional events in a cell population. IMAGEtags can be applied to studies of gene expression from pol II promoters in single cells and in real time. The system is simple, sensitive and applicable to many cell types and experimental conditions.

669 A A Novel Poly(A) RNA-Binding Protein Regulates a Key Subunit of ATP Synthase in Breast Cancer Cells

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Poly(A)-binding proteins, or Pabs, comprise one class of RNA-binding proteins that influence multiple steps in gene expression from polyadenylation within the nucleus to translation in the cytoplasm. All previously characterized Pabs interact with polyadenosine RNA via RNA Recognition motifs; however, a novel family of nuclear Pabs utilizes tandem CCCH zinc fingers for polyadenosine RNA recognition. The human member of this family of zinc finger Pabs is termed Zinc Finger CCCH-type containing #14 (ZC3H14) and has recently been linked to a form of non-syndromic intellectual disability as well as the estrogen receptor status of breast tumors. Although the function of ZC3H14 is unknown, the budding yeast counterpart, Nab2, ensures proper poly(A) tail length and mRNA export from the nucleus, suggesting a role for ZC3H14 in post-transcriptional regulation. In an effort to understand the post-transcriptional function of ZC3H14 as well as to gain insight into the spectrum of targets of nuclear Pabs, we employed a genome-wide microarray analysis of transcripts affected upon knockdown of the novel Pab, ZC3H14, or the well-characterized nuclear Pab, PABPN1. To focus on targets of relevance to breast cancer, experiments used MCF7 breast cancer cells. Knockdown of PABPN1 significantly affected ~17% of expressed transcripts as compared to knockdown of ZC3H14, which affected only ~1% of expressed transcripts. Results from this study show that PABPN1 affects the steady-state level of a large fraction of the transcriptome, which is consistent with the model that PABPN1 is a global polyadenylation factor. In contrast, knockdown of ZC3H14 affected a much smaller fraction of the transcripts, suggesting that ZC3H14 may have specific mRNA targets in these cells. One intriguing target that we selected for further analysis is the ATP Synthase F_o Subunit C, or ATP5G1. ATP Synthase is the central enzyme in oxidative phosphorylation and is responsible for the majority of ATP production in mammalian cells. The steady-state level of ATP5G1 is robustly decreased upon knockdown of ZC3H14, but not PABPN1. Interestingly, our data demonstrates that ZC3H14 specifically affects the steady-state mRNA level of ATP5G1 and not other ATP Synthase subunits, suggesting specificity. We are able to rescue the decrease in ATP5G1 by addition of a siRNA-resistant ZC3H14 plasmid. ATP5G1 is a rate-limiting component in F_o Subunit C assembly and ultimately ATP Synthase activity, and we observe that the effect of ZC3H14 on ATP5G1 results in decreased cellular ATP levels. Interestingly, upon RNA-IP of ZC3H14, we observe enrichment for ATP5G1 as well as other subunit C components, suggesting that ZC3H14 may partner with another factor to specifically regulate ATP5G1 levels. This data suggests that ZC3H14 may play a role in regulating cellular energy levels, which has broad implications for proper neuronal function as well as cancer.

670 B Messenger RNA decay rates feedback to influence transcription and buffer gene expression in mammalian cells

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There is considerable evidence that transcription influences mRNA decay and several models for how this can occur have been put forward. However, we believe that the cell may also have mechanisms by which mRNA decay rates can also feedback and influence transcription. This assertion is based on some recent results generated in our laboratories. First, we examined the differences in mRNA decay between human foreskin fibroblasts (HFF) and genetically identical induced pluripotent stem (iPS) cells that were derived from these HFFs. Not surprisingly, there were many differences in mRNA decay rates, but when we assessed changes in mRNA abundance between the two cells we were surprised to find that there was a negative correlation between decay and abundance. Transcripts that were more stable in iPS cells were frequently of lower abundance than in HFFs. Conversely, destabilized transcripts tended to be more abundant. Next, we examined the effects of perturbing mRNA decay on transcription. We undertook a global analysis of mRNA decay rates in C2C12 myoblasts following depletion of the deadenylase PARN. We determined that several mRNAs were significantly stabilized in a PARN knockdown cell line. Intriguingly, as before, we found that the abundance of these stabilized transcripts either was not altered, or was slightly reduced. The opposite was true for the transcripts that were destabilized in PARN KD cells – their abundance tended to increase. We believe that these destabilized mRNAs are affected through indirect mechanisms making the opposing effects on transcription even more surprising as they are unlikely to be mediated by effects of PARN on either transcription or mRNA decay. To investigate this phenomenon further, we validated the changes in mRNA decay and abundance for four of the stabilized transcripts by qRT-PCR. We then measured the abundance of newly transcribed pre-mRNAs as an indicator of transcription rate. In each case, despite clear decreases in mRNA decay rates, the mRNA abundance and pre-mRNA abundance was surprisingly reduced instead of increased as current models would predict. These results imply that the cell is able to buffer mRNA levels by down-regulating transcription to compensate for reduced mRNA decay.

671 C The human protein Nol12- ribosome biogenesis meets DNA damage and senescence

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It has been shown, in yeast as well as mammalian cells, that ribosome biogenesis and cell proliferation are two intimately linked processes. Defects in either one can lead to disease. To understand the differences between yeast and mammalian ribosome biogenesis and how the latter is integrated into cell division and proliferation pathways in higher eukaryotes, we are investigating the role of different ribosome biogenesis factors in human cells.

Here we focus on Nol12, the human ortholog of yeast Rrp17p, which has recently been described as a 5'-3' exonuclease required for 5′ end processing of 5.8S and 25S rRNAs. Nol12 is a member of a conserved family of proteins and Nol12 orthologs have been related to dMyc-stimulated cell growth, proliferation and eye development in *Drosophila*, nucleolar architecture and function in rat as well as ribosome biogenesis in mouse.

We have studied the impact of Nol12 knockdown in human cells, using shRNAs, siRNAs, biochemical assays and proteomics. Cells lacking Nol12 are defective not only in pre-60S processing, as previously observed in yeast, but interestingly also in the maturation of 40S precursor RNAs. Moreover, we found that in the absence of Nol12, nucleolar architecture was severely affected, a hallmark for ribosome defects in mammalian cells. In addition, cells were not progressing through the cell cycle, accumulating in G1, a checkpoint that has been closely associated with defects in ribosome biogenesis; However, these cells were also unable to efficiently undergo G2/M progression, resulting in an accumulation of cyclin D1 and depletion of cyclin B1. In the past it has been shown that altered ribosome biogenesis is responsible for p53 stabilization through the action of several ribosomal proteins and MDM2, leading to G1 arrest, making this a p53-dependent response. However, in cells absent of Nol12, the effects on pre-rRNA processing, nucleolar architecture and G1 block seemed to occur in a p53-independent manner, while block of cells in G2/M was not observed in $HCT^{p53\ -/-}$ cells; c-myc was also not upregulated in these cells. Interestingly, a similar observation has been made in cells where disruption of 40S and 60S ribosome biogenesis was shown to lead to the activation of a novel G2/M checkpoint, while p53 was induced in these cells. In addition to cell cycle checkpoints, the DNA damage response pathway was also activated in the absence of Nol12 and cells went into senescence 48hrs after knockdown.

Our results suggest that the exonuclease Nol12 may have additional function to that during ribosome biogenesis, involving DNA damage, which in its absence leads to an activation of G1 checkpoint in a p53 as well as ribosome biogenesis-independent manner.

672 A The tissue specific and eco-responsive transcriptome of Drosophila

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Integrative analysis of RNA sequencing data from *Drosophila melanogaster* reveals an animal transcriptome of unprecedented complexity, comprising discrete, tissue-specific transcripts. To decipher the information encoded in and probe the dynamics of the transcriptome, we generated and analyzed strand-specific poly(A)+ RNA-seq, CAGE, and cDNA sequences from 30 developmental time-course samples, 29 tissues, and 21 environmental perturbations in *D. melanogaster* which we call the Fly LifeMap. Although 55% of all genes and 66% of spliced genes may encode more than one protein isoform, a set of 47 largely neural-specific genes are the targets of RNA editing, extensive alternative mRNA splicing and have the potential to encode more than 1000 transcript isoforms each. The magnitudes of RNA splicing changes are much larger between tissues than throughout development or in response to environmental perturbations, and the majority of sex-specific splicing is gonad-specific. The gonads produce comparatively few protein isoforms per gene but express hundreds of previously unknown protein-coding and noncoding genes. The gonads also express antisense transcripts at key conserved developmental genes, including *eve*, *Dcr-2*, and *CTCF*. In neural tissue, antisense transcription is largely due to extension of 3'UTRs resulting in overlap with neighboring genes. In gonads, antisense transcription is due to independent transcription initiating at gonad-specific promoters. Most RNA-seq reads are accounted for within transcripts models, and we observe little evidence of pervasive transcription outside of primary transcripts. In summary, the *Drosophila* transcriptome is substantially more complex than previously recognized and arises from tissue-specific, combinatorial usage of well-defined promoter elements, splice sites, and polyadenylation sites.

673 B Modeling the RNA-binding specificity of GLD-1 suggests a function of coding region-located sites in translational repression

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To understand the function of RNA-binding proteins (RBPs) encoded in animal genomes, it is important to identify their target RNAs. Although it is generally accepted that the binding specificity of an RBP is well described in terms of the nucleotide sequence of its binding sites, other factors such as the structural accessibility of binding sites or their clustering, to enable binding of RBP multimers, are also believed to play a role. Here we focus on GLD-1, a translational regulator in Caenorhabditis elegans, whose binding specificity and targets have been studied with a variety of methods such as CLIP (crosslinking and immunoprecipitation), RIP-Chip (microarray measurement of RNAs associated with an immunoprecipitated protein), profiling of polysome-associated mRNAs and biophysical determination of binding affinities of GLD-1 for short nucleotide sequences. The latter approach appears to allow an accurate reconstruction of the sequence specificity of the protein and may be applied to uncover the specificity and function of other RBPs. Taking into account the accessibility of putative target sites significantly improves the prediction of our GLD-1 binding model, in particular due to a more accurate prediction of binding in the transcript coding regions. Relating GLD-1 binding to translational repression and stabilization of its target transcripts we find that binding sites along the entire transcripts contribute to functional responses.

674 C Beyond the ribosome, antagonistic functions played by a pair of ribosomal proteins paralogs

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While ribosomal proteins are increasingly found to play a role beside their involvement in protein synthesis within the ribosome. It is also still controversial whether their paralogs have essential or distinct functions, and this has not been explored in vertebrates. Previous work from our lab showed that the Ribosomal Protein Large Subunit 22 (RPL22), or rather, its absence, interfered with T-cell development in a lineage-specific manner. Unlike the lack of most other ribosomal proteins that lead to deleterious effects, RPL22 deletion in mice specifically inhibits T-cell commitment to the α/β, but not the γ/δ lineage. RPL22 paralog RPL22-Like1 does not rescue this lineage-specific defect. Strikingly, despite being highly homologous, RPL22 and RPL22-Like1 appear to play distinct and antagonistic roles. The focus of this research is to understand the roles of RPL22 and RPL22-Like1 in hematopoietic development. We found that they regulate the emergence of hematopoietic stem cells by controlling expression of Smad1. Emergence of T-cell lineage and hematopoeitic stem cells were investigated using zebrafish embryos, as well as *in vitro* RNA-proteins interactions to address this first example of ribosomal protein paralogs performing antagonistic functions in a tissue-restricted manner.

675 A Clindamycin ribosome interactions: a molecular dynamics study

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Antibiotics are drugs that treat the diseases of bacterial and fungal origin. Secondary uses include strengthening the immune system in cases of lowered immunity. Clindamycin is one of the antibiotics from the lincosamide class which are used to treat diseases caused mostly by Gram-positive bacteria and against some protozoal diseases. Lincosamidesinteract with the bacterial large (50S) ribosomal subunit and inhibit the process of protein synthesis leading to bacterial cell death. The increase of resistance of many bacterial strains against known antibiotics is caused by the expanded use of antibiotics in medical practice and veterinary. This is a very important reason for continuous work to find new, better and more effective antibacterial drugs.

Mutations of the antibiotic target are one of the common modifications that lead to bacterial resistance because such alterations typically prevent proper binding of the antibiotic in the targeted site. Currently, there are three structures of clindamycin in the complex with the 50S1,2,3 ribosomal subunit available in the Protein Data Bank coming from different organisms. Interestingly, two of the structures show significantly different conformations of the drug. The aim of this study was to compare the dynamic properties of the clindamycin binding site in the 50S subunit with and without the A2058G mutation to understand why this nucleotide substitution blocks the binding of lincosamides. To achieve this goal we applied full-atom

molecular dynamics. Using the NAMD [4] package we performed four types of simulations: (1) the complex of clindamycin with the fragment of the 50S subunit of the ribosome as well as (2) the unbound ribosome fragment, (3) the complex of clindamycin with the mutated ribosome fragment and (4) the unbound mutated ribosome fragment.

To prepare the starting systems for the simulations we chose the 3OFZ 50S subunit structure from *Escherichia coli*, which consists of ribosomal RNA, ribosomal proteins, one clindamycin molecule, magnesium and zinc ions and crystal waters. For our simulations, we cut a sphere with the radius of about 20Å around clindamycin to account for the long-range interactions of the antibiotics in the 50S subunit. We added 228 K^+ ions to neutralize the charge and approximately 27000 TIP3P water molecules to solvate the system shaped in a truncated octahedron around the complex. The effect of the mutation on clindamycin positioning in the binding cleft resulting from these molecular dynamics simulations will be discussed.

676 B Global gene regulation mediated by intron retention during T cell activation

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Large-scale genomics studies have shown that gene activation and/or repression, as determined at the steady-state mRNA level, are often demarcated by corresponding changes in variant histone marks (e.g. H3K4me3, H3K9me3). Such concordance, however, is lacking for transcripts differentially expressed between resting and activated human CD4+ T cells, suggesting posttranscriptional gene regulation might be involved. To solve this conundrum, strand-specific RNA-seq was employed to profile T-cell transcriptomes before and after extracellular stimulation. Our results showed that resting T cells exhibit a higher than expected level of intronic tags, the proportion of which is significantly reduced upon T cell activation. Intron retention in fact is a widespread phenomenon as demonstrated by IRI (intron retention index) analysis, a newly devised approach to monitor intron retention at the individual gene level. In addition, by integrating a large collection of ChIP-seq data, we further demonstrated that intron-retained transcripts are unstable and possibly degraded by RNA surveillance complex in the nucleus. More strikingly, fold change in intron retention level is negatively correlated with that of steady-state mRNA level, indicating that global intron retention may serves as a major contributor for gene regulation during T cell activation. Supporting this notion, majority of the genes upregulated in activated T cells are accompanied with a significant reduction in intron retention. Of them, 267 genes are likely to be regulated solely at the posttranscriptional level, and are highly enriched in the pathwyas that are essential for proper T cell proliferation and cytokine release. Taken together, our study uncovered a novel posttranscriptional mechanism mediated by global intron retention. It can bypass the requirement for *de novo* transcript synthesis and therefore shorten the responding time to extracellular stimuli such as acute infection.

677 C Large-scale analysis of eukaryotic RNA-binding protein binding preferences and exploration of their roles in post-transcriptional gene regulation

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Hundreds of genes in eukaryotic genomes encode RNA-binding proteins (RBPs) but few have well-defined RNA-binding preferences or experimentally defined RNA targets. We have developed a combined biochemical and computational approach, termed RNAcompete, to analyze the RNA-binding preferences of RBPs (Ray, Kazan *et al.* Nature Biotechnology 2009). Motifs identified using RNAcompete haven been shown to be comparable to RNA-binding data from *in vitro* selection (e.g. SELEX) and immunoprecipitation (e.g. CLIP) experiments. Here, we use RNAcompete to conduct the first large-scale analysis of RNA-binding preferences for 207 RBPs from 20 diverse kingdoms across eukarya. We find that most RBPs do not have strict requirements for RNA secondary structure and bind to short ssRNA sequences. Furthermore, the binding motifs of homologous RBPs display deep evolutionary conservation, such that the recognition preferences for a large fraction of metazoan RBPs can be inferred from the sequences of their binding domains. We are also able to utilize RNAcompete-derived motifs during transcriptome analyses in metazoans to facilitate identification of RBP-RNA interactions enriched in mRNA subsets and associated with specific post-transcriptional processes. The collection of motifs derived from our RNAcompete experiments will be invaluable for understanding the interactions between RBPs and RNA, as well as their functional relevance.

678 A RNase disruption of transcriptional positive auto-regulation is essential for energy-efficient phenotypic switching

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The distinct response kinetics of transcriptional and post-transcriptional regulation, and their non-uniform use across genes and environments, suggests that selective interplay of these mechanisms might be an evolved strategy to generate characteristic responses[1–4]. On the transcriptional level, the arrangement of transcription factors and their target genes into recurring network motifs generates response dynamics that are suited to particular information-processing functions[5]. Characteristic network topologies have also been observed at the post-transcriptional level with noncoding RNAs preferentially organized in feedforward loops[6]. Here, we have investigated whether the architecture of ribonuclease interactions in a gene regulatory network has any significant implication for environmental response strategies of an organism. We have discovered that RNases act in a characteristic regulatory motif that conserves energy by mediating efficient conditional phenotypic switching in both bacteria and archaea. Through analysis of the *E. coli* gene regulatory network, we made the intriguing observation that RNases preferentially target operons that are regulated by a transcriptional positive auto-regulation (PAR) loop, which is known to mediate bistable phenotypic switching[5]. A mathematical model for this regulatory motif predicted that relative to transcriptional repression, RNase-disruption of a PAR loop (RPAR) mediates significantly faster repression of target genes. Indeed, we find the RPAR motif to be essential for favorable bioenergetics of the salt-in strategy of salinity adaptation by extremely halophilic archaea. Specifically, the RPAR motif coordinates diverse metabolic functions to shift potassium uptake from a proton-gradient symport to a ATP-driven process in response to a wide array of environmental changes that alter membrane potential. Our results demonstrate that interplay of transcriptional and post-transcriptional regulation in a RPAR motif is a general principle by which prokaryotes regulate critical energy-consuming metabolic functions.

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679 B A Potential Role for snoRNAs in PKR Activation during Metabolic Stress

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PKR (Protein Kinase RNA activated) is a member of the stress-response kinase family. Catalytically active PKR inhibits translation by phosphorylating the eukaryotic initiation factor, eIF2a. PKR contains a regulatory dsRNA-binding domain at its N-terminus and a catalytic kinase domain at its C-terminus.

PKR has long been known to be activated by viral dsRNA as part of the mammalian immune response. However, PKR kinase activity is also stimulated under conditions of metabolic stress in mice. This stimulation requires a functional kinase domain, as well as a functional dsRNA-binding domain. However, the cellular dsRNA required for PKR activation during metabolic stress is unknown. We investigated this question using Mouse Embryonic Fibroblast (MEF) cells expressing wildtype PKR (PKR_{WT}) or PKR with a point mutation in each dsRNA-binding motif (PKR_{RM}). Cells were incubated in the presence or absence of palmitic acid (PA), to mimic a high-fat or regular diet, respectively, followed by immunoprecipitation of PKR. PKR immunopurified RNAs from two different sets of three biological replicates were subjected to high-throughput sequencing. Since it is predicted that PKR_{RM} does not bind to RNA activator(s), RNAs enriched in both immunopurified PKR_{WT} and PKR_{RM} after PA treatment were excluded from our analysis.

We found that 122, and 90, exons were enriched by $\geq$ 2-fold in PKR_{WT} samples after PA treatment (FDR $\leq$ 5%) in the first and second datasets, respectively. Interestingly, 43% and 78% of the enriched exons encode snoRNAs in the first and second datasets, respectively. An alternative bioinformatics approach using a sliding window across the genome showed that 224 regions were enriched in PKR_{WT} after PA treatment (Fold increase $\geq$ 2, q-value FDR $\leq$ 5%). Of these, 112 were snoRNAs; the large majority of the remaining regions were annotated mRNA transcripts, but a few unannotated regions and lncRNAs were also observed.

Immunoprecipitation of PKR in extracts of UV-crosslinked cells, followed by RT-qPCR, was used to confirm that snoRNAs specifically associated with PKR_{WT} after PA treatment. These studies showed that snoRNAs were enriched in PKR_{WT} after PA treatment, but not in the PKR_{RM} samples.

CHO cells haploinsufficient for the spliceosomal protein SmD3 maintain pre-mRNA splicing, but show reduced levels of intronic snoRNAs[1]. Consistent with the idea that snoRNAs trigger PKR activation after PA treatment, wildtype CHO cells, but not SmD3 haploinsufficient cells, showed increased PKR phosphorylation after PA treatment. Further, the decrease in PKR phosphorylation in mutant cells correlated with a decrease in JNK phosphorylation.

Using gel-shift assays we also find that PKR_{WT}, but not PKR_{RM}, binds directly to snoRNAs.

Our results support an unprecedented and unexpected model whereby snoRNAs play a role in the activation of PKR under metabolic stress.

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680 C RNAs in silico: learning from accelerated molecular dynamics

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Ribonucleic acid (RNA) is acquiring a large importance in cell biology, as more functions that it accomplishes are discovered. However, experimental characterization of RNAs dynamical behavior at atomistic level is difficult. Molecular simulations at atomistic detail, in combination with state-of-the-art free-energy techniques, can bridge the gap providing an unparalleled perspective on the mechanism and dynamics of RNA folding, conformational transitions, and of RNA/protein interactions. Two recent applications of these techniques will be discussed. The first is focused on a characterization of the zipping and unzipping mechanisms for a RNA double strand [1]. Results are compared with experimental findings, including analysis of X-ray data [2], ultrafast spectroscopy [3] and thermodynamic data [4]. Implications on the directionality of RNA processing enzymes are also discussed. The second application is a study of the interaction between TAR RNA from HIV and a cyclic binding peptide of pharmaceutical relevance [5]. This is done by introducing a suitable acceleration technique that allows for a blind prediction of the bound structure. Results are in nice agreement with previous NMR experiments [6].

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681 A DNA methylation level is differentially correlated with the evolutionary features of coding exons in different genic positions

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DNA cytosine methylation is a central epigenetic marker. It is usually mutagenic, and may increase the level of sequence divergence. However, methylated genes have been reported to evolve more slowly than unmethylated genes. Hence, there is a controversy on whether DNA methylation is correlated with increased or decreased protein evolutionary rates. We hypothesize that this controversy has resulted from the differential correlations between DNA methylation and the evolutionary rates of coding exons in different genic positions. To test this hypothesis, we compare human-mouse and human-macaque exonic evolutionary rates against experimentally determined single-base-resolution DNA methylation data derived from multiple human cell types. We show that DNA methylation is significantly related to within-gene variations in evolutionary rates. First, DNA methylation level is more strongly correlated with C-to-T mutations at CpG dinucleotides in the first coding exons than in the internal and last exons, although it is positively correlated with the synonymous substitution rate in all exon positions. Second, for the first exons, DNA methylation level is negatively correlated with exonic expression level, but positively correlated with both nonsynonymous substitution rate and the sample specificity of DNA methylation level. For the internal and last exons, however, we observe the opposite correlations. Our results imply that DNA methylation level is differentially correlated with the biological (and evolutionary) features of coding exons in different genic positions. The first exons appear more prone to the mutagenic effects, whereas the other exons are more influenced by the regulatory effects of DNA methylation.

682 B Exome-Wide Computational Prediction of Evolutionarily Conserved CIS-Regulatory G-Quadruplex Motifs

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G-quadruplex structures formed by guanine rich nucleic acids have been implicated in important biological processes, human disease, and as therapeutic targets. Recently there has been much interest in studying the potential roles of RNA G-quadruplexes as *cis*-regulatory elements in post-transcriptional gene expression. Due to the technical limitations of experimental approaches, genome-wide computational analyses are needed for large-scale studies of G-quadruplexes. However, most computational methods have difficulty validating their predictions without laborious wet laboratory testing. We have developed a new method to map evolutionarily conserved G-quadruplex motifs in nucleic acid sequences. Cross-species motif conservation provides evidence for their biological relevance. We have implemented our method to develop a web-based software application and an exome-wide database of G-quadruplex motifs conserved across species. We have used these tools to study homologous G-quadruplex motifs in the context of 5′-, 3′-UTRs (untranslated regions) and coding sections of aligned mRNA sequences.

Our database contains more than 400,000 predicted homologous G-quadruplexes in the exomes of *Homo sapiens, Mus musculus, Pan troglodytes, Canis lupus familiaris, Danio rerio, Caenorhabditis elegans,* and *Kluyveromyces lactis,* constituting over 90,000 total genes. Conserved G-quadruplexes were mapped in the 5'- and 3'-UTRs of a large variety of genes, including which are involved in apoptosis, brain development, epigenetics, cell proliferation, oncogenesis, and transcription. We are using the database to study G-quadruplex composition, as well as distribution in the untranslated and translated exome regions with a view to investigate their biological roles as *cis*-regulatory elements.

683 C Determining optimal flanking regions of RNA secondary structures for experimental analysis

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Multiple sequence and structural alignments are often used to perform more accurate RNA secondary structure predictions than folding single sequences. As such they provide useful consensus structures for different experiments, e.g. structure probing. However, design of probing or other experimental analysis often require considering the structure of a single sequence in question and in addition a sequence flanking the predicted structure. Both aspects contribute to the structure of a single sequence to deviate from the corresponding consensus structure extracted from the structural alignment that contains the comparative information. To address this, we have developed a tool, RNAcfold (RNA context folding), that determines optimal flanking regions and can cope with arbitrary requirements for the minimum size of flanking regions up- and downstream of the specific structure in question. This is done by making use of constrained folding as implemented in the ViennaRNA Package while selecting the size of the flanking regions so that the probability for observing the structure in the region corresponding to the extracted structure from the structural alignment is maximized. In more detail, the probability is determined by the fraction of the partition function over all allowed secondary structures which satisfy the constraints compared to the partition function over all allowed secondary structures without constraints.

The Rfam database 11.0 was processed for number of sequences and structure content resulting in 324 suitable structure alignments. Next, the similarity between consensus structures and minimum free energy structures corresponding to optimal flanking regions or to fixed sized flanking regions was evaluated for each sequence. We evaluated the agreement between the folded structures (with and without optimal extension of flanking regions, respectively) to the consensus structure from Rfam using Matthews correlation coefficients (MCC). We report improvements for all combinations of minimum flanking regions in the range 0 to 50 (in steps of 10) for 5' and 3' UTRs. For example, a typical set up of a minimum 0 nt in 5'UTR and 30nt 3'UTR, the average improvement is 0.05 in MCC and for 15.74% of the families an improvement of more than 0.1 in MCC is obtained.

684 A EASANA: RNA-Seq and Affymetrix HTA2 data analysis, visualization and interpretation

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Recent advances in technologies allow to study the transcriptome as we never could before but also increase amount and complexity of data (up to several billion). Thus, analyzing such "big data" is challenging, time consuming, and needs the development of efficient and user-friendly tools. In this context, for public or private organization researchers, it is quite difficult to manage these new bioinformatics issues.

We developed a dedicated analysis and visualization tool for transcriptomics data named EASANA (publicly available at https://www.easana.com). This tool allows to display gene intensity taking into account the genomic context at the exon level. EASANA was first applied to Affymetrix Exon Array data (Jia et al., Cell 2012; Ameyar-Zazoua et al., Nat Struct Mol Biol 2012; Moreira et al., EMBO Mol Med 2013; Gandoura et al., J Hepatol 2013) and was recently improved to handle exon-exon junction intensity regulation information (Shen et al., Nucleic Acids Res. 2013). EASANA now enables the analysis and visualization of data from the last generation expression technologies. In particular, EASANA can now manage data from RNA-Seq experiments and from the last generation of splicing-sensitive microarray that include exon-exon junction probes (Xu et al., PNAS 2011): the Affymetrix HTA2 (Human Transcriptome Array 2.0).

EASANA handles all main types of RNA-Seq data (Illumina, SOLiD, 454…) and provide results at both gene and exon levels. For exon level analysis, EASANA analyzes regulation of alternative splicing as well as alternative first and terminal exons, known or not in publicly available gene annotations (i.e., new exon-exon junctions can be found). In addition, detection analysis of fusion transcripts and SNP/indel are also included.

A comparison between Illumina RNA-Seq and HTA2 using Human Muscle vs. Liver tissues will be presented: Detection sensibility, number of regulated genes and splicing pattern regulation will be detailed.

In March 2013, Affymetrix launched its new expression array named HTA2. As for RNA-Seq data, EASANA allows the analysis and visualization for data from this new generation array. Analyses at both gene and exon levels are provided and we currently develop algorithm to detect potential fusion transcripts from high-density expression microarray such as HTA2 (Affymetrix named GenoSplice as its preferred data analysis service provider for HTA2 data).

In addition to analyze genomics data, we provide help in the experimental design (e.g., number of replicates, sequencing depth), help in the biological interpretation of data (e.g., functional analysis of results), and follow-up until publication of results (scientific article and/or patent).

685 B Terminator: a method for precise detection of ncRNA ends and terminal stem-loops using chimeric reads from RNA-seq

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Non-coding (nc)RNAs play important roles in many aspects of gene regulation, and other cellular processes. The functions of ncRNAs are determined by both primary sequence and secondary structure, which typically act via base-pairing with other nucleic acid targets, or interacting with proteins and small molecules. Recent years have seen the development of a variety of computational and experimental methods that characterize novel ncRNA sequence and structure. One of these methods, RNA-seq, has been widely used for its high sensitivity and versatility.

Within RNA-seq data sets, we have discovered that many of the unmappable reads are chimeric. That is, these reads consists of two parts, one from the 5' or 3' end of the RNA, the other from the internal part of the opposite strand. This phenomenon clearly suggests self-priming from, and ligation with, terminal stem-loops during cDNA library preparation. Using the chimeric reads from existing RNA-seq data, we developed a program, called Terminator, to precisely determine the ends of ncRNAs and provide support for the predicted terminal stem-loops.

To obtain chimeric reads, we first use a short read mapper capable of partial mapping (not end-to-end) to analyze the original RNA-seq data, and select the partial reads. We then map the unmappable parts to the vicinity of the mapped locations, on the opposite strand. Finally, reads with two parts mapped to opposite strands are used to construct an ncRNA sequence/structure map. The terminally mapped half-reads indicate terminal stem-loops, whereas the internally mapped parts indicate single-stranded regions.

We have tested our method in several species (including *Drosophila* and human) in datasets that contain self-primed ncRNA reads. We confirmed the terminal stem loops and ends of well-studied ncRNAs, and also refined the precise ends for many newly discovered ncRNAs.

The combination of fast short-read mappers (we used bowtie2) and rapid local mapping of normally unmappable fragments allows efficient processing of large numbers of datasets in a short time, and therefore increases the sensitivity of our method. Terminal stem-loops and internal single-stranded regions are common features of most snRNAs and snoRNAs, and many other types of ncRNAs. With the ever-increasing amount of publicly available RNA-seq datasets, Terminator will be a useful tool for studying ncRNAs.

686 C Uncovering markers of cell identity change from transcriptome profiles

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Understanding the control of cellular reprogramming is crucial to improve and create protocols for its induction, to find out about the mechanisms that lead to pathological or failed changes of cell identity in cancer and developmental diseases, and to better understand cell pluripotency and differentiation. There is increasing evidence that modifications in common regulatory networks are required to facilitate changes of cell identity such as induced reprogramming, cancer and differentiation. For example, genes related to the epithelial to mesenchymal transition (EMT) are modified both during reprogramming and cancer. Our goal is to find genes expressed in cells that are undergoing changes in identity through the examination of gene expression profiles from cancer, reprogramming and differentiation.

687 A A new and systematic approach to analyse the population of sRNAs at the genome scale.

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The deep sequencing of small RNAs (sRNAs) provides a snapshot of RNA populations in the cells in specific conditions or tissues. In eukaryotes, these data usually reveal a complex mixture of miRNAs, siRNAs and degradation products, detected at different states of maturation or degradation, and mainly enriched for the sRNAs stabilised by their interaction with proteins or by their interaction with complementary RNAs. Despite the apparent noise created by the overlapping of these sub-populations of sRNAs, classical tools are able to characterize some of them using the rules of maturation described in the last decade. For instance, the sRNA sizes, the 2nt 3' overhangs and the 2D structures allow the detection of miRNAs, whereas the sizes, the 2nt 3' overhangs and their grouping along several hundred nucleotides defines siRNA clusters. These clusters occasional reveal phased sRNAs, illustrating DICER processivity.

In plants, the number of DICER and AGO proteins increases the complexity of this categorization and to bypass this problem we are forced to work with complex mutants. Moreover, the typical rules of sRNA maturation seem often insufficient to completely understand the observations.

By integrating tools classically used to discover correlations and periodicities in signal processing, our work focuses on the development of a new methodology to analyse in-depth the sRNA-seq data at the genome scale. The final goal is to achieve a more resolutive analysis with the current maturation rules and to discover new rules or signatures linked to the processivity of the DICERs and the known functional interactions between the proteins involved in silencing.

For instance in *A. thaliana*, RDR6 long double stranded RNA products are known to be mainly processed by DCL4 into 21nt siRNAs; from deep sequencing data analysis however, we also observed processing at a lower rate into 24nt siRNAs by DCL3. By the measure of cross-correlation of the genomic positions of these 21 and 24mers, our approach allowed us to propose a model for the ordered processing of these dsRNA substrates by both DICER proteins.

688 B Stepwise co-evolution between bacterial CRP/FNR-type transcription factors and their transcriptional networks

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Transcription is a key system in all living organisms. Transcription factors (TFs), in particular, play a central regulatory role(s) in this system, although the evolutionary scenarios of TFs and their coevolution with their target genes are poorly understood. Determining the relationships between the TFs and their target genes from an evolutionary perspective should contribute to our understanding of not only the variety of transcription regulation systems but also the archaic transcriptional networks.

The CRP/FNR-type TFs are members of a well-characterized global TF family in bacteria and have two conserved domains: the N-terminal ligand-binding domain for small molecules (e.g., cAMP, NO, or O_2) and the C-terminal DNA-binding domain. Although the CRP/FNR-type TFs recognize very similar consensus DNA target sequences, they can regulate different sets of genes in response to environmental signals. To clarify the evolution of the CRP/FNR-type TFs throughout the bacterial kingdom, we undertook a comprehensive computational analysis of a large number of annotated CRP/FNR-type TFs and the corresponding bacterial genomes. Based on the amino acid sequence similarities among 1,455 annotated CRP/FNR-type TFs, spectral clustering classified the TFs into 12 representative groups, and stepwise clustering allowed us to propose a possible process of protein evolution. Although each cluster mainly consists of functionally distinct members (e.g., CRP, NTC, FLP, and FixK), FNR-related TFs are found in several groups and are distributed in a wide range of bacterial phyla in the sequence similarity network. This result suggests that the CRP/FNR-type TFs originated from an ancestral FNR protein, involved in nitrogen fixation. Furthermore, a phylogenetic profiling analysis showed that combinations of TFs and their target genes have fluctuated dynamically during bacterial evolution. A genome-wide analysis of TF-binding sites also suggested that the diversity of the transcriptional regulatory system was derived by the stepwise adaptation of TF-binding sites to the evolution of TFs.

We are now expanding the survey objects and analyzing the transcriptional networks using the whole set of known TFs (approximately 300 proteins) in *Escherichia coli*. These results will be discussed in this conference.

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689 C The Conserved Structures of Right-Handed Polymerases

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Viral RNA-dependent RNA polymerases (vRdRp) have the classical right-handed polymerase structure containing three conserved subdomains: thumb, palm and fingers. The catalysis reaction is run by two catalytic ions in the palm subdomain. Interestingly, in the vRdRp of the phage φ6, a manganese ion has been observed around 6 Å from the catalytic ion binding site. This non-catalytic ion was later shown to be needed for the template binding, nucleotide coordination and catalysis. An ion in a similar position has also been observed in several other vRdRps. Now, we have identified the non-catalytic ion binding site by using structural alignment. Our results suggest that the non-catalytic ion binding site is common for positive-stranded and double-stranded RNA polymerases. Furthermore, we have studied the common structural features and evolutionary relationships of the right-handed polymerases by using a novel structural classification method. This study covers vRdRps, single-subunit DNA-dependent RNA polymerases, RNA-dependent DNA polymerases and DNA-dependent DNA polymerases. These results provide information about the structural and functional conservation of the right-handed polymerases.

690 A Discovering Conserved CIS-Regulatory G-Quadruplex Motifs in the Transcripts of Human CHD8 Gene Involved in Autism

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The human CHD8 (Chromodomain helicase DNA binding protein 8) is a chromatin remodeling agent. CHD8 represses transcription by enlisting the help of histone H1 at target genes where it remodels the chromatin. CHD8 binds to beta-catenin and suppresses the activity of p53/TP53-mediated apoptosis, thus negatively regulating the Wnt-signaling pathway. Mutations in the transcribed region of the human CHD8 gene have been linked to autism. Studying regulation of human CHD8 gene expression is expected to enhance our understanding of its function and role in human disease.

G-quadruplexes are highly stable three-dimensional structures formed in guanine rich DNA and RNA sequences. G-quadruplex structure consists of square coplanar arrays of G-tetrads. RNA G-quaduplexes have received significant attention because of their importance in biological processes such as regulation of protein synthesis and mRNA turnover.

The goal of this project has been to study the role of G-quadruplex forming motifsin regulating gene expression of human CHD8.

Using computational tools developed in our lab, we adopted a bioinformatics approach to map evolutionarily conserved G-quadruplexes in five orthologs of the humanCHD8 gene: chimpanzee, dog, bovine, mouse and rat. We discovered three highly conserved G-quadruplex motifs in the 3'-UTR of CHD8 mRNA. Two of these motifs were associated with microRNA target sites and one of them was found in close proximity to the polyadenylation signal. We found evolutionarily conserved G-quadruplex motifs in the 5" flanking region of the alternatively transcribed human CHD8 isoform 1. Conserved G-quadruplexes were also found near the splice sites of alternative isoforms.

Our analysis suggests that conserved G-quadruplexes could potentially regulate translation efficiency, mRNA stability, and polyadenylation of the CHD8 mRNAs. Our results also suggest that G-quadruplexes could play a role in alternative transcription, as well as, alternative splicing.

691 B IntEREst: Intron-Exon Retention Estimation using RNA-seq data

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RNA sequencing is widely used today for studying gene expression analysis as well as identifying and quantifying various mRNA processing intermediates in the cells. To estimate the levels of the partially spliced or unspliced transcripts in a cell from RNA-seq data we have developed IntEREst (Intron-Exon Retention Estimation) pipeline in R. The IntEREst pipeline can be run in parallel to increase the performance.

In this study we employed IntEREst to investigate the global analysis of the nuclear processing of the unspliced U12-type introns by the exosome, a ribonuclease complex involved in RNA turnover using ABI's SOLiD 4 transcriptome sequencing data. TheU12-type introns are a distinct subset of nuclear introns and constitute >0.5 % of all introns (~800 introns in humans). They are removed via a separate spliceosome (so called U12-dependent spliceosome) while the normal U2-type introns are removed by the U2-dependent spliceosome. Earlier studies have shown that the splicing rate of the U12-type introns in the nucleus is slower than that of the U2-type, suggesting that the U12-type introns can regulate the levels of their "host" mRNAs in the cell. Consistently, an elevated level of unspliced U12-type introns have been detected in the steady-state mRNA populations in various organisms. Here we investigated the hypothesis that due to slower splicing, the unspliced mRNAs containing U12-type introns in the nucleus are preferentially degraded by the exosome complex. Exosome is a ribonucleoprotein complex that participates in numerous RNA processing and trimming steps but also degrades RNAs that either aberrant or accumulate in wrong compartments. We compared the retention levels of the U12- and U2-type introns in nuclear and cytoplasmic fractions in control cells and in cells in which the exosome function has been disabled (e.g. knockdown of the Rrp41 and Dis3 subunits). Analyzing more than 100,000,000 paired reads (50 bps + 35 bps, with ~100-200 bps library) that were mapped to U12 intron containing genes, we discovered that exosome inactivation stabilizes unspliced U12-type introns as opposed to the U2-type introns. Moreover, the effects of Rrp41 and Dis3 knockdowns were not identical, suggesting different regulatory roles for the two subunits. Finally, we also confirm in genome-wide fashion that unspliced U12-type introns are indeed overrepresented as compared to U2-type introns.

692 C Understanding without reading: analog encoding of physico-chemical properties of proteins in cognate messenger RNA

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Being related by the genetic code, messenger RNAs (mRNAs) and cognate proteins are polymers with mutually interdependent compositions, which further imply the possibility of a potential connection between their physico-chemical properties. How efficiently do different characteristics of mRNA coding regions reflect the features of cognate proteins and is it possible for the cell to obtain information about proteins from their mRNAs without first reading them on the ribosome? We address these issues in a theoretical proteome-wide analysis and show that average protein hydrophobicity, calculated from either sequences or 3D structures, can be encoded in an analog way by many different mRNA sequence properties with the only constraint being that pyrimidine and purine bases should be clearly distinguishable. Moreover, average characteristics of mRNA sequences allow for a reasonable discrimination between human proteins with different cellular localization and, in particular, cytosolic and membrane proteins, even in the absence of signal-peptide-based mechanisms. We discuss our findings in the context of protein and mRNA localization and propose that this cellular process may be partly determined by basic physico-chemical rationales and interdependencies between the two biomolecules.

693 A cWords – systematic microRNA regulatory motif discovery from mRNA expression data

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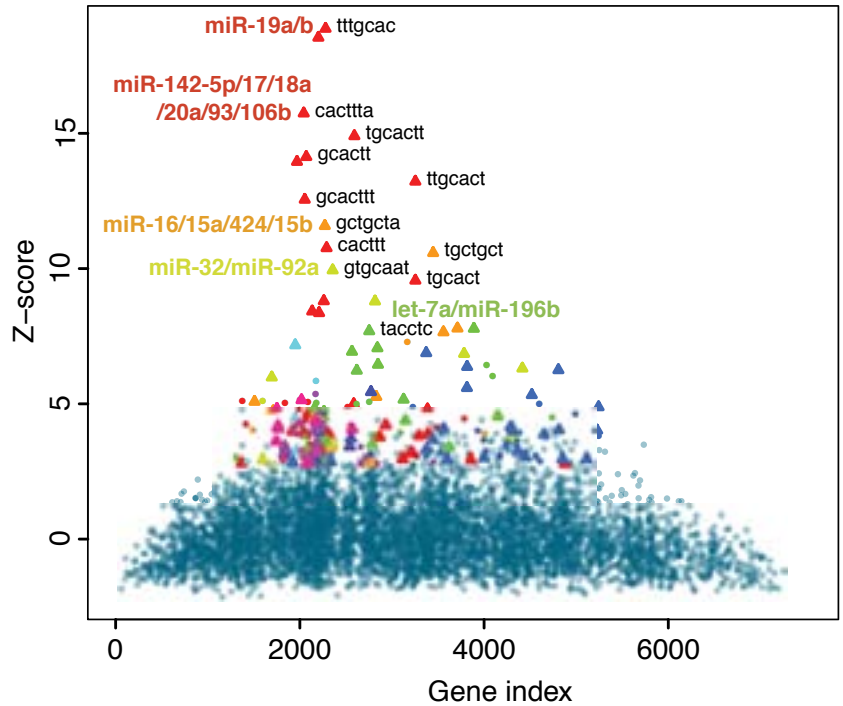
Background: Post-transcriptional regulation of gene expression by small RNAs and RNA binding proteins is of fundamental importance in development of complex organisms, and dysregulation of regulatory RNAs can influence onset, progression and potential treatment of many diseases. Post-transcriptional regulation by small RNAs is mediated through partial complementary binding to messenger RNAs leaving nucleotide signatures or motifs throughout the entire transcriptome. Computational methods for discovery and analysis of sequence motifs in high-throughput mRNA expression profiling experiments are becoming increasingly important tools for the identification of post-transcriptional regulatory motifs and the inference of the regulators and their targets.

Results: cWords is a method designed for regulatory motif discovery in differential case-control mRNA expression datasets. We have improved the algorithms and statistical methods of cWords resulting in at least a factor 100 speed gain over the previous implementation. On a benchmark dataset of 19 microRNA (miRNA) perturbation experiments cWords showed equal or better performance than two comparable methods, miREDUCE and Sylamer. We have developed rigorous motif clustering and visualization that accompany the cWords analysis for more intuitive and effective data interpretation. To demonstrate the versatility of cWords we show that it can also be used for identification of potential siRNA off-target binding. Moreover, cWords analysis of an experiment profiling mRNAs bound by Argonaute (AGO) ribonucleoprotein particles discovered endogenous miRNA binding motifs (Figure 1). The binding sites that occur in the mRNAs most bound by AGO as reported by cWords are shown in Figure 1A and these words correspond to the target sites of most expressed miRNAs in HEK293 cells, which are ranked in Figure 1B.

Conclusions: cWords is an unbiased, flexible and easy to use tool designed for regulatory motif discovery in differential case-control mRNA expression datasets. cWords is based on rigorous statistical methods that demonstrate comparable or better performance than other existing methods. Rich visualization of results promotes intuitive and efficient interpretation of data. cWords is available as a stand-alone Open Source program at Github https://github.com/simras/cWords and as a web-service at: http://servers.binf.ku.dk/cwords/.

A



B

miRNA	HEK293 expression	Seed site
miR-16	0.097	tgctgct
miR-196b	0.090	actacct
miR-15a	0.077	tgctgct
miR-142-5p	0.067	actttat
miR-19a	0.061	tttgcac
miR-18a	0.047	gcacctt
miR-17	0.042	gcacttt
miR-19b	0.041	tttgcac
miR-32	0.038	gtgcaat
miR-20a	0.033	gcacttt
miR-10a	0.031	acagggt
miR-424	0.026	tgctgct
miR-92a	0.023	gtgcaat
miR-15b	0.020	tgctgct
miR-93	0.018	gcacttt
let-7a	0.013	ctacctc
miR-30d	0.012	tgtttac
miR-106b	0.010	gcacttt
miR-140-5p	0.010	aaccact
miR-191	0.010	ttccgtt

Figure 1. **Word enrichment of AGO bound mRNAs.** (A) cWords word cluster plot showing 7mer 3'UTR words correlated with AGO binding in HEK293 cells. The top 10 words are annotated with IDs of the most abundant miRNAs in HEK293 cells. (B) Top 20 most abundant miRNAs in HEK293 cells, listing expression (relative clone frequency) and seed site (position 2-8) for each miRNA.

694 B Analysis of structural and functional impact of UTR single nucleotide variants identified in the non-small cell lung cancer by RNA-sequencing

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Non-small cell lung cancer (NSCLC) is the most common form of lung cancer, with high prevalence of mutations in KRAS gene. Tumors harboring these mutations tend to be aggressive and resistant to chemotherapy. In a recent study, the transcriptome of the tumors cells harboring mutant KRAS and wild-type KRAS have been compared to identify the differentially expressed genes, alternative splicing and single nucleotide variations (SNVs). About 40% of the total SNVs (73,717) identified were mapped to the UTR regions of mRNAs, however, their effects on the UTR region have not been analyzed due to the lack of comprehensive computational pipeline. It is known that the UTR regions of mRNA harbor sequence and structural motifs that are involved in the post-transcriptional regulations of gene expression. SNVs mapped to these regions may cause sequence or structural changes that can interfere with the functions of UTR. Thus, we designed a pipeline to predict the effect of SNVs on the secondary structure of UTR (using RNAsnp) as well as miRNA target sites within the UTR regions of mRNAs (using TargetScan, miRanda and RIsearch). With our comprehensive computational pipeline, we predicted 492 out of 29,290 UTR SNVs have significant effect on the local RNA secondary structure of the UTR regions (corresponding to 429 genes). Of these 492 SNVs, 117 SNVs were present in 104 genes involved in cancer related pathway. On the other hand, 302 out of 29,290 UTR SNVs were predicted to cause changes in the miRNA target site within the UTRs of 289 genes. Of these 302 SNVs, 73 were present in 67 genes involved in cancer related pathway. On these 67 genes, 85 miRNAs were predicted to bind either in the wild- type or mutant. We found 54 out of these 85 miRNAs overlap with lung cancer associated miRNAs from PhenomiR database, while six miRNAs are associated specifically with NSCLC according to microRNA body map database.

695 C Searching the coding region for microRNA targets

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Finding microRNA targets in the coding region is difficult due to the overwhelming signal encoding the amino acid sequence. Here we introduce an algorithm (called PACCMIT-CDS [1]) that finds potential microRNA targets within coding sequences by searching for conserved motifs that are complementary to the microRNA seed region and also overrepresented in comparison with a background model preserving both codon usage and amino acid sequence. Precision and sensitivity of PACCMIT-CDS are evaluated using PAR-CLIP and proteomics datasets. Thanks to the properly constructed background, the new algorithm achieves a lower rate of false positives and better ranking of predictions than do currently available algorithms, designed to find microRNA targets within 3'UTRs.

1. R. M. Marín, M. Šulc, and J. Vaníček, RNA, in press (2013), http://rnajournal.cshlp.org/lookup/doi/10.1261/rna.035634.112.

696 A PyCRAC CLIP data analyses predict a prominent role for Nrd1 and Nab3 in regulation of protein coding gene expression in yeast

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RNA-binding proteins play crucial roles in the synthesis, processing and degradation of RNA in a cell. To better understand the function of RNA-binding proteins, it is important to identify their RNA substrates and the sites of interaction. This would help to better predict their function and lead to the design of more focused functional analyses. The development of CLIP and related techniques has made it possible to identify direct protein-RNA interactions *in vivo* at nucleotide resolution.

The analysis of the high-throughput sequencing datasets, however, can be daunting, and demands more than a basic knowledge in bioinformatics and computer programming skills. The development of pyCRAC was driven by a requirement for a set of user-friendly, flexible and coherent set of tools tailored to handle CLIP and related RNA sequencing data. With a large number of valuable CLIP datasets now publically available, pyCRAC provides a comprehensive set of easy to use tools that allow the less experienced researcher to (fully) exploit these data.

To illustrate the functionality of pyCRAC, we have used the toolset to reanalyse recently published Nrd1 and Nab3 PAR-CLIP data, and we have also experimentally validated some of our findings. We found that Nrd1-Nab3 bound between 20 to 30% of protein-coding transcripts and we speculate that the proposed Nrd1-Nab3 "fail-safe" transcription termination mechanism is commonly used to prevent transcriptional read-through of mRNA coding genes. Nrd1 and Nab3 targets were also significantly enriched for enzymes and permeases involved in nucleotide/amino acid uptake and proteins involved in mitochondrial organization, indicating Nrd1-Nab3 function is tightly integrated with the nutrient response. Notably, transcripts encoding mRNA export and turnover factors were also frequently targeted. We conclude that Nrd1 and Nab3 play a prominent role in the regulation of protein coding gene expression, in particular genes involved in the nutrient response. Results from our *in silico* and experimental analyses will be presented.

697 B Segmentation of Proximal RNA Binding Sites from High-throughput Sequencing Data Using a Density-Based Clustering Approach

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[1]University of Vienna

High-throughput sequencing has been widely used to find novel protein-binding RNAs, utilizing methods such as CLIP-Seq and Genomic SELEX. Typical analyses of the high-throughput data employ the use of a histogram of the aligned read count per base, the intensity of which indicates, for a specific region of an RNA, the underlying sequence's relative occurrence of binding events in the experiment. This technique can, however, obscure the precise locations of binding sites in close proximity to each other, which would be found when analyzing high-throughput data for enzymes whose activity require multiple RNA binding sites, as well as for overlapping distinct ncRNA loci derived from processing events. We present a density clustering approach based on the OPTICS algorithm which can detect any number of RNA binding sites indicated by the data without prior knowledge of the expected length or number of binding sites in the transcript. Combined with histogram analysis as post-processing, the algorithm can detect minimal-length proximal binding sites of RNAs binding yeast RNA Polymerase II derived from Genomic SELEX data. Band-shift assays show that output of the algorithm corresponds both to the correct length as well as the binding affinity. The algorithm represents a novel approach to processing read data and is fast enough to run to large data sets after alignment of the reads is performed. It can be applied to many types of high-throughput sequencing data to deconvolute proximal DNA and RNA binding sites encoded in the genome. A C implementation of the algorithm will be available at http://alu.abc.univie.ac.at/hillfinder

698 C Full-Length Transcript Sequencing: Looking Beyond the ENCODE data

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Most RNA-seq experiments to date use short cDNA reads to assemble and count putative transcripts. These methods can infer alternative events such as splicing or transcription start sites, but cannot correlate events that are far apart on the original transcript since short reads cannot truly assemble the original, single RNA molecule. To address this deficiency, we applied the long read length capabilities of the PacBio® *RS* to sequence full-length cDNA molecules derived from human H1 stem cell polyA RNA. This cell line, a Tier 1 sample for the ENCODE project, has been extensively characterized by multiple 2nd generation sequencing approaches, including RNA-seq to assist in transcript assembly. We hypothesized that the long reads would help not only to assign the most common full-length mRNA isoforms in the H1 line, but also assist in assembling transcripts that are difficult with current algorithms.

We demonstrate that several full-length library preparation methods generate sequencing libraries that are highly enriched in full-length cDNA molecules. This was shown using a reference-based approach of aligning putative full-length cDNA reads to known transcripts in the Gencode set. By this alignment method, we detect many known full-length transcripts spanning a range from 500 bp to 6000 bp in length. We also present genome-based alignment approaches using gapped alignment methods that account for splicing events. To assist the genome-based approach, we have developed an error correction method, LSC, which uses short-read data to improve the alignments of long full-length cDNA sequences. Using both long and short reads, we developed an isoform detection and prediction pipeline. We report our findings on detecting novel splicing events and the discovery of new ncRNAs that are not apparent from Cufflinks short-read assembly alone. As an unintended benefit, this methodology also captures transcript maturation in action, as retained intron events can often be seen in the full-length reads. The methods and algorithms for long-read cDNA sequence determination will assist researchers to better characterize the transcriptome's true form and help unlock combinatorial RNA processing regulation that cannot be observed in previous RNA-seq data sets.

699 A Comprehensive identification of RNA 5′ ends in E. coli

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Recent prokaryotic transcriptome studies, led by advances in sequencing that have improved the technique's sensitivity, report antisense transcription to be more prevalent than previously expected. As many as 70% of annotated genes have been reported to have antisense counterparts that are regularly expressed at low levels. Although function of these widespread antisense transcripts is not known, they are often considered to be product of background or pervasive transcription.

We adapted and modified the standard RNA-seq library preparation method in order to discriminate between pervasive transcription products and stable, likely regulated, transcripts with a defined 5' end. The modification of the library preparation allowed us to specifically enrich for 5' ends resulting in most comprehensive overview of *E. coli* transcripts featuring stable 5' ends. Moreover, we were able to distinguish between primary transcriptional start sites (TSSs) with 5' triphosphate and stable 5' ends resulting from ribonuclease processing, bearing a monophosphate. We identified alternative TSSs of known genes as well as novel TSSs giving rise to unexpected short, mostly antisense RNAs. In addition, we monitored the influence of well-known RNA chaperone Hfq on TSSs profile. Expression of novel short RNAs, as well as their dependence on Hfq were confirmed by Northern blot analysis, further supporting the sequencing data. Overall, our data suggests strands opposite to annotated genes have potential to code for regulated RNAs with discrete 5' ends.

700 B Engineered "restriction RNases" for sequence-specific cleavage of dsRNA and RNA in DNA-RNA hybrids

Janusz Bujnicki[1], Krzysztof Skowronek[1], Agata Sulej[1], Dariusz Pianka[1], Irina Tuszynska[1], Marcin Nowotny[2], Justyna Czarnecka[1]

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Ribonucleases (RNases) are valuable tools applied in the analysis of RNA sequence, structure and function. Their substrate specificity is limited to recognition of single bases or distinct secondary structures in the substrate. Thus far, there have been no RNases available for purely sequence-dependent fragmentation of RNA, analogous to restriction enzymes for DNA. We have therefore searched for existing RNases that could be engineered to become sequence-specific. Using a combination of bioinformatics methods and experimental protein engineering we have obtained prototypes of two sequence-specific "restriction RNases" (RRNases): one that cleaves both strands of dsRNA within a target sequence, and one that cleaves RNA within DNA-RNA hybrids at a particular distance from the target sequence.

Based on structural analysis of enzymes from the RNase III superfamily we identified loops that could be extended to make specific contacts with bases in the dsRNA substrate. Biochemical characterization of selected members that possess extended versions of such loops revealed that some of them indeed exhibit sequence specificity. For one of such enzymes we constructed a structural model of a protein-RNA complex, and used it to guide site-directed mutagenesis aimed at elucidating the molecular basis of specificity and to increase the selectivity of cleavage. The obtained prototype RRNase recognizes a partially degenerated hexanucleotide target sequence and is capable of cleaving individual sites in long dsRNA molecules.

A prototype RRNase that cleaves the RNA strand in DNA-RNA hybrids 5 nucleotides from a nonanucleotide recognition sequence was constructed by fusing two functionally distinct domains: a non-specific RNase HI and a zinc finger that recognizes a sequence in DNA-RNA hybrids. The optimization of the fusion enzyme's specificity was guided by a structural model of the protein-substrate complex and involved a number of steps, including site-directed mutagenesis of the RNase moiety and optimization of the interdomain linker length.

For both types of RRNases we implemented methods of specificity engineering, to enable generation of variants specific for other target sequences, making it feasible to acquire a library of enzymes that recognize and cleave a variety of sequences, much like the commercially available assortment of restriction enzymes. Potentially, RRNases may be used in vitro for production of RNA molecules with defined length and termini, which may be a cheaper alternative to chemical synthesis; they may be also used *in vivo* for targeted RNA degradation.

701 C miR-Direct: RT-qPCR analysis of plasma microRNAs without prior RNA extraction

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Circulating microRNAs (miRNAs) have great potential as biomarkers, but current methods for their detection are hampered by inconsistent RNA recovery during isolation, by the difficulty of eliminating RT-PCR inhibitors, and by the low concentration at which most miRNAs appear in blood. Here we describe a novel method (miR-Direct™) in which miRNAs or other small RNAs (or fragments of large RNAs) of interest can be enriched from plasma samples and directly quantified by RT-qPCR without prior extraction of total RNA. miR-Direct consists of the following steps: (1) treatment of plasma with agents that release miRNAs from lipid and/or protein complexes; (2) capture of miRNAs of interest by hybridization with specific probes attached to magnetic beads; (3) washing the captured miRNAs to remove inhibitors of amplification reactions present in plasma and the release buffer; (4) release of the captured miRNAs into solution; and (5) detection of the released miRNAs using an RT-qPCR assay. We compared two such assays, SomaGenics' miR-ID® and Life Technologies' TaqMan® microRNA assay, for detection of three circulating miRNAs (hsa-miR-16, hsa-miR-148a and hsa-miR-125b) as well as a spike-in control (cel-miR-39). In this comparison, miR-ID detection produced significantly lower cycle threshold (Ct) values than TaqMan detection, and Ct values showed the expected dependence on starting plasma volume only with miR-ID. The miR-Direct method reduces the variable loss of small RNAs that is usually seen with standard total RNA isolation conditions. Because sample processing (up to the qPCR step) is a one-tube process, the method is highly amenable to automation. Because irrelevant RNA and DNA is removed prior to the amplification steps, background is low and sensitivity high, resulting in accurate expression profiling of small RNAs and determinations of absolute copy numbers.

702 A Comparing the transcriptome of mouse and human using RNA sequencing

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[1]National Institutes of Health; [2]Diagnostics Discovery Department, Genentech, Inc.

Mice are often used as model organisms for human disease. There are many cases, however, in which mouse models do not recapitulate all of the symptoms present in the relevant human disease. This is especially true in models of neurological disease. The brain is an incredibly complex organ and understanding what makes the human brain unique and what it shares with its rodent models may help us to understand why some models are have better face validity than others. RNA sequencing (RNA-Seq) is uniquely applicable for direct comparison of gene expression between species as it does not require the use of sequence-specific probes, as is the case with microarray. We have used RNA-Seq to create a high-resolution transcriptome data set of mouse cerebral cortex and human frontal cortex. We found a large number of genes more highly expressed in one species as compared to the other. We validated a number of these findings using independent techniques. Taking advantage of the sequencing information found with RNA-seq we also noted that conserved A-to-I RNA editing sites were nonsynonymous coding changes in all but one case. We propose this dataset as a helpful resource for interrogating gene expression differences between humans and mice.

703 B Genome-wide profiling of RNA secondary structure in planta

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[1]Pennsylvania State University

RNA structure plays critical roles in regulating various post-transcription events involved in translation, splicing, and polyadenylation. However, lack of genome-wide *in vivo* RNA structural data limits our understanding of how RNAs fold and regulate gene expression *in vivo*. Herein we develop a platform to probe RNA structures on a genome-wide scale *in vivo*. With the application of next generation sequencing, we establish high-throughput *in vivo* RNA secondary structure profiling in *Arabidopsis thaliana*, a model plant species and modern eukaryote. This platform probes the RNA secondary structures of more than 10,000 transcripts, provides even coverage of each transcript, and gives excellent agreement on rRNA structures as a benchmark. This platform provides an accurate and quantitative genome-wide RNA structural map *in vivo* that reveals native RNA structural features that relate to numerous biological processes including translation initiation and regulation, alternative polyadenylation, and alternative splicing. This platform can be applied to different organisms, different tissues and different treatments which will allow the role of RNA structures in gene regulation to be understood on a genome-wide scale.

704 C Post-transcriptional regulation of mitochondrial gene expression

Aleksandra Filipovska[1], Ganqiang Liu[4], Stefan Siira[1], Timothy Mercer[3], Anne-Marie Shearwood[1], Dimitar Azmanov[1], Stefan Davies[1], John Mattick[2], Oliver Rackham[1]

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Human mitochondria contain a small and compact genome that is transcribed as long polycistronic transcripts that encompass each strand of the genome, which are processed into mature mRNAs, tRNAs and rRNAs within the mitochondrial matrix. Recently we provided the first comprehensive map of the human mitochondrial transcriptome by near-exhaustive deep sequencing of long and small RNA fractions from purified mitochondria (1). We have identified previously undescribed transcripts, including small RNA and long non-coding RNAs encoded by the mitochondrial genome (2). Furthermore despite their common polycistronic origin, we observed wide variation between individual tRNAs, mRNAs, and rRNA amounts, indicating the importance of RNA-binding proteins in the regulation of mitochondrial gene expression (3). We have investigated the roles of the mammalian pentatricopeptide repeat (PPR) proteins and found that these RNA-binding proteins are all localized to mitochondria where they regulate mitochondrial gene expression. Mammalian PPR proteins have diverse roles in RNA metabolism and translation that are important for mitochondrial function and cell health. To investigate the importance of RNA-binding proteins in mitochondria globally we have established new methods for massively parallel sequencing and analyses of RNase-accessible regions of human mitochondrial RNA. We have identified specific regions within mitochondrial transcripts that are bound by RNA-binding proteins. These mitochondrial protein footprints indicate that RNA-binding proteins as well as small RNAs play a significant role in the regulation of mitochondrial gene expression.

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705 A Investigating ligation bias in small RNA library construction for high-throughput sequencing and the effect of different 3′ and 5′ adapters

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[1]**New England Biolabs**

High-throughput sequencing (HTS) has become a powerful tool for the detection of and sequence characterization of microRNAs (miRNA) and other small RNAs (sRNA). Unfortunately, the use of HTS data to determine the relative quantity of different miRNAs in a sample has been shown to be inconsistent with quantitative PCR and Northern Blot results. Several recent studies have concluded that the major contributor to this inconsistency is bias introduced during the construction of sRNA libraries for HTS and that the bias is primarily derived from the adapter ligation steps; specifically when single stranded adapters are sequentially ligated to the 3' and 5'-end of sRNAs using T4 RNA ligases. In our current study we investigate the effects of ligation bias by using a defined mixture of 962 miRNA sequences and several combinations of adapters in HTS library construction. Our results provide insight about the nature of ligation bias and allowed us to design adapters which reduce ligation bias and produce HTS results that more accurately reflect the actual concentrations of miRNAs in the defined starting material.

706 B tRid, a method for eliminating tRNAs without sequence information of tRNAs

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We here report a unique method, designated as tRid, for eliminating tremendous amount of tRNAs from any organism's RNA without knowing the sequence information of tRNAs. The key component of tRid is a flexible tRNA-acylation ribozyme, known as flexizyme. This catalyst is able to recognize the 3'-end CCA of tRNA, which is common sequence to all tRNAs used in organisms, and adds any one of a diverse repertoire of natural/non-natural amino acids to the 3'-OH of tRNA. Therefore, selective removal of tRNA would be possible through the aminoacylation using a biotinylated amino acid (Fig. 1).

For demonstrating tRid, HeLa S3 and *E. coli* <200 nt RNAs were treated by modified flexizyme, whose 3'-end nucleotide is oxidatively cleaved by sodium periodate for suppressing self-contamination of flexizyme (Fig. 2). After the treatment, 70-100 nt RNAs were purified and sequenced. As a result, the ratio of tRNAs derived from HeLa S3 and *E. coli* were decreased from 89% to 35% and 85% to 30%, respectively. This result means that tRid does not need the information of tRNA sequences so that it can eliminate tRNAs from any kinds of organism's RNA. For application of tRid, we tried to discover novel tRNA-sized RNAs from 70-100 nt RNAs treated by tRid. The ratio of tRNA-sized RNAs derived from HeLa S3 and *E. coli* were increased from 11% to 65% and 15% to 70%, respectively. We discovered several novel sRNAs from the enriched *E. coli* tRNA-sized RNAs and elucidated that these sRNAs are transcribed as similar size of tRNA by northern blotting. This result demonstrates tRid facilitates the efficient exploration of novel tRNA-sized RNAs. For another application of tRid, we purified <200 nt RNAs from pond water, which contains various kinds of microorganisms, and eliminated tRNAs by tRid. As a result, the ratio of tRNAs was decreased from 90% to 46% and the ratio of tRNA-sized RNAs was increased from 10% to 54%. This result means that tRid can be applied to metatranscriptome analysis.

In conclusion, tRid is a general-purpose method, which can eliminate any organism's tRNAs without the sequence information of tRNAs and can be applied various studies that is difficult to do because of the tremendous amounts of tRNAs.

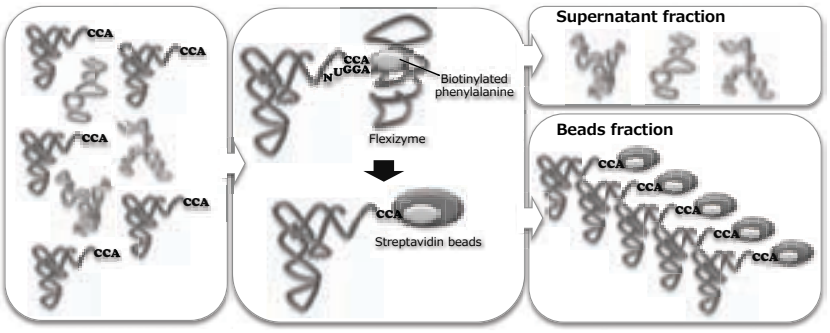

Fig. 1 Schematic illustration of tRid

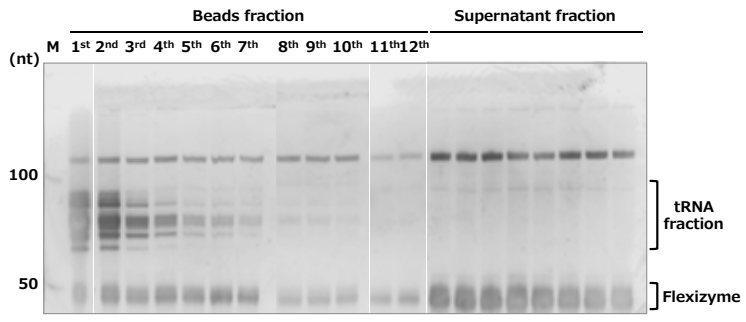

Fig. 2 The demonstration of tRNA elimination from <200 nt RNAs by tRid. *E. Coli* <200 nt RNAs were treated by flexizyme, biotinylated phenylalanine and streptavidin beads twelve times.

707 C Abstract Withdrawn

708 A Dynamic responses of the hepatocytic mRNA interactome to metabolic reprogramming

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mRNAs are regulated at multiple steps throughout their lifetime by RNA-binding proteins (RBPs) which collectively represent the "mRNA interactome" of a cell. Interestingly, many unexpected proteins such as metabolic enzymes were found to bind mRNAs in vivo [1, 2], potentially playing important roles interconnecting posttranscriptional regulation with cellular metabolism [3]. IRP1/aconitase represents a well studied example, which, depending on intracellular iron levels, switches between functions as an enzyme or an RBP controlling mRNAs involved in iron metabolism. As liver cells represent a metabolically critical cell type, we determined the mRNA interactome of hepatocytic HuH-7 cells by in vivo RNA-protein crosslinking, isolation of mRNP complexes by oligo-d(T) chromatography and analysis by quantitative mass spectrometry [4]. We identified 726 proteins, including known RBPs, expected RBPs that are expressed in the hepatic cells, and many previously unknown RBPs. Interestingly, especially enzymes of central energy metabolism pathways display mRNA binding, notably of the glycolytic pathway and the TCA cycle. Next, cells were treated with the glycolytic inhibitor 2-deoxyglucose or left untreated prior to interactome capture and comparative quantitative proteomic analysis. We will describe the responses of the HuH-7 cell mRNA interactome to altered glycolysis, and report the development of comparative, quantitative interactome capture as an informative experimental approach to characterize the states and responses of cellular systems.

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709 B Cytoplasmic mRNA Capping and the Implications of Cap Homeostasis

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Until recently, the removal of an mRNA's 5' cap structure was thought to be an irreversible step leading to the degradation of the decapped mRNA. Our lab has discovered and begun to characterize the cytoplasmic capping enzyme (cCE) complex, which restores the 5' cap onto certain decapped mRNAs. Results of experiments aimed at identifying recapping targets led to the discovery of cap homeostasis, a process by which some mRNAs undergo cyclical decapping followed by recapping. The regulated decapping and recapping of these mRNAs may function as mechanism for cells to survey and regulate their translation. Our previous work described the development of cells that are stably transfected with a tetracycline-inducible dominant negative form of cCE. Expressing dominant negative cCE increased the population of uncapped transcripts, and these were shifted from polysomes to non-translating mRNPs. Importantly, this is specific to recapping targets as the translation of control transcripts was unchanged. Interestingly, 5' RACE experiments performed with RNA from cells inhibited for cytoplasmic capping identified transcripts that were missing portions of their 5' ends, raising the possibility that cytoplasmic capping may enable the translation of N-terminally truncated proteins as well as full-length proteins from the same parent transcript. We couple informatics approaches with 5' RACE and sequencing to assess the diversity of these validated cCE targets and to pinpoint the locations where these transcripts are recapped. Finally, we are adapting ribosome profiling for use in our dominant negative cCE cell line as a way to ascertain the prevalence and functional effects of cap homeostasis on a transcriptomic level.

Supported by NIH grant GM084177 to DRS. DLK is supported by a Pelotonia postdoctoral fellowship and by NIH grant T32 CA0093338-34.

710 C Screening of the RNase-sensitive subnuclear structures identified the Sam68 nuclear body that was built on RNA with novel protein components

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The mammalian cell nucleus contains membraneless suborganelles characterized by a distinct set of resident proteins and referred to as nuclear bodies. The nuclear bodies are thought to serve as the sites for biogenesis of various RNA species, the storage and assembly of ribonucleoprotein complexes and the retention of specific RNA species. We have been studying on the nuclear paraspeckle that is formed around the architectural NEAT1 long noncoding RNA (lncRNA) (Sasaki et al., PNAS 2009, Naganuma et al., EMBO J 2012). Here, we searched for additional nuclear bodies that were built on unidentified architectural RNAs by screening of the "RNase-sensitive nuclear bodies" using 10432 fluorescence-tagged human full-length (FLJ) cDNA clones (Goshima et al., Nat methods 2008). First, we chose 463 FLJ cDNA clones whose fluorescently tagged-protein products were localized in certain nuclear foci. Subsequently, we explored whether the respective nuclear focus was abolished or diffused upon RNase treatment after cell permeabilization. "The RNase-sensitivity screening" identified 25 tagged proteins that required RNA for their localization in distinct nuclear foci. Immunostaining of the corresponding endogenous proteins confirmed that the Sam68 nuclear body (SNB) was an RNase-sensitive structure. The above screening simultaneously identified five novel SNB proteins (SNB1-5) whose localization in SNB were also abolished by RNase treatment. RNAi of each SNB component revealed that two RNA-binding proteins (Sam68 and SNB3) were required for SNB formation. Our data argue that the SNB structural core is built on certain nuclear RNA(s) that collaborate with at least two RNA-binding proteins.

711 A In vivo capture of RBPs bound to defined RNA species

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Recently, a comprehensive and unbiased method, called mRNA interactome capture, was developed for global identification of RBPs in living cells [1; 2; 3]. The design of this method focuses on determination of complete mRNA interactomes, but cannot be applied to capture RBPs assembled on defined RNA species. Methods to identify RNAs bound by single RBPs were successfully setup before (CLIP, PAR-CLIP, HITS-ClIP [reviewed in 4]). However, identifying RBPs that bind a particular RNA has been challenging so far. Here we present an experimental approach to determine all RBPs bound to a defined RNA species *in vivo,* which should enable studying the plasticity of particular Ribonucleoproteins (RNPs) throughout the different steps of RNA life, under changing cellular conditions (e.g. stress and disease) or different cellular stages. Using the global mRNA interactome capture approach including *in vivo* UV crosslinking as a starting point, we tailor the pull-down protocol by making use of DNA/LNA mixmer oligonucleotides as specific bait. Our proof of principle model is based on luciferase reporter constructs that allow introducing known RNA motifs upstream or downstream of the targeted open reading frame. We will discuss performance parameters of our approach to meet an urgent need in RNA research.

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712 B Discovery of gene expression inhibitors using a high throughput single-cell analysis system

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The many macromolecular machines involved in eukaryotic gene expression have been individually studied in model organisms for decades. These efforts have been enhanced by the use of specific reporter genes that are sensitive to defects in an individual process. In recent years, it has become increasingly evident that gene expression processes in eukaryotes involve communication and coordination between all of the gene expression machineries. We have previously described the development of a versatile gene expression reporter for budding yeast that employs high-throughput flow cytometry. Cells harboring the reporter generate green and red fluorescence from spliced and unspliced transcripts respectively. Our reporter exhibits a unique signature for defects in many gene expression processes including transcription, pre-mRNA splicing, NMD, mRNA export and mRNA decay. Using a high throughput approach we have genetically explored contributions of knockout mutations to gene expression.

Many of the pathways in gene expression are targets for therapeutics and naturally occurring small molecules have provided a wealth of information about those pathways. Further work to identify novel targets and effectors will enhance our understanding of the control of gene expression and, importantly, provide potential new drug candidates. We have adapted our reporter assay to perform high-throughput screening for small molecule inhibitors of specific gene expression processes using an inducible version of our reporter. In our pilot study, we screened hundreds of small molecules that have a history of use in human clinical trials. We have followed these experiments with an expanded library of thousands of small molecules. By means of plate reader and flow cytometry analysis, we have identified many small molecules that modulate gene expression and affect cell growth. We are exploring the nature of the putative inhibition by these compounds which behave like pre-mRNA splicing, RNA decay or NMD inhibitors in our assay. Additionally, by virtue of the single-cell nature of our assay, we have identified molecules that increase cell-to-cell variation in reporter expression. We are currently identifying patterns between our primary hit compounds and the effect on eukaryotic gene expression pathways.

713 C Imaging trinucleotide repeat RNA in live cells using Spinach2, an RNA tag with enhanced brightness and thermostability

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Decades of research have revealed the vast array and crucial roles of RNA at all levels of gene expression. Imaging RNAs in real time in living cells is vitally important for studying these processes, but is technically challenging. A genetically encoded RNA mimic of GFP called Spinach was recently described that provides a markedly simplified approach for imaging RNAs in living systems. Spinach is an aptamer that binds a small molecule chromophore. Separately, both Spinach and the chromophore are nonfluorescent; however, when bound the Spinach-chromophore complex is brightly fluorescent, allowing specific imaging of tagged RNAs in living cells. Although powerful, Spinach suffers from limited sensitivity in mammalian systems due to low thermal stability, poor folding, and ion sensitivity. For this reason, we subjected Spinach to targeted mutagenesis and developed Spinach2. Spinach2 has enhanced brightness, thermal stability, and folding relative to Spinach, and performs robustly in both bacterial mammalian cells. We have used Spinach2 to study toxic CGG-repeat containing RNAs in a model of Fragile-X tremor ataxia syndrome and show that Spinach2 is a versatile new tool for *in vivo* imaging.

714 A Programmable sequence-specific click-labeling of RNA using archaeal box C/D RNP methyltransferases

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Biophysical and mechanistic investigation of RNA function requires site-specific incorporation of spectroscopic and chemical probes. In nature, a particular class of RNA methyltransferases, box C/D ribonucleoprotein complexes (C/D RNPs), direct AdoMet-dependent site-specific 2'-O-methylation to numerous biological sites [1]. Precise base pairing of a guide RNA and the substrate selects the target nucleotide (Fig. 1A). We have combined the specificity of C/D RNP machinery with synthetic AdoMet analogs to incorporate a reactive group which could be further appended with a desired label (Fig. 1B and 1C). Therefore we have *in vitro* reconstituted a C/D RNP from the thermophilic archaeon *Pyrococcus abyssi* and demonstrated its ability to transfer a prop-2-ynyl group from a synthetic cofactor analog [2] to both the wild-type and newly programmed target sites in model tRNA and pre-mRNA molecules. Target selection of the RNP was programmed by changing a 12 nt guide sequence in a 64-nt C/D guide RNA leading to efficient derivatization of three out of four new targets in each RNA substrate. We also show that the transferred terminal alkyne can be further appended with a fluorophore using a bioorthogonal azide-alkyne 1,3-cycloaddition (click) reaction (Fig. 2). The described approach [3] for the first time permits synthetically tunable sequence-specific labeling of RNA with single-nucleotide precision.

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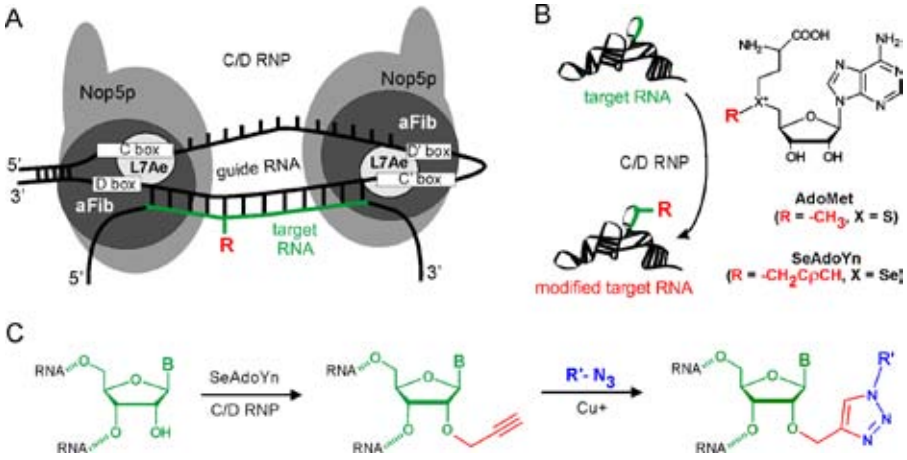

Figure 1. Archaeal C/D RNP-directed sequence-specific modification and labeling of target RNA. (**A**) Schematic structure of a C/D RNP complex with substrate RNA. Core proteins L7Ae, Nop5p and aFib are bound at the C/D and C'/D' sites of a guide RNA. One of the variable guide sequences is shown base-paired to a target sequence (green) of a substrate RNA. Modification occurs at a nucleotide complementary to the 5th position upstream from the D box; (**B**) C/D RNP-directed transfer of an activated side chain (red) from a cofactor S-adenosyl-L-methionine (AdoMet, X=S and R=methyl) or its analog SeAdoYn (X=Se and R=prop-2-ynyl) onto an RNA substrate; (**C**) Two-step labeling of target RNA via a C/D RNP-directed alkynylation, followed by Cu(I)-assisted click-coupling of a fluorophore azide (blue).

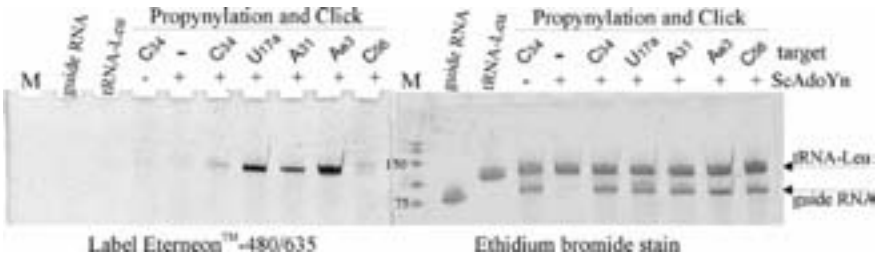

Figure 2. C/D RNP-dependent fluorescent labeling of predetermined sites in a model tRNA substrate via guide RNA-directed enzymatic propynylation and click-coupling of an Eterneon(480/635) azide.

715 B A new technique for live cell RNA detection that enables single cell analysis and live cell sorting

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[1]EMD Millipore

Understanding gene expression provides a window into the regulation of all cellular activity. To better understand and measure gene expression, various methods have been developed. Traditional techniques require long and laborious sample preparations and are prone to potential amplification bias. Other techniques involve transfections, cell fixation and cell lysis which could introduce unwanted change in expression levels.

A method for performing RNA detection in live cells would provide more biologically relevant information. Our technique allows for single-cell detection to accurately measure gene expression levels of living cells without stressful or harmful treatments. Because this technique does not involve harsh chemicals or transfection procedures, the data collected more accurately reflects a natural cell state. Furthermore following detection the cells remain unchanged and available for downstream assays.

This technique utilizes a gold nanoparticle surrounded by a high density oligonucleotide target specific sequence hybridized to complementary probes. Once in the cell, the gold nanoparticle will detect its target and release the fluorescent probe. The unquenched probe can then be accurately measured by flow cytometry for single cell RNA detection.

Here we show experiments using this technique for detection of RNA in single cells and live cell sorting by FACS with correlating qPCR data. In addition we prove this technique does not alter gene expression or affect cell health.

716 C Identification of novel post-transcriptional regulatory sequences

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Post-transcriptional events are essential for an organism's proper function and development, and misregulation of these events often results in cancers and other diseases in humans. Identifying sites within mRNAs that determine their post-transcriptional fates is therefore an essential step towards understanding post-transcriptional regulatory pathways. Such sites are typically found in the gene's untranslated regions (5'UTR and 3'UTRs), where they recruit trans-acting factors to control mRNA localization, decay rates, and translation efficiency. Many UTR regulatory sequences, as well as their mechanisms of action, still remain to be discovered.

One method we utilized was to use comparative genomics to identify preferentially conserved sequences in UTRs. We focused on those sequences that do not match binding sites for known trans-factors, and used traditional reporter assays in cell culture to validate multiple novel motifs. We are currently investigating the mechanisms by which a set of these sequences mediate post-transcriptional regulation. Although our approach has been successful in identifying novel regulatory sequences, it is not practical for large-scale validation of the thousands of conserved sequences observed in human UTRs.

To begin globally assessing 3'UTR sequences for regulatory ability, we developed an assay to test thousands of possible regulatory sequences in parallel. We generated large GFP reporter libraries in which random short sequences were inserted within the 3'UTR. When these reporters were integrated into the genome of cultured human cells, the GFP intensity corresponded to the post-transcriptional regulatory potential of the inserted sequence: cells with low GFP intensity contained a sequence that downregulated expression, and vice versa. We isolated cells undergoing differential expression and identified the proportions of each inserted short sequence in the different cell populations. We performed this screen in the context of three different human 3'UTRs and identified sequences that alter post-transcriptional gene regulation. We will compare this functional data to sequences conserved in human 3'UTRs. Additionally, our approach can identify sites undergoing rapid evolution without prior knowledge of the *trans*-factors. Because of the widespread impact of post-transcriptional regulation, knowing which sequences act in post-transcriptional regulation will help us to better understand human health and disease.

RNA 2013 Late Additions

717 B Maintenance of adult beta–cell identity by microRNAs and transcription factors

Topic: **Non-coding & Regulatory RNAs**

Eran Hornstein, Amitai Mandelbaum, Sharon Kredo-Russo, Tal Melkman-Zehavi

Hornstein lab, Department of Molecular Genetics, Weizmann Institute of Science

Normal physiology depends on defined functional output of differentiated cells. A century of Developmental–Biology studies revealed diverse mechanisms for differentiation, however once 'terminally' differentiated, cells are thought to naïvely stay put. However differentiated cells are surprisingly fragile, for example, phenotypic collapse and de–differentiation of beta–cells was recently discovered in pathogenesis of type 2 diabetes. These discoveries necessitate investigations of mechanisms for maintenance of robust cell–type identity over decades in the adult organism? microRNAs, which are small non-coding RNAs, are known to impart robustness to development. Our investigations reveal that microRNAs are providing means for continuous maintenance of adult beta–cell identity and therefore are important genetic components in metabolic disorders including in diabetes. This provides a new framework for miRNA function in adult tissues and in human disease.

718 C Prostate cancer exosomes offering novel circulating non-coding RNA biomarkers for early cancer diagnosis and prognosis.

Topic: **Non-coding & Regulatory RNAs**

Samuel E. Brennan[1], Nham Tran[2*], Aled Clayton[3], Eileen M. McGowan[1], Paul J. Cozzi[4], Rosetta Martiniello-Wilks[1,2]*

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Prostate cancer (PCa) can be cured in approx. 80% of men presenting with early, organ confined disease following surgery to remove the prostate. The commonly used blood test for prostate-specific antigen (PSA) shows elevated serum levels in men with PCa. Unfortunately, PSA alone is a poor predictor of disease outcome (prognosis) and invasive prostate biopsies are required to determine the PCa stage and prognosis. As a result, poor testing compliance results in most men presenting with advanced disease which is largely incurable. Thus, we are investigating the utility of RNAs enriched in cellular nanovesicles (40-100nm in size) released into the circulation, to develop novel non-invasive biomarker tests for early PCa diagnosis and prognosis.

Total RNA was isolated from human prostate epithelial cells, established PCa cell lines and their culture supernatant exosomes. The microRNA (miRNA) and messenger RNA (mRNA) expression profiles from these sources were analysed using the Affymetrix and Arraystar microarray platforms and the data were mined using Partek Genomics Suite. Biomarker candidates were then validated using qPCR.

The expression profiles revealed a vast number of differentially expressed miRNAs and mRNAs in PCa exosomes that can be readily developed into potential biomarkers. In brief, many miRNAs were up-regulated in PCa exosomes while the majority of mRNAs were down-regulated. Gene set analyses further suggested that differentially expressed exosomal RNAs were associated with various aspects of cancer development and progression. Exosomes show potential as sources for novel circulating biomarker discovery.

719 C Cold shock domain protein functions in reprogramming from differentiated cells to stem cells in the moss Physcomitrella patens

Topic: **Riboregulation in Development**

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Differentiated cells of the moss *Physcomitrella patens* have a high ability of being reprogrammed into stem cells. For instance, after leaf excision, differentiated leaf cells facing the cut are reprogrammed. Here we show that *P. patens* Cold Shock domain Protein (PpCSP), which is a homolog of one mammalian induced pluripotent stem cell (iPS) factor Lin28, plays a positive role in the reprogramming. In Physcomitrella genome, there are four *PpCSP* paralogous genes (PpCSP1 to PpCSP4), which encode highly similar RNA binding proteins. All of these four CSP proteins are localized in cytoplasm. The promoter activity and the localization of fluorescent proteins fused to the native PpCSP proteins showed that all PpCSP proteins accumulate in leaf cells facing the cut after excision and is maintained in reprogrammed stem cells. *PpCSP1* mRNA is regulated by putative degradation activity in its 3' UTR. Stabilized *PpCSP1* mRNA lines by excluding the degradation signal region showed enhanced reprogramming in cells not facing to the cut. Quadruple *PpCSP* mutants exhibited lower and delayed reprogramming. These data indicate that PpCSPs redundantly function as positive factors in the reprogramming. To reveal the molecular function of PpCSPs, transcriptome analysis was performed to seek downstream factors of PpCSP1. We found several candidate genes positively regulated by PpCSP1. Furthermore, to find the interacting factors of cytoplasmic PpCSP1 protein, we performed isobaric Tags for Relative and Absolute Quantitation (iTRAQ). PolyA-binding protein was identified and may have function together with PpCSP1. At last, by using ^{32}P labeled RNA, we confirmed that PpCSP1 could bind with mRNA in general. We currently search for target mRNAs of PpCSP1 by RNA-immunoprecipitation assay.

720 C Equilibrium-dependent ribosomal recoding mechanisms in RNA viruses

Topic: **Translational Regulation**

Carolina Salguero, Michael A. Durney, Victoria M. D'Souza

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RNA viruses have evolved mRNA-mediated recoding mechanisms to regulate gene expression at the translational level. Two of such mechanisms, codon readthrough and programed -1 frameshift, allow the ribosome to bypass a stop codon and synthesize fusion viral proteins. The frequency by which these events occur is important for efficient viral infectivity, and is regulated by domains in the translating mRNA (in the case of the SARScorona virus and the murine leukemia virus, the domains are pseudoknots). Although aspects of frameshifting have received considerable attention, an understanding of the RNA structures and structural rearrangements that influence the efficiency is lacking. Our results from murine leukemia virus indicate that retroviral gene expression is regulated by a dynamic, proton-driven equilibrium between an active, read-through permissive, and an inactive pseudoknot conformation. We are now working towards a complete structural and mechanistic understanding of the frameshifting frequency in SARS-corona virus by combining structural studies with biochemical in-vivo and in-vitro experiments. Specifically, to understand the basis for how the recoding frequency is maintained, we determined the structures of the mRNA signal in both frameshifting conformations: permissive and nonpermissive. These structures allowed us to engineer mutants to test our equilibrium model and generalize the results for equilibrium-dependent recoding to include both readthrough and frameshift mechanisms.

721 C Functional and Structural Characterization of a Thermostable RNA/DNA Dependent RNA Polymerase

Topic: **RNA protein interactions**

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The discovery of RNA interference (RNAi) in the late 1990s has revolutionized biological research with great potential for therapy. Since double-stranded RNAs (dsRNAs) are required for various RNAi applications, there is a need for cost-effective methods for producing large quantities of high-quality dsRNA molecules, including using enzymatic approaches, as an alternative to chemical synthesis. Here, we characterize at the functional and structural level a thermostable RNA polymerase named QT2 that can use either DNA or RNA as a template. We present the crystal structure of the catalytic domain of QT2 (113 kDa) at a resolution of 3.2 Å. The results indicate that the enzyme could form a useful tool to generate large pools of dsRNA molecules. We will present our structural studies with a view to dissect the molecular basis of its various catalytic activities and the molecular basis for its thermostability.

722 A Identification of novel methyltransferases, responsible for N-1 methyl-adenosine base modification of 25S rRNA in *S.cerevisiae*

Topic: **tRNA, snRNA, snoRNA, rRNA**

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S.cerevisiae contains two N-1 methyl adenosine (m^1A) base modifications in the helix 25.1 and helix 65 of 25S rRNA. The enzymes responsible for these base modifications remained elusive for a long time. We identified ribosomal RNA processing protein 8 (Rrp8) and Bmt2, a previously uncharacterised ORF as a methyltransferases involved in m^1A645 and m^1A2142 modification of 25S rRNA, respectively. The genes were identified by RP-HPLC screening of all deletion mutants of putative RNA methyltransferase and was confirmed by gene complementation and phenotypic characterization. Furthermore, we analysed the significance of m^1A645 and m^1A2142 modification in ribosome synthesis and translation. Intriguingly, the loss of m^1A645 modification retards the cell growth at lower temperature and cause significant defect in 60S assembly. On the other hand loss of m^1A2142 modification confers anisomycin and hydrogen peroxide hypersensitivity to the cells. Our results emphasize the importance of RNA modifications in cellular physiology.

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723 A Functional link between U1 snRNA 5′-end AU di-nucleotides and the mRNA cap-binding complex

Topic: **Splicing Regulation**

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Binding of the U1 snRNP to pre-mRNA 5' splice site (5'ss) plays a critical role in splicing by committing the pre-mRNA substrate to the splicing pathway. The interaction between U1 snRNP and the 5'ss is mediated in part by Watson-and-Crick basepairing of the U1 snRNA's 5'-end (from the 3rd to the 9th positions) to the relatively conserved 5'ss (GUAUGU, in the budding yeast). Intriguingly, despite no apparent participation of the first two AU residues of the U1 snRNA in interacting with the 5'ss, these two positions are highly conserved from fungi to metazoans. To investigate the role of these two residues in splicing, we systematically mutated them, at the U1 snRNA gene (*SNR19*) level, to all possible combinations. The majority of mutants exhibit no statistically significant difference in terms of fitness from that of the wild-type cells, suggesting that the AU dinucleotides may not be crucial. However, the AU-to-UU (AU>UU) mutant is greatly compromised in fitness and exhibits a cold-sensitive phenotype. Primer-extension analysis shows that the otherwise unique transcriptional start of *SNR19* in the AU>UU mutant is dramatically altered and results in multiple 5'-end-truncated and non-functional U1 snRNAs. Quantitative real-time PCR as well as **splicing-sensitive microarray** analysis show a global accumulation of pre-mRNAs, **suggesting that** insufficient production of functional U1 snRNA in the AU>UU mutant significantly compromises splicing. Moreover, genome-wide synthetic-lethal screen uncovered two categories of genes that are functionally related to the AU>UU mutation: splicing as well as transcription-related genes. Detailed analysis pinpoints that the most appealing candidates are the normally nonessential cap-binding proteins, Cbp20p and Cbp80p, which become indispensible upon altering the AU di-nucleotides in essentially all variations. This result thus strongly suggests that the AU di-nucleotides indeed have a role in splicing mediated through the cap-binding complex. The mechanistic details of this novel link are being defined biochemically.

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RNA 2013 Addendum

724-B A Long non-coding RNA regulates chromatin-mediated modulation of alternative splicing

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Recent evidence from numerous laboratories has revealed a novel role for chromatin as a regulator of alternative splicing. Traditionally, chromatin has been suggested to indirectly regulate alternative splicing by modulating the RNA polymerase II elongation rate. We have identified a more direct role of epigenetic modifications in splicing regulation. We find in FGFR2, a paradigm of PTB-regulated alternative splicing, distinctive signatures of histone marks that correlate with alternative splicing outcome. Modulation of these modifications induces splice site switching in a predictable manner. The molecular mechanism linking chromatin to the splicing machinery involves a chromatin/splicing adaptor system in which a chromatin-binding protein, MRG15, reads the histone marks and binds specifically to methylated H3K36. In turn, MRG15, by protein-protein interaction, favors the recruitment of the splicing regulator PTB specifically to its RNA splice site, inhibiting exon inclusion. On the other hand, enrichment of H3K27me counteracts the effect of H3K36me in FGFR2 splicing regulation by inhibiting recruitment of the chromatin-adaptor complex to FGFR2. Importantly, we show here that the high H3K27me / low H3K36me chromatin signature is established by a long non-coding RNA antisense to FGFR2. This asFGFR2 promotes recruitment of the Polycomb complex PRC2 and an H3K36 demethylase to FGFR2, which creates the splicing-specific chromatin signature characteristic of exon inclusion. These findings identify a lncRNA as a novel class of alternative splicing regulators and they point to cross-talk between histone marks that regulate alternative splicing in a combinatorial way. We propose that the epigenetic memory contained in histone modification patterns is not only used to determine gene activity, but also to establish and maintain cell-specific alternative splicing programs.

725-A DNA-damage induced regulation of splicing of MDMX-mRNA in ovarian carcinomas

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MDMX is an essential regulator of p53 activity during development and tumorigenesis. Among many different spliced transcripts known of MDMX, the variant MDMX-S has a prognostic relevance in different cancers. We opted to analyze the splicing mechanism underlying the generation of MDMX-S transcript. We also searched for a connection between the DNA-damage checkpoint kinases ATM and ATR with the alternative spliced MDMX-S transcript. The expression of MDMX splice variants, especially the ratio of FL-MDMX and MDMX-S was analyzed in 33 ovarian carcinoma samples. Furthermore, we investigated the role of ATM/ATR-pathway in the alternative splicing of exon 6 in an ovarian carcinoma cell line (OAW-42). RNA-Pull-Down was used to identify splice proteins which could be involved in the alternative splicing of exon 6. Finally, we knocked down a main NMD protein (Upf1) to see, whether MDMX-S is a target of the NMD.

The MDMX-S transcript was detected in 18 of 33 ovarian carcinoma samples. Increased level of MDMX-S compared with FL-MDMX associated with a decreased overall survival. We observed a significant increase of the MDMX-S transcript level upon treatment of OAW-42 cells with cisplatin. This could be prevented by inhibition of ATM/ATR and p53. Therefore, we conclude that DNA-damage pathways mediate the alternative splicing of the MDMX-mRNA. In addition, we identified two splicing proteins (PUF60 and hnRNP-C1/2) which seem to be involved in splicing of exon 6 of MDMX-mRNA. The inhibition of NMD by knock down of Upf1 resulted in the increase of the MDMX-S transcript level. This suggests that transcripts lacking exon 6 are subjected to degradation by NMD. In summary, according to the data presented here the MDMX-S transcript associates with an aggressive phenotype in ovarian carcinomas. However, since MDMX-S is subjected to degradation by NMD, the mechanism underlying this observed phenotype is still unknown.

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