2019

RNA

24th Annual Meeting of the RNA Society

Kraków / Poland • June 11 - June 16

RNA 2019

The 24th Annual Meeting of the RNA Society

PROGRAM & ABSTRACTS

June 11 - 16, 2019
ICE Kraków Congress Centre
Kraków, Poland

Witold Filipowicz – *Friedrich Miescher Inst, Switzerland*
Brenda Bass – *University of Utah, USA*
Elena Conti – *Max Planck Inst of Biochemistry, Germany*
Tetsuro Hirose – *Hokkaido University, Japan*
Artur Jarmolowski – *Adam Mickiewicz University, Poland*
Gene Yeo – *University of California San Diego, USA*

GENERAL INFORMATION

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

NO UNAUTHORIZED PHOTOGRAPHY IN SESSIONS: To encourage sharing of unpublished data at the RNA Society Meeting, taking of photographs and/or videos during scientific sessions (oral or poster), or of posters outside of session hours, is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

USE OF SOCIAL MEDIA: The official hash tag of the 24th Annual Meeting of the RNA Society is #RNA2019. The organizers encourage attendees to tweet about the amazing science they experience at the meeting, so that those who could not come to Kraków can join in from afar. However, please respect these few simple rules when using the #RNA2019 hash tag or talking about the meeting on Twitter and other social media:

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

Front Cover Image: The cover is based on traditional Polish folk papercut art. Probably modelled on traditional Jewish papercuts, Polish wycinanki (pronounced vee-chee-non-kee) originated as an inexpensive means of decorating the homes of Polish peasants. They were generally made by women using sheep-shearing scissors and any readily available paper sheets, and replaced each spring when homes were whitewashed. In traditional Polish papercuts floral (flowers, cereals, fruits, vegetables, etc.) and animal (wild birds, roosters, peacocks, etc.) motifs dominated. The cover of the RNA 2019 abstract book was designed by Lucyna Talejko-Kwiatkowska, a well-known Polish graphic artist who illustrates books and designs covers for many publishers.

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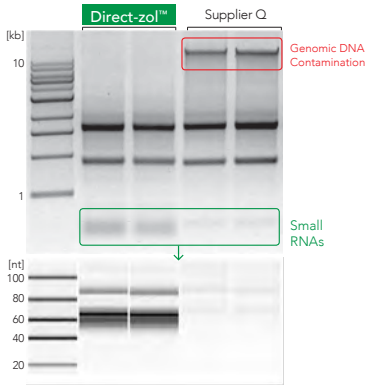

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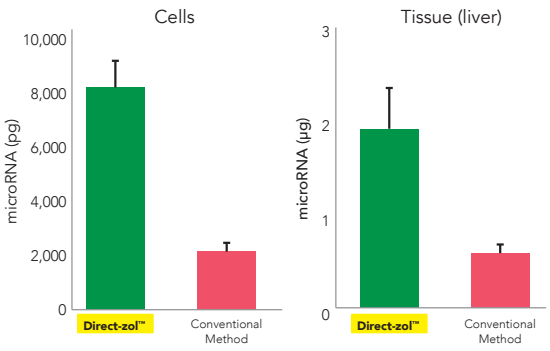

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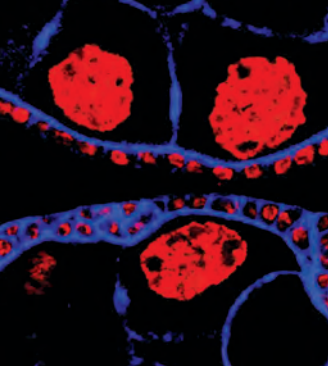

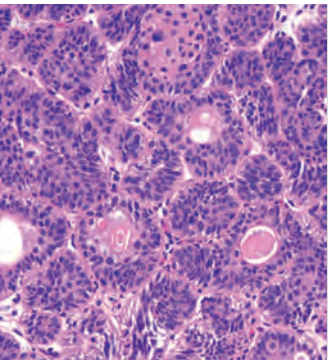

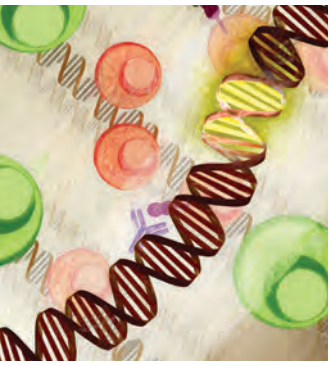

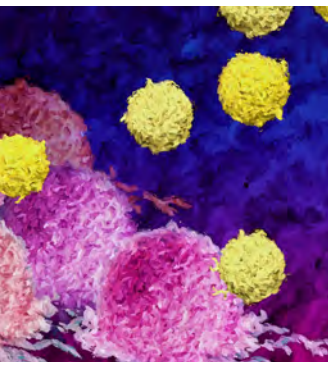

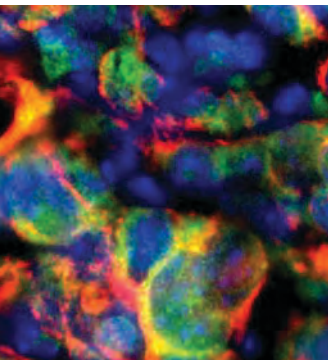

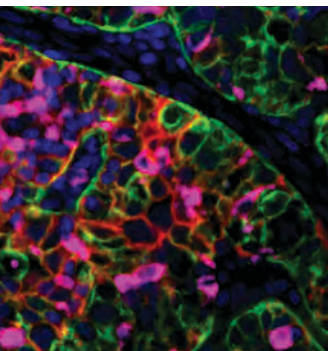

2019 SCIENTIFIC CONFERENCES

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AACR International Conference
New Horizons in Cancer Research
Program Committee Cochairs:
Elizabeth M. Jaffee and Hong Wu
May 3-5, 2019 | Shenzhen, China

The Hippo Pathway:
Signaling, Cancer, and Beyond
Conference Cochairs: Fernando Camargo, Anwesha Dey, and Kun-Liang Guan
May 8-11, 2019 | San Diego, CA

Bladder Cancer: Transforming the Field
Conference Cochairs: Charles G. Drake, Jason A. Efstathiou, Donna E. Hansel, Dan Theodorescu, and Ellen C. Zwarthoff
May 18-21, 2019 | Denver, CO

International Conference on
Malignant Lymphoma (ICML)
June 18-22, 2019 | Lugano, Switzerland

Environmental Carcinogenesis:
Potential Pathway to Cancer Prevention
Conference Cochairs: Margaret L. Kripke, Ernest T. Hawk, and Timothy R. Rebbeck
June 22-24, 2019 | Charlotte, NC

Immune Cell Therapies for Cancer:
Successes and Challenges of CAR T Cells and Other Forms of Adoptive Therapy
Conference Cochairs: Crystal L. Mackall and Patrick Hwu
July 19-22, 2019 | San Francisco, CA

Pancreatic Cancer:
Advances in Science and Clinical Care
Conference Cochairs: Dafna Bar-Sagi, Luis A. Diaz, Elizabeth M. Jaffee, Ben Z. Stanger, and Brian M. Wolpin
September 6-9, 2019 | Boston, MA

Advances in Ovarian Cancer Research
Conference Cochairs: Carol Aghajanian, David D. L. Bowtell, George Coukos, Alan D. D'Andrea, and Karen H. Lu
September 13-16, 2019 | Atlanta, GA

Advances in Pediatric Cancer Research
Conference Cochairs: Crystal Mackall, David Malkin, Stefan Pfister, and Kimberly Stegmaier
September 17-20, 2019 | Montreal, Quebec, Canada

12th AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
Conference Chair: Laura Fejerman
September 20-23, 2019 | San Francisco, CA

Fifth CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference:
Translating Science into Survival
Conference Cochairs: Christoph Huber, Guido Kroemer, Ellen Puré, and Giorgio Trinchieri
September 25-28, 2019 | Paris, France

Cancer Research UK-AACR Joint Conference:
Engineering and Physical Sciences in Oncology
Conference Cochairs: Sangeeta N. Bhatia, Kevin M. Brindle, Joe W. Gray, and Molly Stevens
October 15-17, 2019 | London, England

Learn more and register at
AACR.org/Calendar

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The RNA Society

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RNA 2020

The 25th Annual Meeting of the RNA Society will be held in Vancouver, BC, Canada, from May 26-31, 2020, at the Vancouver Convention Centre.

2018 Organizers

Sarah Woodson, *Johns Hopkins University, USA*
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Invitation to Membership

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

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

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

Benefits of RNA Society membership include:

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

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

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

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rna@faseb.org
www.rnasociety.org

April 29, 2019

Dear Participants:

Welcome to RNA 2019 in the historic center of Krakow, Poland! This year, the most exciting developments in RNA science are being celebrated in a vibrant nation with a long history of scholarly accomplishment. The Jagiellonian University in Krakow is among the oldest in the world (founded in 1364), and its many accomplished graduates include Nicolaus Copernicus. Poland itself has played a key role in RNA science, with a tradition of innovation in nucleotide chemistry, mechanistic enzymology, structural biology and bioinformatics of RNA. It is an honor to host the meeting in this special place, and I am grateful to the organizers for assembling a stellar program that reflects the breadth and growth of RNA science today. A large number of generous meeting sponsors have helped to ensure that we can support broad participation, lively discussion and good times together as a community. And most importantly, you are here! Thanks for coming to RNA 2019 and joining in the celebration.

Enjoy the meeting!

Anna Marie Pyle
RNA Society President, 2019-2020

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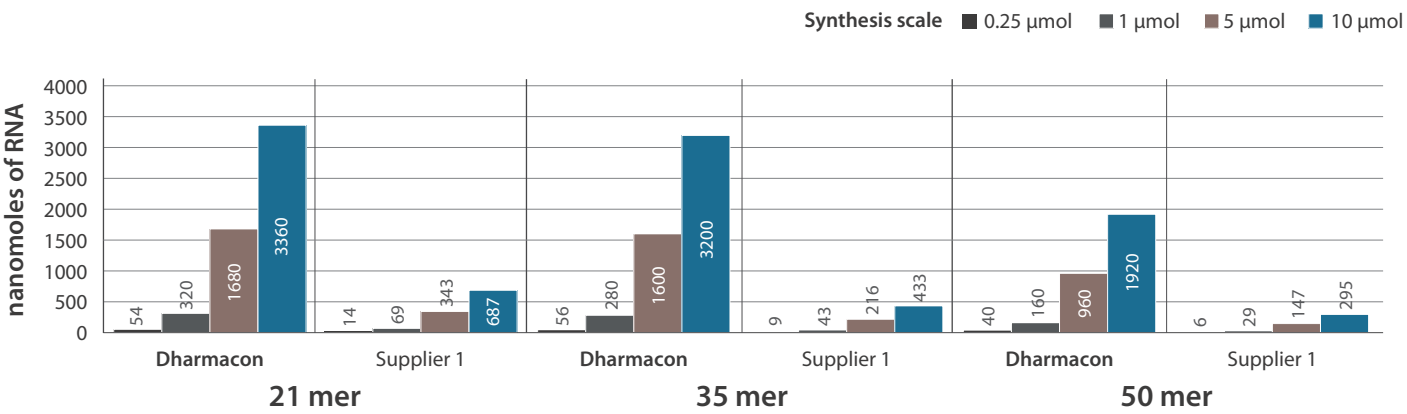
RNA yield (HPLC-purified)



The yield for RNA oligos of three different lengths was compared for all available synthesis scales between Dharmacon and a competitor.

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NOTES

PROGRAM—RNA 2019

The 24th Annual Meeting of the RNA Society
Kraków, Poland
June 11 – June 16, 2019

Tuesday, June 11

14:00 – 20:00	Registration	Level 0 Foyer
18:00 – 20:00	**Opening Session**	Auditorium Hall
	Welcome	
	Keynote 1 (Abstract 1) *Sponsored by RNA Research Center in Poznan (Adam Mickiewicz Univ and the Inst of Bioorganic Chemistry PAS).* *Phillip Sharp, Massachusetts Inst of Technology*	
	Piano Concert, Szymon Nehring	
20:00 – 22:00	Welcome Reception	Levels 1 & 2 Foyers

Wednesday, June 12

07:15 – 08:45	**Sponsored Seminar: Full-length RNA sequencing with nanopores (page 17)** *Sponsored by Oxford Nanopore Technologies*	Park Inn Hotel
08:00 – 19:00	Registration	Level 0 Foyer
09:00 – 10:30	**Plenary Session 1: Mechanisms of RNA Splicing (2-11)** *Sponsored by Academia Europaea* *Chair: Magda Konarska, Univ of Warsaw, Centre for New Technologies*	Auditorium Hall
10:30 – 11:00	Coffee Break	Levels 1 & 2 Foyers
11:00 – 11:45	**Plenary Session 1 continues**	
11:45 – 12:30	**Keynote 2 (12)** *Tom Cech, Univ of Colorado, Boulder*	Auditorium Hall
12:30 – 14:00	Lunch	Levels 1 & 2 Foyers
14:00 – 16:15	**Concurrent Session 1: Regulation of mRNA Splicing (13-22)** *Chair: Woan-Yuh Tarn, Academia Sinica*	Auditorium Hall
	Concurrent Session 2: RNA Localization & Transport (23-32) *Chair: Jeffrey Chao, Friedrich Miescher Inst for Biomedical Research*	Theatre Hall
16:15 – 16:45	Coffee Break	Levels 1 & 2 Foyers
16:45 – 19:00	**Plenary Session 2: Regulatory RNAs and RNPs (33-41)** *Dedicated to the memory of Aaron Klug* *Chair: Yukihide Tomari, Univ of Tokyo*	Auditorium Hall
19:00 – 20:00	Junior Scientists Social	Theatre Hall
19:00 – 20:30	Dinner	Levels 1 & 2 Foyers
19:00 – 20:30	Meetings Committee Dinner/Meeting	Level 0 Meeting Room

20:30 – 23:00 **Poster Session 1 (even numbers)**

Number	Topic Categories	Conference Hall, S4 Level 3
174 - 211	Biology and Mechanism of Small RNAs	
212 - 234, 867	Biology and Mechanism of Transcription	
235 - 243	Chemical Biology of RNA	
244 - 269	Emerging and High-throughput Techniques	
270 - 285	Interconnected RNA Processes	
286 - 308	Long Non-coding RNAs including Circular RNAs	
309 - 324	Regulatory RNAs in Bacteria	
325 - 331	Regulatory RNAs in Eukaryotes	
332 - 352	Ribosome Biogenesis and Structure	
353 - 362	RNA and Cellular Granules & Phase Separation	
363 - 364	RNA and Cellular Immunity	
365 - 375	RNA and Chromatin & Epigenetics	
376 - 405	RNA Binding Proteins and Helicases	
406 - 432, 868, 869	RNA Bioinformatics and Modeling	
433 - 452	RNA Catalysis and Riboswitches	
453 - 497, 866	RNA Modification and Editing	
498 - 532	RNA Structure and Folding	
533 - 537	RNA Synthetic Biology and Systems Biology	
538 - 551	RNA Transport and Localization	

Number	Topic Categories	Level 0 Foyer
552 - 597	RNA Turnover	
598 - 638	RNA-Protein Interactions	
639 - 685	RNAs in Disease	
686 - 698	RNPs: Biogenesis, Structure & Function	
699 - 713, 870	Splicing Mechanism	
714 - 759, 871	Splicing Regulation including Alternative Splicing	
760 - 773	Targeting RNA for Therapy	
774 - 817	Translational Mechanism and Regulation	
818 - 849	tRNA: Processing and Function	
850 - 865	Viral RNAs	

Thursday, June 13

07:45 – 08:45 **Sponsored Seminar: Applying Lexogen RNA-Seq technologies (page 19)** Park Inn Hotel
Sponsored by Lexogen

08:00 – 13:30 Registration Level 0 Foyer

09:00 – 10:30 **Concurrent Session 3: Synthesis and Processing of RNA (42-51)** Auditorium Hall
Chair: Karla Neugebauer, Yale Univ

Concurrent Session 4: RNA in Disease and Therapy (52-61) Theatre Hall
Sponsored by Storm Therapeutics
Chair: Matthew Disney, Scripps Research Inst

Workshop 1: The tRNA World beyond Translation (62-72) Chamber Hall - S3, Level 3
Organizers: Sebastian Glatt, Jagiellonian Univ; Sebastian Leidel, Univ of Bern

10:30 – 11:00 Coffee Break Levels 1 & 2 Foyers

11:00 – 11:45	**Concurrent Sessions 3 & 4 and Workshop 1 continue**	
11:45 – 13:15	**Panel Discussion: RNA Research and RNA Therapeutics: Past Insights and Future Prospects** *Panelists: Tom Cech, Univ of Colorado, Boulder; Matthew Disney, Scripps Research Inst; Gideon Dreyfuss, HHMI / Univ of Pennsylvania; Anastasia Khvorova, Univ of Massachusetts Medical School; Adrian Krainer, Cold Spring Harbor Laboratory; Anna Marie Pyle, Yale Univ; Joan Steitz, Yale Univ / HHMI*	Auditorium Hall
13:15 – 13:30	Lunch to Go – free afternoon and evening	Levels 1 & 2 Foyers

Friday, June 14

07:40 – 08:40	**Sponsored Seminar: Mapping RNA binding sites using eCLIP (page 21)** *Sponsored by Eclipse BioInnovations*	Park Inn Hotel
08:00 – 19:00	Registration	Level 0 Foyer
09:00 – 10:30	**Plenary Session 3: Mechanisms of Translation (73-81)** *Dedicated to the memory of Tom Steitz and Sidney Brenner* *Chair: Nahum Sonenberg, McGill Univ*	Auditorium Hall
10:30 – 11:00	Coffee Break	Levels 1 & 2 Foyers
11:00 – 11:45	**Plenary Session 3 continues**	
11:45 – 12:30	**Keynote 3 (82)** *Maria Carmo-Fonseca, Inst of Molecular Medicine, Univ of Lisboa*	Auditorium Hall
12:30 – 14:00	Mentoring Lunch *Organized by Nancy Greenbaum, Hunter College, CUNY*	Park Inn Hotel
12:30 – 14:00	Lunch	Levels 1 & 2 Foyers
14:00 – 16:15	**Plenary Session 4: RNA Modification and Editing (83-92)** *Dedicated to the memory of Marie Öhman* *Chair: Chuan He, Univ of Chicago and Peking Univ*	Auditorium Hall
16:15 – 16:45	Coffee Break	Levels 1 & 2 Foyers
16:45 – 19:00	**Concurrent Session 5: Non-coding RNAs: Long & Short, Linear & Circular (93-102)** *Chair: Julia Salzman, Stanford Univ School of Medicine*	Auditorium Hall
	Concurrent Session 6: Translation Regulation (103-112) *Chair: Andrea Berman, Univ of Pittsburgh*	Theatre Hall
	Workshop 2: Computational Modeling of RNA Structure and Complexes (113-122) *Organizers: Janusz Bujnicki, International Inst of Molecular and Cell Biology in Warsaw; Francois Major, Univ of Montreal; Eric Westhof, Univ of Strasbourg*	Chamber Hall - S3, Level 3
19:00 – 20:30	Dinner	Levels 1 & 2 Foyers
19:00 – 20:30	Board of Directors Dinner/Meeting	Level 0 Meeting Room

20:30 – 23:00 **Poster Session 2 (odd numbers)**

Number	Topic Categories	Conference Hall, S4 Level 3
174 - 211	Biology and Mechanism of Small RNAs	
212 - 234, 867	Biology and Mechanism of Transcription	
235 - 243	Chemical Biology of RNA	
244 - 269	Emerging and High-throughput Techniques	
270 - 285	Interconnected RNA Processes	
286 - 308	Long Non-coding RNAs including Circular RNAs	
309 - 324	Regulatory RNAs in Bacteria	
325 - 331	Regulatory RNAs in Eukaryotes	
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353 - 362	RNA and Cellular Granules & Phase Separation	
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498 - 532	RNA Structure and Folding	
533 - 537	RNA Synthetic Biology and Systems Biology	
538 - 551	RNA Transport and Localization	

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686 - 698	RNPs: Biogenesis, Structure & Function	
699 - 713, 870	Splicing Mechanism	
714 - 759, 871	Splicing Regulation including Alternative Splicing	
760 - 773	Targeting RNA for Therapy	
774 - 817	Translational Mechanism and Regulation	
818 - 849	tRNA: Processing and Function	
850 - 865	Viral RNAs	

Saturday, June 15

08:00 – 19:00 Registration — Level 0 Foyer

09:00 – 11:00 **Concurrent Session 7: Interconnected RNA Processes (123-131)** — Auditorium Hall
Chair: Andrzej Dziembowski, Inst of Biochemistry & Biophysics, Polish Academy of Sciences, Warsaw

Concurrent Session 8: RNA Catalysis, Folding and Structure (132-140) — Theatre Hall
Chair: Rick Russell, Univ of Texas at Austin

11:00 – 11:30 Coffee Break — Levels 1 & 2 Foyers

11:30 – 13:00 **Workshop 3: Membrane-less Granules and Liquid-Liquid Phase Separation (141-148)** — Auditorium Hall
Organizer: Dominique Weil, CNRS, Sorbonne Univ

Junior Scientists Workshop: Publishing and the Editorial Process (page 23) — Theatre Hall
Organized by the RNA Junior Scientist Committee
Chair: Eleonora de Klerk, Univ of California, San Francisco

11:30 – 13:00	**Workshop 4: Computational Methods for RNA Data Analysis (149-153)** *Sponsored by Locana, Inc.* *Organizers: Eugene Yeo, Univ of California, San Diego; Yoseph Barash, Univ Pennsylvania*	Chamber Hall – S3, Level 3
13:00 – 14:30	Lunch	Levels 1 & 2 Foyers
14:30 – 16:45	**Concurrent Session 9: RNA Turnover (154-163)** *Chair: Oliver Mühlemann, Univ of Bern*	Auditorium Hall
	Concurrent Session 10: New Research Trends and Technologies (164-173) *Chair: Jernej Ule, Francis Crick Inst*	Theatre Hall
16:45 – 17:15	Coffee Break	Levels 1 & 2 Foyers
17:15 – 18:45	**Awards Ceremony** *Join us in acknowledging our award winners, as well as our colleagues who have passed away in the past year.*	Auditorium Hall
18:45 – 19:30	Transfer to Stara Zajezdnia on own	
19:30 – 24:00	Reception/Entertainment/Dinner/Dance	Stara Zajezdnia

Sunday, June 16

Conference concludes

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SPONSORED SEMINARS

Wednesday, June 12, 2019 07:15 - 08:45

Location: Park Inn Hotel
Theme: Full-length RNA sequencing with nanopores
Chairperson: Oliver Hartwell, Oxford Nanopore Technologies Ltd.
Sponsored by: Oxford Nanopore Technologies Ltd.

This session includes two speakers discussing transcriptomics using nanopore sequencing and an update from Libby Snell, Oxford Nanopore Technologies Ltd about the latest advances in nanopore technology.

To find out more and to register visit: register.nanoporetech.com/rnasociety2019_seminar

The detection of 7-deazaguanine in RNA using nanopore sequencing: progress towards detection of naturally occurring G-quadruplexes in cells

Carika Weldon
Wellcome Centre for Human Genetics, University of Oxford, UK

G-quadruplexes (G4s) are RNA/DNA secondary structures, which are made from G-quartets. Several researches confirm their existence in promoter region of several medically important genes such as c-MYC and KRAS. Their role has also been shown to affect several post-transcriptional processes, including splicing. Previously we have shown that our novel method, FoLDeR (Footprinting Of Long Deazaguanine RNA), is able to identify two G4s that regulate the alternative splicing of the apoptotic regulator Bcl-X[1]. However, the presence of 7-deazaguanine substitutions prevents RNA strands to be spliced. To resolve this, incorporation of 7-deazaguanine must be restricted to functionally relevant regions. To achieve this aim, a method is required to detect 7-deazaguanine in RNA. By using Direct RNA nanopore sequencing we were able to gather sequencing data of both normal and fully substituted 7-deazaguanine RNA. Initial results indicated that 7-deazaguanine causes a massive reduction in basecalling rates. Further analysis via Tombo showed that this is due to detectable changes in the ionic current on 7-deazaguanines, as well as surrounding nucleotides. This allows us to programme the Tombo basecaller to relate the changes in currents to 7-deazaguanine. Ultimately this will provide a new method to study the biological occurrence and function of G4s in splicing and transcriptional control in several disorders such as cancer and cardiovascular diseases.

Nanopore sequencing reveals the transcriptional complexity of neuropsychiatric disease genes

Tomasz Wrzesinski
Earlham Institute

Mutations leading to aberrant splicing are increasingly associated with human diseases and disorders. So far, we have relied on short read sequencing to assess splicing diversity and transcriptional convolution. However, the accurate annotation and quantification of full-length transcript using short read sequencing still remains a challenging task. Fortunately, recent developments have now made it possible to sequence full length cDNA on the Oxford Nanopore platform enabling the in-depth annotation and analysis of alternatively spliced transcripts. We specifically focused on splicing events within complex genes (>40 exons) such as

the voltage-gated calcium channels (VGCCs - *CACNA1A*, *CACNA1C*, *CACNA1D*, *CACNA1S*) as many of these genes have been associated with human cardiac and neuropsychiatric diseases. Among those genes, *CACNA1C* has been robustly linked with bipolar disorder through genome, transcriptome and methylome studies. We generated full length sequences for VGCC transcripts, in 3 individuals and 6 different regions of human brain to quantify transcript diversity and identify novel functional splicing events. In *CACNA1C* and *CACNA1D*, we were able to determine 38 and 33 novel exons, respectively, as well as novel frame-conserving splice junctions and micro-exons. Our approach allowed to further annotate 83 and 44 novel high-confidence isoforms in *CACNA1C* and *CACNA1D*, respectively, which abundance clearly distinguish between different brain tissues. Our data demonstrate that long read sequencing including Oxford Nanopore allows to uncover unprecedented transcriptional complexity, leading to more accurate annotations which in turn facilitates the understanding of the functional implications of mutations altering splicing.

LEXOGEN
Enabling complete transcriptome sequencing

Thursday, June 13, 2019 07:30 - 08:45

Location: Park Inn Hotel
Theme: Applying Lexogen RNA-Seq technologies to determine transcription & mRNA turnover, RNA-protein interactions, and poly(A)$^+$ & poly(A)$^-$ RNA 3' end abundances
Chairperson: Lukas Paul (Senior Manager of Scientific Affairs, Lexogen, Vienna, Austria)
Sponsored by: Lexogen

While NGS-based RNA sequencing has established itself as a powerful tool for transcriptome analysis, it actually refers to diverse platforms, applications, and protocols. In this seminar, three stories will be presented that reveal how selected technologies from Lexogen –SLAMseq metabolic RNA labeling, the CORALL Total RNA-Seq library preparation protocol, and the QuantSeq 3' RNA-Seq method – can be applied to distinct research questions.

Characterization of mRNA dynamics under hypoxia using SLAMseq

Rebekka Bauer[1], Dominik C. Fuhrmann[1], Tobias Schmid[1], Bernhard Brüne[1]
[1]Institute of Biochemistry I, Faculty of Medicine, Goethe-University Frankfurt, Germany

Reduced oxygen availability, i.e. hypoxia, is an important feature of numerous pathophysiological conditions. In addition to short-term hypoxic episodes, as observed e.g. in the case of ischemic stroke, various disease states, including tumors and inflammatory diseases, are characterized by chronic or intermittent hypoxia. Many cellular responses to low oxygen tensions are mediated by the hypoxia-inducible factors (HIFs), a family of heterodimeric transcription factors consisting of a shared, constitutively expressed β-subunit and one of three α-subunits. Yet, there is increasing evidence that post-transcriptional mechanisms play an important role in the adaptation to hypoxia as well.

In the present project, we aimed to determine the dynamics of mRNA expression changes under acute and chronic hypoxia. To assess the impact of both transcriptional and post-transcriptional (i.e. mRNA stability) regulatory mechanisms in myeloid cells, we employed the recently developed SLAM-seq (thiol-linked alkylation for the metabolic sequencing of RNA) method (Herzog et al., 2017). Specifically, we labelled monocytic THP1 cells with 4-thiouridine (4SU) under acute and chronic hypoxia and analyzed changes in *de novo* synthesis as well as in the stability of mRNAs in a transcriptome-wide manner.

Using this approach, we were able to assign altered mRNA expression levels in response to hypoxia to changes in *de novo* transcription, in mRNA stability, or a combination of both. Importantly, our findings shed further light on the exact molecular mechanisms facilitating the adaptation to hypoxia.

Reference:
Herzog VA, Reichholf B, Neumann T, Rescheneder P, Bhat P, Burkard TR, Wlotzka W, von Haeseler A, Zuber J, Ameres SL. (2017) Thiol-linked alkylation of RNA to assess expression dynamics. Nature Methods 14: 1198-1204.

Key words:
RNA dynamics, hypoxia

Translational regulation of IL32 in the inflammatory tumor microenvironment

Anica Scholz[1], *Nicola Boeffinger*[1], *Denise Aigner*[1], *Bernhard Brüne*[1], *Tobias Schmid*[1]
[1]Institute of Biochemistry I, Faculty of Medicine, Goethe-University Frankfurt, Germany

Translation is one of the most energy-demanding cellular processes, and therefore is tightly regulated. Changes in translation are commonly observed during tumor development, contributing to various hallmarks of cancer. Tumors are closely associated with inflammation. Specifically, tumors do not only arise in inflammatory sites, they acquire the ability to evade anti-tumor responses and instead corrupt certain immune cells, such as macrophages, to support tumor growth and progression.

Here, we aimed to further characterize the translational changes in tumor cells within an inflammatory tumor microenvironment. To this end, we stimulated human MCF7 breast cancer cells with interleukin 1β (IL1β) and identified translationally regulated targets via ribosome profiling analyses. Among these, the pro-inflammatory cytokine IL32 emerged as an interesting candidate. Interestingly, while translation regulatory mechanisms are commonly mediated by features within the 5' untranslated regions (5'UTRs) of transcripts, this was not the case for IL32, despite the strong changes in translation in response to IL1β. Instead, corroborated by massively altered protein occupancies, the 3'UTR of IL32 appeared to confer the regulatory properties.

Key words:
translation, tumor microenvironment

Simultaneous Measurement of Transcriptional and Post-transcriptional Parameters by 3'end RNA-seq

Manfred Schmid[1], *Agnieszka Tudek*[2], *Torben Heick Jensen*[1]
[1]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark; [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Cellular RNA levels are determined by transcription and decay rates, which are fundamental in understanding gene expression regulation. Measurement of these two parameters is usually performed independently, complicating analysis as well as introducing methodological biases and batch effects that hamper direct comparison. We developed a simple approach of concurrent sequencing of S. cerevisiae polyA$^+$ and polyA$^-$ RNA 3'ends to simultaneously estimate total RNA levels, transcription and decay rates from the same RNA sample [1]. The transcription data generated correlate well with reported estimates and also reveal local RNA polymerase stalling and termination sites with high precision. Although the method by design uses brief metabolic labeling of newly synthesized RNA with 4-thiouracil, the results demonstrate that transcription estimates can also be gained from unlabeled RNA samples. These findings underscore the potential of the approach, which should be generally applicable to study a range of biological questions. We recently applied the same experimental strategy to analyze nuclear RNA exosome-dependent decay pathways in human cells. Highlights from those efforts will be presented at the end.

[1] Schmid M, Tudek A, Jensen TH. (2018) *Simultaneous Measurement of Transcriptional and Post-transcriptional Parameters by 3'End RNA-Seq.* Cell Reports, 24, 2468-2478

ECLIPSE BIOINNOVATIONS

Friday, June 14, 2019 07:40 - 08:40

Location: Park Inn Hotel
Title: Robust transcriptome-wide mapping of RNA binding sites using eCLIP.
Sponsored by: Eclipse Bioinnovations

The essential role of RNA processing in all aspects of human and model organism biology has been shown through the ever-increasing number of RNA regulatory events linked to key developmental steps and the initiation and progression of both inherited and infectious diseases. More recently, the genomics revolution has led to the development of a myriad of methods to profile many aspects of RNA processing, including regulatory interactions, RNA splicing changes, translation rates, RNA modification sites, and others. However, many of these protocols are highly complex, and remain challenging to pick up and perform by non-expert labs.

Eclipse Bio was founded in 2017 to bring simplified and robust RNA genomics technologies to aid researchers studying RNA biology. Our lead platform product is enhanced crosslinking and immunoprecipitation (eCLIP), which builds upon previous CLIP-seq methods to enable robust identification of RNA binding protein targets (Van Nostrand et al., Nature Methods 2016). With upcoming products designed to directly identify miRNA targets with chimeric-eCLIP and profile RNA modifications such as m6A, as well as products which improve the efficiency, reproducibility and robustness of data for researchers doing whole genome pooled library screens, Eclipse will continue to lead the development of new techniques to aid RNA researchers.

Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP)

Dr. Peter Chu, CEO, Eclipse Bioinnovations, and Heather Foster, Senior Product Specialist
Eclipse Bioinnovations

RNA binding proteins play key roles in regulating RNA processing by interacting with RNA primary sequence and secondary structures to control splicing, RNA stability, translation initiation and elongation, and localization. With the discovery of more and more RNA binding proteins associated with disease or other biological processes, it is often essential to be able to directly map which RNAs are bound by the protein of interest. Crosslinking and immunoprecipitation (CLIP) methods enable this by immunoprecipitating the protein of interest along with crosslinked RNA, followed by library preparation and high-throughput sequencing to identify regions that show significantly enriched signal.

Recently, improved CLIP-seq methods have been described that dramatically improve these experiments by increasing the conversion of RNA into high-throughput sequencing library. In particular, eCLIP (developed in the Yeo laboratory at UCSD and described in Van Nostrand et al., Nature Methods 2016) has been shown to be highly robust, with over 150 RNA binding proteins profiled to date. In this presentation, we will describe the eCLIP method and show how eCLIP can be used to profile the targets of an RNA binding protein of interest in an unbiased, transcriptome-wide manner. We will also discuss how the use of proper controls (input, IgG-only, and wild-type versus tagged protein) can provide significantly improved signal to noise in detecting true signal and removing at abundant RNAs. Finally, we will show how the basic eCLIP method can be modified to more deeply probe individual aspects of RNA biology, by showing how adapted eCLIP methods have been used to directly profile miRNA targets (through chimeric ligation of miRNA with their mRNA targets) and RNA modifications (using an m6A-specific approach).

Eclipse Bioinnovations eCLIP services

Heather Foster, Senior Product Specialist, and Ines Rabano, Marketing Manager
Eclipse Bioinnovations

In this presentation we will provide specific examples of how eCLIP can be adapted into individual projects through the use of Eclipse eCLIP kit and service products. For expert users, we will discuss details of how different sample types can be incorporated into eCLIP workflows, what reagents and optimizations need to be performed before beginning full eCLIP experiments, and what controls provide useful comparisons for analysis. We will further discuss options for non-expert users, including Eclipse-provided service options for both eCLIP experiments as well computational data analysis support.

Unexpected insights into RNA processing from crosslinking immunoprecipitation experiments

Marija Dargyte, Jon Howard and Jeremy R. Sanford
Department of Molecular, Cellular and Developmental Biology, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95060, USA

Crosslinking immunoprecipitation and high throughput sequencing (CLIP-Seq) is a powerful approach for studying protein-RNA interactions on a global scale. RNA maps generated by CLIP-seq provide important mechanistic clues to the regulatory logic of post-transcriptional gene regulation. One of the most intriguing aspects of this approach is the ability to discover connections between RNA binding proteins and unexpected classes of RNA transcripts. Along these lines, we will highlight our recent CLIP-seq experiments focusing on regulation of exon definition and microRNA processing by alternative splicing factors.

JUNIOR SCIENTIST WORKSHOP

Saturday, June 15th

11:30 – 13:30 **Workshop on "Publishing and the Editorial process"** Theatre Hall
Organized by the RNA Society Junior Scientist Committee
Chair: Eleonora de Klerk, UCSF

The RNA junior scientists are pleased to host the workshop on "Publishing and the Editorial process", which will bring together Chief Editors, Senior Editors and Reviewing Editors to discuss the future of scientific publishing, currently considered a billion-dollar industry.

The workshop is structured as a panel discussion which will cover the following topics:

- differences between open access and standard subscription-based publication
- policies regarding open access (current EU laws versus US laws)
- dilemma between unpublished pre-prints and peer-reviewed articles
- the review process

11:30 – 11:45 Welcoming remarks by Eleonora de Klerk
University of California, San Francisco (UCSF); representing the RNA Society Junior Scientists Committee
11:45 – 11:55 **Timothy W. Nilsen**, Chief Editor, RNA
11:55 – 12:05 **Keith Fox**, Senior Executive Editor, Nucleic Acid Research
12:05 – 12:15 **Andrew Marshall**, Chief Editor, Nature Biotechnology
12:15 – 12:25 **Agnieszka Chacinska**, Reviewing Editor, eLIFE
12:25 – 12:35 **Ines Alvarez-Garcia**, Senior Editor, PLOS Biology
12:35 – 12:45 **Stefanie Boehm**, Editor, EMBO
12:45 – 13:30 Panel Discussion

ADDITIONAL SCHEDULED EVENTS AT RNA 2019

Tuesday, June 11

10:30 – 16:00 **Junior Scientists Pre-Conference Activity** Wieliczka Salt Mine

Open to all graduate students and postdocs. Pre-registration required.

Our pre-conference activity this year will give you the opportunity to explore the beautiful Wieliczka Salt Mine. The morning prior to registration, join us on a tour to the famous mine, an exceptional monument on the UNESCO's World Cultural and Natural Heritage List since 1978. With its magnificent chambers chiseled out in rock salt, amazing underground saline lakes, majestic timber constructions and unique statues sculpted in salt, it is one of the most valuable monuments of material and spiritual culture in Poland. Not surprisingly it is visited each year by more than one million tourists from all over the world. This is a great way to get to know fellow Junior Scientists and explore one of Poland's main attractions!

Registration & Fees: The registration link can be found on the RNA 2019 website (Junior Scientists page). The total price is 25 Euro, which includes transportation and Entrance Ticket. Payments are performed online during registration.

19:30 – 20:00 **Welcome Concert** Auditorium Hall

Open to all attendees.

Following the opening welcome and keynote talk, we are thrilled to present a piano concert by Poland's acclaimed virtuoso, Szymon Nehring. He is one of the most gifted and promising pianists of the younger generation in Poland. He is the only Pole to win First Prize at the Arthur Rubinstein International Piano Master Competition in Tel Aviv, one of the most important piano competitions in the world.

Wednesday, June 12

19:00 – 20:30 **Meetings Committee Meeting** Level 0 Meeting Room

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

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

19:00 – 20:00 **Junior Scientists Social** Theatre Hall

Open to all graduate students and postdocs.

This junior scientist-only social is a great opportunity to meet your peers, unwind, and above all... connect! This year, to make the evening even more fun, we are setting up the "RNA trivia night". There will be winners, but losers will forget their status very quickly! Come have drinks with us!

Friday, June 14

12:30-14:00 **Mentoring Lunch** Park Inn Hotel

Open to all attendees with pre-arrangement. Seating is limited, and advance completion of the pre-conference survey is required.

This lunch is an informal gathering that brings together six or seven graduate students and post docs with one to two academic and industry mentors to answer questions about careers. Topics include the pros and cons of academic vs industry careers, finding jobs, grant applications, and of course lots of interesting science. These lunches are fun for the mentors and hopefully fun and useful for the mentees as well. To the extent possible, mentors and mentees with common career and geographical objectives or experiences are grouped together.

19:00-20:30 **RNA Society Board of Directors Meeting** Level 0 Meeting Room

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

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

Saturday, June 15

17:15-18:45 **Awards Ceremony** Auditorium Hall

Open to all conference attendees.

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

- RNA Society Lifetime Achievement Award
  Adrian Krainer, Cold Spring Harbor Laboratory
- RNA Society Service Award
  Jim McSwiggen, McSwiggen Biotech Consulting
- RNA Society Mid-Career Award
  Ailong Ke, Cornell University
- RNA Society Early-Career Award
  Maria Barna, Stanford University
- RNA Society/Scaringe Award Winners
  Xuebing Wu, Whitehead Institute / MIT / HHMI; Post-doctoral Award
  Michael C. Chen, University of Cambridge & NIH; Graduate Student Award
  Max Wilkinson, MRC Laboratory of Molecular Biology & University of Cambridge; Graduate Student Award
- Poster Prize Winners

19:30-24:00 **Conference Closing Event** Stara Zajezdnia

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

For our closing party, we will transfer on our own by walking or transit to Stara Zajezdnia, Krakow's historic tram depot now operating as a microbrewery and event venue, protected by the UNESCO World Heritage List of Cultural and Natural Heritage. Located approximately 20 minutes walking from ICE Congress Centre, maps will be available at the RNA 2019 registration desk on Saturday, or navigate with your device to Świętego Wawrzyńca 12 (http://starazajezdniakrakow.pl/en).

The evening begins with a reception featuring a cultural dance performance by Trebunie-Tutki, a folk group originating from the village of Bialy Dunajec. They will play the traditional mountain music of the Polish Górale (ethnic highlander) culture.

Following dinner, dance the night away to the live music of No Stress, a popular Krakow cover band which refers to themselves as a harmonious pack of creative and professional musicians – a recipe for fun! No Stress guarantees that everyone will want to dance to their combination of classic and latest hits.

RNA 2019 AWARDS

The RNA Society Lifetime Achievement Award

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

Previous winners include Joan Steitz (2003), Harry Noller (2004), John Abelson (2005), Christine Guthrie (2006), Walter Keller (2007), Norm Pace (2008), Thomas Cech (2009), Fritz Eckstein (2010), Witold Filipowicz (2011), Olke Uhlenbeck (2012), Phillip Sharp (2013), Reinhard Lührmann (2014), Anita Hopper (2015), Eric Westhof (2016), Lynne Maquat (2017), and Jean Beggs (2018).

*Congratulations to **Adrian Krainer**, Cold Spring Harbor Laboratory, who is the winner of the 2019 RNA Society Lifetime Achievement Award.*

The RNA Society Service Award

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

Previous winners include Tim Nilsen (2003), Chris Greer (2004), Jean Beggs (2005), Olke Uhlenbeck (2006), Marvin Wickens (2007), Eric Westhof (2008), Anita Hopper (2009), Lynne Maquat (2010), Evelyn Jabri (2011), Brenda Peculis (2012), Ann Marie Micenmacher (2014), David Lilley (2015), Andrea Barta (2016), Andrew Feig (2017), and Elizabeth Tran (2018).

*Congratulations to **Jim McSwiggen**, CEO of the RNA Society and McSwiggen Biotech Consulting LLC, who is the winner of the 2019 RNA Society Service Award.*

The RNA Society Mid-Career Award

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

Previous winners include Karla Neugebauer (2017), Nils Walter (2017), and Erik Sontheimer (2018).

*Congratulations to **Ailong Ke,** Cornell University, who is the winner of the 2019 RNA Society Mid-Career Award.*

The RNA Society Early-Career Award

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

Previous winners include Wendy Gilbert (2017), Gene Yeo (2017), and Andrei Korostelev (2018).

*Congratulations to **Maria Barna,** Stanford University, who is the winner of the 2019 RNA Society Early-Career Award.*

The RNA Society/Scaringe Award

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

SCARINGE
Supporting the Future

Previous graduate student winners include: Jeff Barrick (2006), Malte Beringer (2007), Qi Zhang (2008), Jeremey Wilusz (2009), John Calarco (2010), Jasmine Perez (2011), Chenguang Gong (2012), Tatjana Trcek Pulisic (2012), Wenwen Fang (2013), David Weinberg (2014), Samuel Sternberg (2015), Katherine Warner (2015), Ryan Flynn (2016), Nian Liu (2016), Malik Chaker-Margot (2017), Madeline Sherlock (2018), and Boxuan Zhao (2018).

Previous postdoctoral fellow winners include Megan Talkington (2006), Zefeng Wang (2007), Alexei Aravin (2008), Shobha Vasudevan (2009), Luciano Marraffini (2010), Hani Zaher (2011), Kotaro Nakanishi (2012), Dipali Sashital (2012), Je-Hyun Yoon (2013), Jinwei Zhang (2014), Olga Anczukow-Camarda (2015), Schraga Schwartz (2015), Basil Greber (2016), Thi Hoang Duong Nguyen (2016), Zhipeng Lu (2017), and Fuguo Jiang (2018).

Congratulations to the winners of the 2019 RNA Society/Scaringe Awards:
Graduate students ***Michael C. Chen,*** *University of Cambridge & the NIH, and* ***Max Wilkinson,*** *MRC Laboratory of Molecular Biology & University Cambridge, along with postdoctoral fellow* ***Xuebing Wu,*** *Whitehead Institute / MIT / HHMI.*

Sponsored Poster Prizes

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

baseclick

Baseclick is pleased to sponsor a poster prize in the amount of a $200 cash award.

THE EMBO JOURNAL

The EMBO Journal is pleased to sponsor a 200€ poster prize. A credit certificate will be awarded at RNA 2019, with the prize to be claimed directly from EMBO.

International Journal of *Molecular Sciences*
an Open Access Journal by MDPI

The International Journal of Molecular Sciences, an MDPI open-access journal, is pleased to sponsor a poster prize in the amount of a $300 cash award.

The RNA Society is pleased to provide additional poster prizes for RNA 2019, as needed, depending on the number of outstanding poster presentations. Each prize consists of a $250 cash award.

ORAL ABSTRACT LISTING

TUESDAY, JUNE 11, 2019: 18:00 – 20:00; AUDITORIUM HALL

Opening Session: Keynote 1

Sponsored by RNA Research Center in Poznan (Adam Mickiewicz Univ and the Inst of Bioorganic Chemistry PAS)

Phillip Sharp, Massachusetts Inst of Technology

Abstract 1

1 RNA Biology in Gene Regulation
Phillip Sharp

WEDNESDAY, JUNE 12, 2019: 9:00 – 11:45; AUDITORIUM HALL

Plenary Session 1: Mechanisms of RNA Splicing

Sponsored by Academia Europaea

Chair: Magda Konarska, Univ of Warsaw, Centre for New Technologies

Abstracts 2 – 11

2 Snapshots of the catalytic spliceosomes
Max Wilkinson [Scaringe Award], Sebastian Fica, Wojciech Galej, Clément Charenton, Kiyoshi Nagai

3 Structures of the Catalytically Activated Yeast Spliceosome Reveal the Mechanism of Branching
Ruixue Wan, Rui Bai, Chuangye Yan, Jianlin Lei, Yigong Shi

4 Rearrangements within the U6 snRNA core during the transition between the two catalytic steps of splicing
Katarzyna Eysmont, Katarzyna Matylla-Kulinska, Agata Jaskulska, Marcin Magnus, Magda Konarska

5 Functional Analysis of Cwc24 ZF-domain in 5′ Splice Site Selection
Nan-Ying Wu, Soo-Chen Cheng

6 A Distinct Subset of Human Short Introns with Weak Pyrimidine Tract: U2AF Heterodimer Is Replaced by SPF45/RBM17 as General Splicing Factor
Kazuhiro Fukumura, Rei Yoshimoto, Tetsuro Hirose, Kunio Inoue, Akila Mayeda

7 A human post-catalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation
Sebastian Fica, Chris Oubridge, Max Wilkinson, Andrew Newman, Kiyoshi Nagai

8 CWC27, a new player in EJC assembly by the spliceosome
Virginia Busetto, Isabelle Barbosa, Jérôme Basquin, Emélie Marquenet, Elena Conti, Hervé Le Hir

9 CryoEM structure of spliceosomal E complex and biochemical analyses reveal a unified mechanism for intron definition, exon definition, and back-splicing
Rui Zhao, Xueni Li, Shiheng Liu, Lingdi Zhang, Aaron Issaian, Ryan Hill, Sara Espinosa, Yanxiang Cui, Kirk Hansen, Hong Zhou

10 Cryo-EM Structures of a Group II Intron Reverse Splicing into DNA
Daniel B. Haack, Xiaodong Yan, Cheng Zhang, Jason Hingey, Dmitry Lyumkis, Timothy S. Baker, Navtej Toor

11 Self-regulatory network of the spliceosome
Magorzata Rogalska, Estefania Mancini, Xavier Hernandez Alias, Martin Schaefer, Luis Serrano, Juan Valcárcel

WEDNESDAY, JUNE 12, 2019: 11:45 – 12:30; AUDITORIUM HALL

Keynote 2

Tom Cech, Univ of Colorado, Boulder

Abstract 12

12 RNA binding to a chromatin modifier: structure and function
Yicheng Long, Taeyoung Hwang, Anne Gooding, Karen Goodrich, John Rinn, Thomas Cech

WEDNESDAY, JUNE 12, 2019: 14:00 – 16:15; AUDITORIUM HALL

Concurrent Session 1: Regulation of mRNA Splicing

Chair: Woan-Yuh Tarn, Academia Sinica

Abstracts 13 – 22

13 Rhythmic gene expression is controlled by alternative splicing triggering nonsense mediated decay
Marco Preussner, Alexander Neumann, Stefan Meinke, Florian Heyd

14 A high-throughput chemical screen to identify splicing modulators of nSR100/Srrm4-dependent microexons.
Andrew Best, Hong Han, Justin Lim, Ulrich Braunschweig, Sha Farhangmehr, Adrian Pasculescu, Allesandro Datti, Benjamin Blencowe

15 Modeling and Predicting the Activities of *Trans*-Acting Splicing Factors with Machine Learning
Miaowei Mao, Yue Hu, Yun Yang, Yajie Qian, Huanhuan Wei, Wei Fan, Yi Yang, Xiaoling Li, Zefeng Wang

16 The Alazami Syndrome-associated protein LARP7 guides U6 small nuclear RNA modification and contributes to splicing robustness
Daniele Hasler, Rajyalakshmi Meduri, Maciej Bak, Gerhard Lehmann, Leonhard Heizinger, Xin Wang, Zhi-Tong Li, François Sement, Astrid Bruckmann, Anne-Catherine Dock Bregeon, Rainer Merkl, Reinhard Kalb, Eva Grauer, Erdmute Kunstmann, Mihaela Zavolan, Mo-Fang Liu, Utz Fischer, Gunter Meister

17 Knockout of box H/ACA RNAs that guide the two U1 snRNA pseudouridylations affects specific subsets of human splicing events
Justine Dacanay, Jing Yen Yong, Donald Sim, Xavier Roca

18 Secondary motifs of moderate affinity enable concentration-dependent regulation by Rbfox in development
Bridget E. Begg, Christopher B. Burge

19 Contribution of alternative splicing dynamics to deterministic cell reprogramming: a comparison between B cell and MEFs reprogramming
Claudia Vivori, Panagiotis Papasaikas, Bruno Di Stefano, Ralph Stadhouders, Thomas Graf, Juan Valcárcel Juarez

20 Mechanisms and targets of introns dependent regulation of cell survival and adaptation to starvation
Sherif Abou Elela

21 The RNA helicase DHX34 has a dual role in NMD and in pre-mRNA splicing and is mutated in MDS/AML patients
Nele Hug, Michaela Raab, Stuart Aitken, Ana Rio-Machin, Jude Fitzgibbon, Javier Caceres

22 *Drosophila* Sister of Sex-lethal reinforces a male-specific gene expression pattern by controlling Sxl-lethal-dependent alternative splicing
Rebecca Moschall, Mathias Rass, Oliver Rossbach, Gerhard Lehmann, Lars Kullmann, Norbert Eichner, Daniela Strauss, Gunter Meister, Stephan Schneuwly, Michael Krahn, Jan Medenbach

WEDNESDAY, JUNE 12, 2019: 14:00 – 16:15; THEATRE HALL

Concurrent Session 2: RNA Localization & Transport

Chair: Jeffrey Chao, Friedrich Miescher Inst for Biomedical Research

Abstracts 23 – 32

23 Spatiotemporal organization of the *E. coli* transcriptome: Translation-independence and engagement in regulation
Shanmugapriya Kannaiah, Jonathan Livny, Orna Amster-Choder

24 High-throughput dissection of function and binding preferences of a nuclear localization element common in long RNAs
Yoav Lubelsky, Igor Ulitsky

25 Trypanosomes can initiate nuclear export co-transcriptionally
Carina Goos, Mario Dejung, Ann M Wehman, Elisabeth Meyer-Natus, Johannes Schmidt, Jack Sunter, Markus Engstler, Falk Butter, Susanne Kramer

26 A Deletion in the Mouse Nxf1 Intron 10 CTE Results in Downregulation of Genes Involved in Learning and Synaptic Plasticity
Magdalena Rutkowska, Aleksandra Stamper, Maria Michalak, Wenhao Xu, David Rekosh, Marie-Louise Hammarskjold

27 Integration of protein, RNA and RBP localisation maps to understand subcellular organisation
Eneko Villanueva, Tom Smith, Mohamed Elzek, Rayner M. L. Queiroz, Manasa Ramakrishna, Mariavittoria Pizzinga, Anne E. Willis, Kathryn S. Lilley

28 Alternative 3' UTRs directlocalization of functionally diverse protein isoforms in neuronal compartments
Camilla Ciolli Mattioli, Aviv Rom, Vedran Franke, Koshi Imami, Gerard Arrey, Mandy Terne, Andrew Woehler, Altuna Akalin, Igor Ulitsky, Marina Chekulaeva

29 Makorin 1 controls embryonic patterning by alleviating Bruno-mediated repression of *oskar* translation
Annabelle Dold, Hong Han, Niankun Liu, Andrea Hildebrandt, Mirko Brüggemann, Cornelia Rücklé, Anke Busch, Petra Beli, Kathi Zarnack, Julian König, Jean-Yves Roignant, Paul Lasko

30 Structural basis of RNA transport: Tropomyosin 1 – Kinesin high-resolution structure and its interaction with RNA
Lyudmila Dimitrova-Paternoga, Pravin Kumar Ankush Jagtap, Anna Cyrklaff, Janosch Hennig, Anne Ephrussi, Imre Gaspar

31 Staufen1 reads out structure and sequence features in ARF1 dsRNA for target recognition.
Deepak Kumar Yadav, Dagmar Zigáčková, Maria Zlobina, Tomáš Klumpler, Christelle Beaumont, Monika Kubíčková, Štěpánka Vaňáčová, Peter Josef Lukavsky

32 Cyclin *CLB2* mRNA localization regulates the mitotic progression
Evelina Tutucci, Weihan Li, Robert H. Singer

WEDNESDAY, JUNE 12, 2019: 16:45 – 19:00; AUDITORIUM HALL
Plenary Session 2: Regulatory RNAs and RNPs
Dedicated to the memory of Aaron Klug
Chair: Yukihide Tomari, Univ of Tokyo
Abstracts 33 – 41

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33 RNA-mediated Gene Regulation and Immunity: Structure-function of Riboswitches; Mechanism and Applications of Type I CRISPR.
Ailong Ke [Mid-career Award]

34 Primary sequence relevance in plant microRNAs processing
Arantxa M. L. Rojas, Julieta L. Mateos, Salvador Drusin, Nicolas Bologna, Uciel Chorostecki, Belén Moro, Edgardo G. Bresso, Arnaldo Schapire, Diego Moreno, Rodolfo M. Rasia, Javier F. Palatnik

35 Arabidopsis thaliana mRNA Adenosine Methylase (MTA) is a new player in miRNA biogenesis regulatory pathway.
Susheel Sagar Bhat, Dawid Bielewicz, Natalia Grzelak, Tomasz Gulanicz, Zsuzsanna Bodi, Lukasz Szewc, Mateusz Bajczyk, Jakub Dolata, Dariusz Smolinski, Rupert G. Fray, Artur Jarmolowski, Zofia Szweykowska-Kulinska

36 MicroRNA arm switching regulated by uridylation
Haedong Kim, Jimi Kim, Sha Yu, V. Narry Kim

37 Deciphering target-directed miRNA degradation
Paulina Pawlica, Jessica Sheu-Gruttadauria, Ian MacRae, Joan Steitz

38 AGO2 localizes to mitochondria and regulates mitochondrial function and gene expression
Marissa Holmbeck, Gerald Shadel, Jun Lu

39 Qin: A novel RNA endonuclease of the piRNA biogenesis pathway
Neha Dhimole, Teresa Carlomagno

40 Planarians recruit piRNAs for mRNA turnover in adult stem cells
Iana Kim, Elizabeth Duncan, Eric Ross, Vladyslava Gorbovytska, Stephanie Nowotarski, Sarah Elliott, Alejandro Sánchez Alvarado, Claus Kuhn

41 The dynamics of bacterial small RNA-guided mRNA targeting through Hfq
Ewelina Malecka-Grajek, Subrata Panja, Boyang Hua, Taekjip Ha, Sarah Woodson

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THURSDAY, JUNE 13, 2019: 09:00 – 11:45; AUDITORIUM HALL
Concurrent Session 3: Synthesis and Processing of RNA
Chair: Karla Neugebauer, Yale Univ
Abstracts 42 – 51

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42 Defining the "licence to cut" : structural and functional insights from deconstructing the eukaryotic mRNA 3' end processing machinery
Chris Hill, Vytautė Boreikaitė, Ananthanarayanan Kumar, Ana Casañal, Peter Kubík, Gianluca Degliesposti, Sarah Maslen, Angelica Mariani, Ottilie von Loeffelholz, Mathias Girbig, Mark Skehel, Lori Passmore

43 Cryo-EM studies of the human pre-mRNA 3'-end processing machinery
Yadong Sun, Yixiao Zhang, Keith Hamilton, James Manley, Yongsheng Shi, Thomas Walz, Liang Tong

44 U1snRNP protects transcripts from incorrect poly(A) site usage via modulation of the C-terminal domain of RNA polymerase II
Amelie S. Wachs, Simon A. Krooss, Johannes Kopp, Matthias Geyer, Jörg Langemeier, Jens Bohne

45 CDK11 is required for transcription of replication-dependent histone genes
Pavla Gajduskova, Igor Ruiz de Los Mozos, Michal Rajecky, Milan Hluchy, Jernej Ule, Dalibor Blazek

46 Structural Basis of Transcription: RNA Polymerase backtracking and its reactivation
Mo'men Abdelkareem, Charlotte Saint-André, Maria Takacs, Gabor Papai, Corinne Crucifix, Xieyang Guo, Julio Ortiz, Albert Weixlbaumer

47 Transcription initiation defines both mRNA termini in mitochondria of trypanosomes
Ruslan Aphasizhev, François M. Sement, Takuma Suematsu, Tian Yu, Inna Aphasizheva

48 The transcription and splicing modulator HP1γ tethers pre-mRNA to chromatin via intronic repeated sequences
Christophe Rachez, Rachel Legendre, Jia Yi, Etienne Kornobis, Hugo Varet, Caroline Proux, Christian Muchardt

49 Distinct roles for the polyA+ and polyA- H2a.X mRNA isoforms throughout the cell cycle
Esther Griesbach, William Marzluff, Nicholas Proudfoot

50 HnRNP Proteins Organise Human pre-mRNAs into 40S Ribonucleosome Units
Michal Domanski, Emil Dedic, Walid Gharib, Anne-Christine Uldry, Sophie Braga, Manfred Heller, Jirí Novácek, Frédéric Allain, Oliver Mühlemann

51 RNA-mediated regulation of transcription: epigenetic marks and RNA regulate the histone methyltransferase PRC2 through the same functional centre
Qi Zhang, Nicholas McKenzie, Emma Gail, Sarena Flanigan, Brady Owen, Vitalina Levina, Chen Davidovich

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THURSDAY, JUNE 13, 2019: 09:00 – 11:45; THEATRE HALL
Concurrent Session 4: RNA in Disease and Therapy
Sponsored by Storm Therapeutics
Chair: Matthew Disney, Scripps Research Inst
Abstracts 52 – 61

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52 Precise small molecule cleavage of a r(CUG) repeat expansion in a myotonic dystrophy mouse model
Alicia Angelbello, Suzanne Rzuczek, Kendra McKee, Jonathan Chen, Hailey Olafson, Walter Moss, Eric Wang, Matthew Disney

53 Inhibition of Nonsense-Mediated mRNA Decay in Cystic Fibrosis
Youngjin Kim, Adrian Krainer

54 Reversal of pleotropic effect of toxic RNA with expansion of CGG repeats by short antisense oligonucleotides
Magdalene Derbis, Emre Kul, Daria Niewiadomska, Michał Sekrecki, Katarzyna Taylor, Oliver Stork, Krzysztof Sobczak

55 Ribosome meets RISC at expanded CAG repeat tract - allele-selective RNAi approach for therapy of polyglutamine diseases
Agnieszka Fiszer, Adam Ciesiolka*, Anna Stroynowska-Czerwinska*, Pawel Joachimiak, Agata Luzna, Emilia Kozlowska, Michal Michalak, Wlodzimierz Krzyzosiak*

56 A specialized post-transcriptional program in chemoresistant, quiescent cancer cells
Sooncheol Lee, Douglas Micalizzi, Samuel S Truesdell, Syed IA Bukhari, Min-Kyung Choo, Wilhelm Haas, Shobha Vasudevan

57 RNAi screen revealed mitochondrial RNA degrading and processing enzymes as factors controlling human mitochondrial double-stranded RNA, a potent trigger of inappropriate interferon response
Lukasz Borowski, Aleksander Chlebowski, Zbigniew Pietras, Ewelina Owczarek, Kamila Affek, Anna Kotrys, Ashish Dhir, Nichollas Proudfoot, Andrzej Dziembowski, Roman Szczesny

58 Manipulating RNA processing to enhance or inhibit HIV-1 gene expression/replication
Subha Dahal, Alan Cochrane

59 System-wide identification of the cellular RNA-binding proteins that control the initial steps of HIV-1 infection
Manuel Garcia-Moreno, Caroline Lenz, Marko Noerenberg, Aino Jarvelin, Thibault J.M. Sohier, Jessica Quirke, Emiliano P. Ricci, Shabaz Mohammed, Alfredo Castello

60 Misregulation of splicing factors in breast cancer initiation and metastasis
Shipra Das, Martin Akerman, Anil Kesarwani, Adam Geier, SungHee Park, Mattia Brugiolo, Martin Fan, Carolyn Paisie, Nathan Leclair, Laura Urbanski, Paola Peshkepija, Chenle Hu, Xingan Hua, YoungJin Kim, Kuang-Ting Lin, Joshy George, Senthil Muthuswamy, Adrian Krainer, Olga Anczukow

61 Human-specific circular Tau RNAs as new causes for Neurodegeneration leading to Alzheimer's Disease and Frontotemporal Dementia
Justin Welden Welden, Stephen Dockins, Jessica Blackburn, Peter Nelson, Stefan Stamm

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THURSDAY, JUNE 13, 2019: 09:00 – 11:45; CHAMBER HALL - S3, LEVEL 3

Workshop 1: The tRNA World beyond Translation

Organizers: Sebastian Glatt, Jagiellonian Univ;
Sebastian Leidel, Univ of Bern
Abstracts 62 – 72

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62 Metabolic and chemical regulation of tRNA modification under physiological and pathological conditions
Tsutomu Suzuki

63 Effect of tRNA composition in ORF selection and proteostasis in *Drosophila*
Alvaro Glavic, Cristian Eggers, Esteban Contreras, Jorge Zuñiga

64 Expanded tRNA Diversity in Mammals Indicates New Conserved Functions
Jonathan Howard, Andrew Holmes, Brian Lin, Bryan Thornlow, Patricia Chan, Todd Lowe

65 Discovery of a unique RNA 2',3'-cyclic phosphatase in human cells
Paola H. Pinto, Alena Kroupova, Ameya Khandekar, Alexander Schleiffer, Karl Mechtler, Martin Jinek, Stefan Weitzer, Javier Martinez

66 Modulation of mammalian translation by the tRNA half
Yulia Gonskikh, Johannes Grillari, Norbert Polacek

67 Conserved uridines 54 and 55 of human tRNAs are modified by different enzymes in the nucleus and the cytoplasm
Shaoni Mukhopadhyay, Manisha Deogharia, Ramesh Gupta

68 Time-resolved NMR monitoring of tRNA maturation
Pierre Barraud, Alexandre Gato, Matthias Heiss, Marjorie Catala, Stefanie Kellner, Carine Tisné

69 Crystal structure of an Adenovirus Virus-Associated RNA
Iris Hood, Jackson Gordon, Charles Bou Nader, Frances Henderson, Soheila Bahmanjah, Jinwei Zhang

70 The Elongator subunit Elp3 is a non-canonical tRNA acetyltransferase
Ting-Yu Lin, Nour El Hana Abbassi, Sebastian Glatt

71 Chemical availability of tRNA fragments containing modified nucleosides from 34 and 37 positions of anticodon domain
Grazyna Leszczynska, Karolina Bartosik, Michal Matuszewski, Klaudia Sadowska, Katarzyna Debiec, Agnieszka Dziergowska, Barbara Nawrot, Elzbieta Sochacka

72 A microfluidic-based assay recapitulates eukaryotic ribosomal translation and identifies toxic tRNAs
Ketty Pernod, Laure Schaeffer, Johana Chicher, Eveline Hok, Christian Rick, Renaud Geslain, Gilbert Eriani, Eric Westhof, Michael Ryckelynck, Franck Martin

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FRIDAY, JUNE 14, 2019: 09:00 – 11:45; AUDITORIUM HALL

Plenary Session 3: Mechanisms of Translation

Dedicated to the memory of Tom Steitz and Sidney Brenner
Chair: Nahum Sonenberg, McGill Univ
Abstracts 73 – 81

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73 Ribosome Heterogeneity in Translating the Genetic Code
Maria Barna [Early-career Award]

74 Specialisation of ribosomes in gonads
Tayah Hopes, Michaela Agapiou, Amy Turner, Juan Fontana, Julie Aspden

75 The mechanism of inhibition of cap-dependent translation by the Translational Inhibitory Elements (TIE) a3 and a11 in Hox mRNAs
Fatima Alghoul, Laure Schaeffer, Gilbert Eriani, Franck Martin

76 Dissecting the roles of eIF2 and eIF3 during translation using TCP-seq
Susan Wagner, Nikolay E. Shirokikh, Stuart K. Archer, Anna Herrmannova, Neelam Sen, Nick T. Ingolia, Alan G. Hinnebusch, Thomas Preiss, Leos S. Valasek

77 Distinct H/ACA small nucleolar RNA-guided ribosome modifications in control of lipid metabolism and ribosome dynamics
Mary McMahon, Adrian Contreras, Tamayo Uechi, Mikael Holm, Craig Forester, Xiaming Pang, Bin Chen, David Quigley, Kate Kelley, John Gordan, Ryan Gill, Scott Blanchard, Davide Ruggero

78 Transcriptome-wide sites of collided ribosomes reveal sequence determinants of translational pausing
A. Bulak Arpat, Angelica Liechti, Mara De Matos, Peggy Janich, David Gatfield

79 Mechanisms of mammalian mitochondrial translation
Eva Kummer, Katharina Warinner, Marc Leibundgut, Daniel Boehringer, Nenad Ban

80 Capturing ribosomal translocation by EF-G using cryo-EM
Gabriel Demo, Anna Loveland, Egor Svidritskiy, Howard Gamper, Ya-Ming Hou, Andrei Korostelev

81 Anatomy of a standby site: an essential role for ribosomal protein S1 and a secondary structure element for ribosome binding
Cedric Romilly, Sebastian Deindl, Gerhart Wagner

FRIDAY, JUNE 14, 2019: 11:45 – 12:30; AUDITORIUM HALL

Keynote 3

Maria Carmo-Fonseca, Inst of Molecular Medicine, Univ of Lisboa
Abstract 82

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82 The timing of splicing
Maria Carmo-Fonseca

FRIDAY, JUNE 14, 2019: 14:00 – 16:15; AUDITORIUM HALL

Plenary Session 4: RNA Modification and Editing

Dedicated to the memory of Marie Öhman

Chair: Chuan He, Univ of Chicago and Peking Univ

Abstracts 83 – 92

83 Bacterial and eukaryotic RNA gets an NAD-cap - But How to Remove it?
Katharina Höfer, Andres Jäschke

84 Cap-specific terminal *N*-methylation of RNA by an RNA polymerase Ⅱ-associated methyltransferase
Shinichiro Akichika, Seiichi Hirano, Yuichi Shichino, Takeo Suzuki, Hiroshi Nishimasu, Ryuichiro Ishitani, Ai Sugita, Yutaka Hirose, Shintaro Iwasaki, Osamu Nureki, Tsutomu Suzuki

85 Construction and screening of an RNA binding protein CRISPR/Cas9 knockout library identifies RNA degradation as a specific vulnerability in Myc-dependent cancer
Jaclyn Einstein, Eric Van Nostrand, Julia Nussbacher, Alexander Shishkin, Jitendra Meena, Thomas Westbrook, Gene Yeo

86 New insights into mechanisms and functions of mA-YTH modules: lessons from plants
Laura Arribas-Hernandez, Sarah Rennie, Sara Simonini, Mathias Tankmar, Alexander J.H. Andersen, Carlotta Porcelli, Lars Østergaard, Robin Andersson, Peter Brodersen

87 Mechanisms controlling the dynamics of A to I editing
Mamta Jain, Konstantin Licht, Utkarsh Kapoor, Michael F. Jantsch

88 Purifying Selection of long dsRNA is the first line of defense against false activation of innate immunity
Michal Barak, Gilad Finkelstein, Binyanin Knisbacher, Hagit Porath, Ilana Buchumenski, Erez Levanon, Eli Eisenberg

89 RNA editing - an alternative to DNA editing
Thorsten Stafforst

90 PPR-based Mechanisms of Mitochondrial Editing Surveillance in Trypanosomes
Inna Aphasizheva, Mikhail V. Mesitov, Tian Yu, Takuma Suematsu, Ruslan Aphasizhev

91 Expanded functional repertoire of tRNA modifying enzymes
Govardhan Reddy Veerareddygari, Emily Soon, Sarah Schultz, Laura Keffer-Wilkes, Ute Kothe

92 Structure and Function of the eukaryotic Elongator Complex
Sebastian Glatt

FRIDAY, JUNE 14, 2019: 16:45 – 19:00; AUDITORIUM HALL

Concurrent Session 5: Non-coding RNAs: Long & Short, Linear & Circular

Chair: Julia Salzman, Stanford Univ School of Medicine

Abstracts 93 – 102

93 Identification of large noncoding RNA-protein interactions and their effects on stress response in bacteria
Kimberly Harris, Zhiyuan Zhou, Danielle Widner, Michelle Peters, Sarah Wilkins, Nicole Odzer, Ronald Breaker

94 Xist deletional analysis reveals an inter-dependency between Xist RNA and Polycomb complexes for spreading along the inactive X
Hongjae Sunwoo, David Colognori, Andrea Kriz, Chen-Yu Wang, Jeannie Lee

95 Riboregulation of mammalian autophagy by the non-coding vaultRNA 1-1
Magdalena Büscher, Rastislav Horos, Florence Baudin, Rozemarijn Kleinendorst, Anne-Marie Alleaume, Abul K. Tarafder, Thomas Schwarzl, Dziuba Dmytro, Christian Tischer, Elisabeth M. Zielonka, Asli Adak, Alfredo Castello, Wolfgang Huber, Carsten Sachse, Matthias W. Hentze

96 You can't teach an old dog new tricks? Story about newly discovered lincRNA involved in seed dormancy.
Sebastian Sacharowski, Grzegorz Brzyzek, Szymon Swiezewski

97 Characterization of *Arabidopsis thaliana UGT73C6* natural *cis*-antisense long non-coding RNAs and analysis of their role in leaf size modulation.
Shiv Kumar Meena, Ammar Jaber, Susanne Engelmann, Tebbe de Vries, Sven-Eric Behrens, Steffen Abel, Selma Gago Zachert

98 Nopp140-mediated concentration of scaRNPs in Cajal bodies is required for telomere length regulation and spliceosomal snRNA modification
Jonathan Bizarro, Svetlana Deryusheva, Joseph G. Gall, U. Thomas Meier

99 Nuclear export of circular RNA
Linh Ngo, Wanqiu Li, Tobias Williams, Kirsty Carey, Greg Goodall, Vihandha Wickramasinghe

100 Insights into the biogenesis and potential functions of exonic circular RNA
Chikako Ragan, Gregory Goodall, Nikolay Shirokikh, Thomas Preiss

101 Expression, alternative splicing and function of circRNAs in neuronal development and disease
Jorgen Kjems, Karim Rahimi, Daniel Dupont, Morten Venø, Sabine Seeler, Maria Andersen, Mark Denham

102 Large Scale Screenings of lncRNA Functions and Structures
Jordan Ramilowski, Chi Wai Yip, Saumya Agrawal, Chung Chau Hon, Masayoshi Itoh, Takeya Kasukawa, Suzuki Harukazu, Ken Yagi, Michiel de Hoon, Jay Shin, Piero Carninci

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FRIDAY, JUNE 14, 2019: 16:45 – 19:00; THEATRE HALL
Concurrent Session 6: Translation Regulation
Chair: Andrea Berman, Univ of Pittsburgh
Abstracts 103 – 112

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103 Ribosomes in unfamiliar territory: consequences of translation in noncoding sequences
Xuebing Wu [Scaringe Award]

104 Non-canonical translation initiation in yeast generates a cryptic pool of mitochondrial proteins.
Anna Mińcicka, Geoffray Monteuuis, Alexander Kastaniotis, Joanna Kufel

105 mRNA Translation Landscape Shapes the Biology of MYC in Cancer and Immune Response
Kamini Singh, Jianan Lin, Yi Zhong, Antonija Burčul, Prathibha Mohan, Man Jiang, Agnes Viale, Justin R. Cross, Liping Sun, Vladimir Yong-Gonzalez, Ronald C. Hendrickson, Gunnar Rätsch, Zhengqing Ouyang, Hans-Guido Wendel

106 mTORC1 coordinates mRNA translation and mitochondrial dynamics and functions through 4E-BP
Masahiro Morita, Sakie Katsumura, Ivan Topisirovic, Heidi McBride, John Bergeron, Nahum Sonenberg

107 Baseline translation ensures maintenance of a stable population of TOP mRNAs under starvation conditions
Cornelius Schneider, Florian Erhard, Joerg Vogel, Utz Fischer

108 LARP1 is a novel mTORC1 substrate and a key repressor of TOP mRNA translation
Bruno Fonseca, Jian-Jun Jia, Michael Solgaard, Anne Hollensen, Roni Lahr, An-Dao Yang, Marius Niklaus, Roberta Pointet, Huy-Dung Hoang, Izabella Pena, Ewan Smith, Jaclyn Hearnden, Xu-Dong Wang, Giovanna Celucci, Tyson Graber, Christopher Dajadian, Yonghao Yu, Christian Damgaard, Andrea Berman, Tommy Alain

109 Loss of tRNA modification iA_{37} leads to mitochondriopathy and increased +1 frame-shifting in mice
Simon Bohleber, Noelia Fradejas Villar, Wenchao Zhao, Ulrich Schweizer

110 Novel cross talk between tRNA and mRNA processes exerted by Aminoacyl tRNA synthetases
Ofri Levi, Yoav Arava

111 A large-scale functional tethering screen identifies UBAP2L as a translational enhancer protein to rescue neurodevelopmental defects in Fragile X Syndrome
En-Ching Luo, Jason Nathanson, Frederick Tan, Soojin Lee, Sara Kosmaczewski, Joshua Schwartz, Archana Shankar, Sebastian Markmiller, Eric Van Nostrand, Gabriel Pratt, Duy Duong, Yuanchi Ha, Lindy Barrett, Fen-Biao Gao, Stefan Aigner, Gene Yeo

112 Structural insights into Ssd1p, a spatial and temporal regulator of mRNA translation in budding yeast
Uma Jayachandran, Aleksandra Kasprowicz, Rosey Bayne, Edward Wallace, Atlanta Cook

FRIDAY, JUNE 14, 2019: 16:45 – 19:00; CHAMBER HALL - S3, LEVEL 3

Workshop 2: Computational Modeling of RNA Structure and Complexes

Organizers: Janusz Bujnicki, International Inst of Molecular and Cell Biology in Warsaw; Francois Major, Univ of Montreal; Eric Westhof, Univ of Strasbourg

Abstracts 113 – 122

113 A learning model for RNA secondary structure prediction using SHAPE mapping and sequence alignment data
Nicola Calonaci, Francesca Cuturello, Giovanni Bussi

114 RNA structural ensembles at the exon-intron boundary *in vivo*
Lela Lackey, Jayashree Kumar, Alain Laederach

115 Improved boundary definition of RNA structures using multiple sequence alignments and its application on RNA motif detection.
Stefan E Seemann, Radhakrishnan Sabarinathan, Christian Anthon, Jan Gorodkin

116 The importance of being RNASt
Marco Pietrosanto, Marta Adinolfi, Fabrizio Ferrè, Gabriele Ausiello, Manuela Helmer-Citterich

117 Automated and customizable identification of 3D modules and prediction of RNA 3D structures
Jerome Waldispuhl, Roman Sarrazin-Gendron, Vladimir Reinharz, Carlos G. Oliver, Francois Major, Nicolas Moitessier

118 New classification of tetrads and quadruplexes in DNA and RNA structures
Marta Szachniuk, Joanna Miskiewicz, Mariusz Popenda, Joanna Sarzynska, Tomasz Zok

119 Rosetta modeling and M2-seq for coordinate inference in rapidly determined RNA-only cryo-EM maps
Kalli Kappel, Kaiming Zhang, Zhaoming Su, Grigore Pintilie2, Wah Chiu, Rhiju Das

120 Integrating SAXS, chemical probing, motif-modeling and cryo-EM fitting: two-dimensional and three-dimensional structure-function relationships for individual long non-coding RNAs
Karissa Sanbonmatsu

121 Modeling of ribonucleic acid-ligand interactions
Filip Stefaniak, Pietro Boccaletto, Janusz M. Bujnicki

122 Computational approaches to targeting RNA with small molecules
Donovan Chin

SATURDAY, JUNE 15, 2019: 09:00 – 11:00; AUDITORIUM HALL

Concurrent Session 7: Interconnected RNA Processes

Chair: Andrzej Dziembowski, Inst of Biochemistry & Biophysics, Polish Academy of Sciences, Warsaw

Abstracts 123 – 131

123 An Integrator 'Phosphatase Module' Remodels the RNAPII CTD to Attenuate Transcription
Kai-Lieh Huang, Nathan Elrod, Lauren Mascibroda, Telmo Henriques, Deidre Tatomer, Jeremy Wilusz, Karen Adelman, Eric Wagner

124 Gene-Specific Variation in Co-Transcriptional Pre-mRNA Processing
Tara Alpert, Korinna Straube, Lydia Herzel, Fernando Carrillo Oesterreich, Karla Neugebauer

125 Isoform-specific translational control is conserved across higher primates
Julia Philipp, Jolene Draper, Sol Katzman, Sofie Salama, David Haussler, Jeremy Sanford

126 MCL1 alternative 3'UTRs play a role in Mcl-1 protein localization and function
Isabel Pereira-Castro, Beatriz C Garcia, Alexandra Moreira

127 Post-translational modifications of Rpb4 are required for the linkage between mRNA synthesis, translation and decay
Steve Richard, Lital Gross, Shira Urim, Tamar Ziv, Mordechai Choder

128 Defining requirements for PKR activation by snoRNAs
Sarah Safran, Brenda L. Bass

129 The role of RNA-binding proteins in regulating the RIG-I/interferon type I signalling pathway
Gregory Heikel, Rute Maria Dos Santos Pinto, Lucia Timm, Nila Roy Choudhury, Eleanor Gaunt, Paul Digard, Gracjan Michlewski

130 Ubiquitination efficiency of E3 ligase TRIM25 is regulated by RNAs
Kevin Haubrich, Sandra Anett Augsten, Bernd Simon, Pawel Masiewicz, Kathryn Perez, Mathilde Lethier, Stephen Cusack, Frank Gabel, Janosch Hennig

131 Cytoplasmic polyadenylation regulates the innate immune response in animals
Vladyslava Liudkovska, Pawel Krawczyk, Seweryn Mroczek, Aleksandra Bilska, Olga Gewartowska, Jakub Gruchota, Ewa Borsuk, Jonathan Ewbank, Krzysztof Drabikowski, Andrzej Dziembowski

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SATURDAY, JUNE 15 2019: 09:00 – 11:00; THEATRE HALL
Concurrent Session 8: RNA Catalysis, Folding and Structure
Chair: Rick Russell, Univ of Texas at Austin
Abstracts 132 – 140

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132 Structural basis of G-quadruplex unfolding by the DEAH/RHA helicase DHX36
Michael C. Chen [Scaringe Award], Ramreddy Tippana, Natalia A. Demeshkina, Pierre Murat, Shankar Balasubramanian, Sua Myong, Adrian R. Ferré-D'Amaré

133 Alternative catalytic strategies employed by the nucleolytic ribozymes
Timothy Wilson, Yijin Liu, David Lilley

134 An atomic structure of RNase MRP
Ming Lei

135 Riboswitch-ligand structure determination using an integrative structural biology approach
Almudena Ponce-Salvatierra, Radoslaw Pluta, Ewa Skowronek, Magdalena Orlowska, Blazej Baginski, Malgorzata Kurkowska, Elzbieta Purta, Pritha Ghosh, Tomasz K. Wirecki, Filip Stefaniak, Janusz M. Bujnicki

136 *In vivo* identification of critical structural motifs in Influenza A virus mRNAs
Lisa Marie Simon, Edoardo Morandi, Anna Luganini, Giorgio Gribaudo, Luis Martinez-Sobrido, Douglas H. Turner, Salvatore Oliviero, Danny Incarnato

137 Structural and functional studies of a novel interaction between poly(A) and ENE elements
Seyed F. Torabi, Anand T. Vaidya, Suzanne J. DeGregorio, Kazimierz T. Tycowski, Mei-Di Shu, Jimin Wang, Thomas A. Steitz, Joan A. Steitz

138 Bespoke mechanisms of RNA remodelling by viral RNA chaperones
Jack Bravo, Alexander Borodavka, Anders Barth, Antonio Calabrese, Vitalina Levina, Emma Gail, Don Lamb, Chen Davidovich, Roman Tuma

139 Requirement for multi-protein recruitment during co-transcriptional assembly of nascent ribosomal RNA
Margaret Rodgers, Sarah Woodson

140 Real-time Imaging of Cotranscriptional Folding and Translational Regulation
Jonathan Grondin, Adrien Chauvier, Patrick St-Pierre, Jean-François Nadon, Cibran Perez-Gonzalez, Sébastien Eschbach, Anne-Marie Lamontagne, Juan-Carlos Penedo, Daniel Lafontaine

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SATURDAY, JUNE 15, 2019: 11:30 – 13:00; AUDITORIUM HALL
Workshop 3: Membrane-less Granules and Liquid-Liquid Phase Separation
Organizer: Dominique Weil, CNRS, Sorbonne Univ
Abstracts 141 – 148

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141 Hidden codes of NEAT1 lncRNA for biophysical properties of phase-separated paraspeckles
Tomohiro Yamazaki, Sylvie Souquere, Hiro Takakuwa, Hyura Yoshino, Archa H. Fox, Charles S. Bond, Shinichi Nakagawa, Gerard Pierron, Tetsuro Hirose

142 Structural studies of the role phase-separating gene regulatory proteins and the formation of membraneless organelles
Charles Bond

143 New repeat-enriched RNAs regulating subcellular localization and activity of RNA-binding proteins
Eugene Makeyev, Karen Yap

144 DEAD-box ATPases are global regulators of phase-separated organelles and RNA flux
Maria Hondele, Ruchika Sachdev, Stephanie Heinrich, Juan Wang, Beatriz M.A. Fontoura, Karsten Weis

145 Bacterial RNP-bodies organize substeps of mRNA decay
Nadra Al-Husini, Dylan Tomares, Nisansala Muthunayake, W. Seth Childers, Jared Schrader

146 Regulation of RNA Granules by caliciviruses, from hijacking to novel paracrine induction
Valentina Iadevaia, Michèle Brocard, Carla Moller-Levet, James Burke, Roy Parker, Nicolas Locker

147 GC content shapes mRNA storage in human P-bodies
Maite Courel, Yves Clement, Michel Kress, Racha Chouaib, Hugues Roest Crollius, Nancy Standart, Dominique Weil

148 mRNA localization to translation factories for regulation, complex formation or inheritance
Christian Bates, Mariavittoria Pizzinga, Fabian Morales-Polanco, Jennifer Lui, Gabriella Forte, Emma Linney, Mark Ashe

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SATURDAY, JUNE 15, 2019: 11:30 – 13:00; CHAMBER HALL - S3, LEVEL 3

Workshop 4: Computational Methods for RNA

Sponsored by Locana, Inc.
Organizers: Eugene Yeo, Univ of California, San Diego; Yoseph Barash, Univ Pennsylvania
Abstracts 149 – 153

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149 Direct RNA sequencing offers a comprehensive view of polyadenylation status in mammals thanks to an unprecedented range of poly(A) tail lengths measurement.
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150 Poly(A)-ClickSeq as a tool enabling simultaneous genome-wide poly(A)-site identification and differential expression analysis.
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160 Analysis of *upf1*-null mutants in Arabidopsis revealed central role of UPF1 in transcriptional and translational homeostasis
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210 Spatial differences of miRNA genes expression in the early stages of colorectal cancer
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291 Are neuronal cytoplasmic lncRNAs associated with polysomes and translated?
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292 Neuronal circular RNAs in a mouse model of autism spectrum disorders
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293 Identification and characterisation of circular RNAs by multiple displacement amplification
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294 Relationship between human centromeric satellite I noncoding RNA and m6A RNA modification
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295 A natural antisense long non-coding RNA overlapping the UGT76E12 gene of Arabidopsis thaliana regulates its expression by influencing local chromatin state in cis
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296 Structure and degradation of circular RNAs regulate PKR activation in innate immunity
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297 Expression and functions of hypoxia regulated long non-coding RNA HABON in hepatocellular carcinoma cells
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298 The susceptibility of sea-island cotton recombinant inbred lines to *Fusarium oxysporum* f. sp. *vasinfectum* infection is characterized by altered expression of long noncoding RNAs
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299 Structural characterization of group II introns and long non-coding RNAs
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300 Molecular functions of circRNAs in human myoblast proliferation and in rhabdomyosarcoma
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301 A novel long noncoding RNA lincSox2-AS regulates the cancer stem cell population in glioblastoma multiforme
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302 A computational method for precise circular RNA isoform quantification
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303 lncRNAs as triggers of RNA editing in breast cancer
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304 Lnc-ing RNA structure to function
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305 The novel long noncoding RNA lncNB1 promotes tumorigenesis by interacting with ribosomal protein.
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306 The up-regulated circRNA production blast-resistance rice is associated with alternative splicing complexity
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307 Long noncoding *OIP5-AS1* acts as expression regulator of *OIP5* gene
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310 Cold sensing by a group II intron fine-tunes host gene expression
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311 Analysis of FinO targets in *Salmonella* Typhimurium
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312 A census of RNA and protein complexes in a model Gram-positive bacterium reveals exonuclease-mediated stabilization of small RNAs in the competence regulon
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313 Mimicking cotranscriptional riboswitch folding via a superhelicase unwinding assay
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314 The contribution of the Hfq protein to the annealing of Class II sRNAs to their mRNA targets
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315 Control of the *tpiA* mRNA abundance by RNase G contributes to glycolysis upon oxygen availability in *Escherichia coli*
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316 New method for unbiased quantification of riboswitch transcriptional activity
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317 Unveiling the Hfq-mediated RNA-RNA interactome of Salmonella Typhimurium
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318 Comprehensive sequence analysis of group II introns and their phylogenetic profiles in bacteria
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319 Exploiting nucleotide signaling molecules for antibiotic development by using riboswitch sensors
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320 A draft RNA landscape of the colorectal cancer-associated germ *Fusobacterium nucleatum*
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321 A Cas12a-mediated CRISPRi screen for small noncoding RNAs in the human commensal *Bacteroides thetaiotaomicron*
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322 The transcriptomic landscape of the dominant human gut commensal, *Bacteroides thetaiotaomicron*
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323 The recognition of small RNAs and regulated mRNAs by *E. coli* ProQ protein
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324 RNA-seq-based approaches for the characterization of small proteins involved in *Salmonella* Typhimurium virulence
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326 Study of the existence and relevance of human microRNA-encoded peptides (miPEPs) in hematological malignancies.
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329 Exosome-mediated MIR211 modulates tumor microenvironment via the DUSP6-ERK5 axis and contributes to BRAFV600E inhibitor resistance in melanoma.
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330 FUS controls the processing of snoRNAs into smaller RNA fragments that can regulate gene expression
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331 The orphan C/D box snoRNA SNORD116 missing in Prader-Willi syndrome regulates microexon usage
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334 Genome-wide RNAi screen identifies new players in 60S subunit biogenesis in human cells
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335 Molecular mechanism of stress response ribosome-binding trGTPase, BipA.
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336 Structural modular evolution of the ribosome through extensive local duplications
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337 Pseudouridylation enzyme RsuA influences 30S ribosome assembly
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338 Structural basis of alternative ribosome rescue by ArfA and RF2
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339 Strategies for structure determination of a poorly characterized and extremely complex mitochondrial ribosome from trypanosomes
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340 Cryo-EM visualizes translation elongation with authentic substrates and reveals continuous mRNA decoding
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341 Modulation of rRNA processing pathways conserves energetic reserves and maintains nucleolar structure during stress
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342 Unexpected requirements for nucleolar structure and function revealed by genome-wide RNAi screen for increased nucleolar number in human cells
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343 Structural basis for the auto-inhibition of the DEAH-box helicase Dhr1
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344 Ribosome maturation factor Nop53 controls association of the RNA exosome with pre-60S particles
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345 The kinase Rio1 regulates the entry of nascent pre-40S ribosomes into the translating pool
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346 Mapping rRNA 2'-*O*-Methylation by RiboMethSeq and characterization of snoRNAs in *Arabidopsis*
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347 Pol5 is required for assembly of the large ribosomal subunit and the recycling of small subunit biogenesis factors
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348 Iron-dependent cleavage of ribosomal RNA during oxidative stress in the yeast *Saccharomyces cerevisiae*.
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349 YBEY is a ribosome assembly factor required for the mitochondrial small subunit biogenesis
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350 Dynamics of the early steps in ribosome biogenesis.
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351 Comparative genomics analysis of CPR bacteria with their complete genomes: Minimized profiles of ribosomal proteins and ribosomal RNAs
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354 A specific mRNA scaffold promotes *cis*-translational interaction of proteins important for P-body assembly.
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355 A novel tudor-domain protein promotes germline differentiation through post-transcriptional gene regulation in cytoplasmic RNA granules
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356 Regulating the dynamicity of stress induced cytoplasmic ribonucleoprotein granules via liquid-liquid phase separation.
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357 Identification of the first small-molecule regulator of mRNA decapping and processing-body turnover.
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358 TIAR specifies stress-induced nuclear compartments during G2/M checkpoint activation
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359 Molecular mechanisms of the paraspeckle formation as distinct nuclear bodies
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360 De novo missense changes in the helicase DDX6 cause intellectual disability and dysmorphic features by disrupting RNA regulation and P-body assembly.
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362 Phase separation behaviour of the *Arabidopsis* SERRATE protein
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368 Deep into cellular dark matter – role of rDNA array in stress response
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369 Exploring the role of chromatin decondensation at a gene locus in stochastic gene expression
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370 DOT1L suppresses nuclear RNAi originating from enhancer elements in *Caenorhabditis elegans*
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371 Differential epigenetic silencing of nuclear tRNA gene repeats in *Arabidopsis thaliana*
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372 An attempt to reveal differences in RNA methylation of transcriptome of colon cancer model cell line (LoVo) as a result of azacitidine action.
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373 Links between chromatin status and 3'-end mRNA processing
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374 Splicing influences Set2 recruitment and H3K36me3 at intron-containing genes in *Saccharomyces cerevisiae*
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375 Genome surveillance by non-coding transcription
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377 The roles of RNA helicases during large ribosomal subunit biogenesis
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378 Role of the RNA-binding protein Csde1 in oncogene-induced senescence
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381 Discovering RNA-binding proteins involved in T-cell acute lymphoblastic leukemia
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383 RNA-binding protein MSI-1 affects *let-7* microRNA-regulated stem cell-like division of the epithelial seam cells in *Caenorhabditis elegans*
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384 Coregulation of primary microRNA processing by the SR proteins SRSF1 and SRSF3
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385 Structural Determination of Dicer-2 Complexes
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386 Characterizing the stimulation of spliceosomal DEAH-box helicases by G-patch proteins
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387 Loss of DHX36 helicase activity leads to accumulation of translationally inactive target mRNAs with G-rich structures in untranslated regions
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388 RNA translocation mechanism of spliceosomal DEAH-box ATPases
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389 Global analysis of molecular interactions underlying functions of NANOS1 and NANOS3 proteins in human germ cell model
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390 Identification of novel RNA-binding proteins in human mitochondria with focus on poly(A)-binding proteins
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391 Development a FRET-based RNA biosensor.
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392 Nuclear and Cytoplasmic Cofactors of the RNA Exosome Play Unique Roles in Regulation of RNA Transcripts in Conjunction with RNA-Binding Protein Nab2
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395 A non-canonical RNA binding domain in splicing factor SF3A1
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396 Dissecting the role of ARS2 in nonsense mediated decay
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397 Modulation of G-quadruplex structural integrity by hnRNP HF controls cellular stress response linked to tumor progression in glioblastoma
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398 A Viral RNA-Binding Protein with a Divergent RNA Sequence Preference
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399 Structural RNA components affect ZFP36L2-RNA interaction
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400 Identification of Pentatricopeptide repeat proteins that bind organellar transcripts in plants to coordinate nuclear and organellar gene expression
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401 Grad-Seq in mycobacteria
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402 Long LARP1 isoform is dominantly expressed in a wide range of human cancer cell lines
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403 RNA-binding protein NANOS1 is necessary for regulation of mRNAs encoding apoptotic pathways in human germ cells
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404 Hypoxia-induced response of RNA binding proteins in leukemia cells
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405 CRKL regulates alternative splicing of cancer-related genes in cervical cancer samples and HeLa cell
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407 RNApdbee 2.0: a web platform for comprehensive RNA structure annotation
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412 Developing an accurate all-atom fixed-charge force field for RNA with implicitly polarized charges
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414 rnaSMART - tool for identification of Structural Motifs Across RNA Transcripts
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415 rG4-seeker enables high confidence identification of novel rG4 motifs from rG4-seq experiment via platform-specific noise modeling
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416 CRISPR-Cas9 on-target activity is more efficient in absence of local off-targets and strong or weak binding energies
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417 SAXS data-driven modeling of RNA 3D structures
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418 Theoretical pKa determinations of modified pyrimidines using an implicit-explicit solvation model
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423 Comprehensive computational analysis reveals noncanonical reverse transcriptases in CPR bacteria
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425 QM/MM Calculations on Protein-RNA Complexes: Understanding Limitations of Classical MD Simulations and Search for Reliable Cost-Effective QM Methods.
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426 A computational method for detecting novel classes of trans RNA-RNA interactions
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427 cTRAP: identification of candidate causal perturbations from differential expression data
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428 Kinetic Pathways of Metal Cation Binding to RNA – Accurate Exchange Rates and Atomistic Insights from MD Simulations
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429 Autosomal monoallelic expression of protein-coding genes overlapping at 5 end for adenocarcinoma cell lines
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431 DesiRNA - method for RNA secondary structure-based sequence design
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434 Characterization of orphan riboswitches using a genetic screen
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435 Biochemical validation of a second tetrahydrofolate riboswitch class in bacteria
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436 Local-to-global signal transduction at the core of a Mn^{2+} sensing riboswitch
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439 Fitness study of Azoarcus Group I mutants under a gradient of magnesium chloride concentration - Construct of Seascapes
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440 Structure and ligand binding of the SAM-V and glutamine-II riboswitches
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441 Can we predict a riboswitch activity with different ligands by calculations?
Marta Kulik, Takaharu Mori, Yuji Sugita, Joanna Trylska

442 Evidence that the *nadA* Motif is a Riboswitch for the Ubiquitous Enzyme Cofactor NAD^+
Sarah Malkowski, Tara Spencer, Ronald Breaker

443 Cellular Small Molecules Contribute to Twister Ribozyme Catalysis by Indirect Proton Transfer
Kyle Messina, Philip Bevilacqua

444 How chemically functionalized ligands trigger engineered $preQ_1$ riboswitches in *E.coli*
Eva Neuner, Marina Frener, Alexandra Lusser, Ronald Micura

445 Hatchet ribozyme structure and implications for cleavage mechanism
Luqian Zheng, Christoph Falschlunger, Dinshaw Patel, Ronald Micura, Aiming Ren

446 Implementation of Fluorescent Cross Correlation Spectroscopy (FCCS) to Analyze Single Molecule Riboswitch Performance in Eukaryotic Cells.
Jessica Roberts, Eric Hayden, Matthew Ferguson

447 An aminoacylation ribozyme evolved from a natural tRNA sensing riboswitch
Naohiro Terasaka, Satoshi Ishida, Takayuki Katoh, Hiroaki Suga

448 The asymmetry and cooperativity of tandem glycine riboswitch aptamers
Chad Torgerson, David Hiller, Scott Strobel

449 *corA* : A new riboswitch in *Escherichia coli*
Anne-Sophie Vézina Bédard, Daniel Lafontaine, Maude Guillier, Mélanie Geffroy, Francisco Martin, Éric Massé

450 One transcript, distinct stabilities: The methionine biosynthesis operon in *Staphylococcus aureus*
Freya D. R. Wencker, Sonja M. K. Schoenfelder, Sandra Maaß, Dörte Becher, Wilma Ziebuhr

451 Expeditious Validation of Multiple Orphan Riboswitch Candidates
Neil White, Gayan Mirhana Arachchilage, Ronald Breaker

452 Successful design of highly active HDV ribozymes by a combined computational and experimental study
Ryota Yamagami, Mohammad Kayedkhordeh, David Mathews, Philip Bevilacqua1

Poster Topic: RNA Modification and Editing

Abstracts 453 – 497 + 866

453 RNA methylation states in CD8+ T-lymphocyte differentiation
Paulo A. Gameiro, Iosifina Foskolou, Charlotte Capitanchik, Igor Ruiz de los Mozos, David Bargiela, Randall Johnson, Jernej Ule

454 Understanding the role of the m^6A methyltransferase METTL3 in breast cancer
Cyrinne Achour, Devi Prasad Battharai, Francesca Aguilo

455 Characterization of RNA post-transcriptional modifications in *Staphylococcus aureus* tRNAs during infection and antibiotic stresses
Laura Antoine, Philippe Wolff, Eric Westhof, Pascale Romby, Stefano Marzi

456 Exploring the role of 5 methyl cytosine modification in RNA metabolism of mitochondria
Dhiru Bansal

457 Analysis of cap-associated adenosine modifications in eukaryote mRNA
Eleanor Bellows, Francis Barron, Susanne Kruse, Silin Zhong, Zsuzsanna Bodi, Helen Knight, Christopher Hayes, Rupert Fray

458 Epitranscriptomic regulation of HIV-1 gene expression by m^5C and the novel m^5C reader MBD2
David Courtney, Kevin Tsai, Hal Bogerd, Edward Kennedy, Brittany Law, Ann Emery, Ronald Swanstrom, Christopher Holley, Bryan Cullen

459 Rational design of novel H/ACA snoRNAs for targeted pseudouridylation
Dominic Czekay, Ute Kothe

460 Role of N6-methyladenosine in the metabolism of circular RNAs
Gaia Di Timoteo, Dario Dattilo, Ivano Legnini, Mariangela Morlando, Francesca Rossi, Irene Bozzoni

461 p.p1 {margin: 0.0px 0.0px 0.0px 0.0px; font: 12.0px Times} mRNA methylation complex components and methylation outcomes in a model plant system.
Alexander Bridgen, Cameron Grundy, Samuel Chak, Doha Albalawi, Mi Zhang, Kamil Ruzicka, Yka Helariutta, Zsuzsanna Bodi, Gordon Simpson, Rupert Fray

462 The human Pus10 produces pseudouridine 54 in select tRNAs where its recognition sequence contains a modified residue
Manisha Deogharia, Shaoni Mukhopadhyay, Archi Joardar, Ramesh Gupta

463 Withdrawn

464 Novel analogues of N3-CMC with enhanced stability for analysis of pseudouridine in cellular RNA
Maral Halladjian, Yuri Ermolovich, Tomasz Wróbel, Emil Märcher-Rørsted, Fredrik Björkling, Henrik Nielsen

465 A simple screening system for inhibitors of m6A-regulatory enzymes
Miki Imanishi, Akiyo Suda, Shiroh Futaki

466 Protein-mediated enrichment and characterization of 5′ end of RNA polymerase III transcripts
Jingmin Jin, Bo Yan, Nan Dai, Laurence Ettwiller, Ivan Correa Jr, Erbay Yigit, Larry McReynolds

467 Increased endosomal microautophagy suppresses CNS defects caused by loss of ADAR RNA editing in *Drosophila*
Anzer Khan, Simona paro, Leeanne McGurk, Nagraj Sambrani, Marion C. Hogg, James Brindle, Giuseppa Pennetta, Mary A. O'Connell, Liam P. Keegan

468 HBV hijacks TENT4 to stabilize its RNA via mixed tailing
Dongwan Kim, Jinah Yeo, Young-suk Lee, Soo-jin Jung, V. Narry Kim

469 Surveying the landscape of tRNA modifications by combined tRNA sequencing and RNA mass spectrometry
Satoshi Kimura, Peter Dedon, Matthew Waldor

470 A chemical toolbox to study NAD-capped RNAs
Agnieszka Mlynarska-Cieslak, Anais Depaix, Ewa Grudzien-Nogalska, Pawel Sikorski, Marcin Warminski, Mike Kiledjian, Jacek Jemielity, Joanna Kowalska

471 RiboMeth-seq analysis of diffuse large B-cell lymphoma cell lines and patient samples
Nicolai Krogh, Fazila Asmar, Christophe C@me, Kirsten Grønbæk, Henrik Nielsen

472 Global reprogramming of poly-(A) tail metabolism in macrophage immune response
Yeonui Kwak, Hojoong Kwak

473 Chemical synthesis of oligoribonucleotides comprising the trypanosomic cap-4
Josef Leiter, Andrea Rentmeister, Ronald Micura

474 Profiling snoRNA Essentiality in Lung Cancer via a Targeted CRISPR Knockout Screen
Cole J.T. Lewis, Goshu Zakusilo, Wendy V. Gilbert

475 A high resolution A-to-I editing map in the mouse identifies editing events controlled by pre-mRNA splicing
Konstantin Licht, Utkarsh Kapoor, Fabian Amman, Ernesto Picardi, David Martin, Prajakta Bajad, Michael Jantsch

476 Detection of 5-carboxylcytosine in RNA
Janina Licyte, Egle Godliauskaite, Audra Ruksenaite, Viktoras Masevicius, Saulius Klimasauskas, Edita Kriukiene

477 Modulation of Serotonin 2C Receptor RNA Editing by Alterations in Energy Balance
Turnee Malik, Ronald Emeson

478 Theoretical pKa Calculations of Modified Purine Nucleobases using Explicit Waters and a Polarizable Continuum Model
Alan Mlotkowski, Evan Jones, Sebastien Hebert, H. Bernhard Schlegel, John SantaLucia Jr., Christine Chow

479 Role of the RNA modification 5-methylcytosine on ribozyme catalytic activity
Dylan Mooijman, Anne Ephrussi

480 Non-canonical poly(A) polymerase TENT5C regulates immunoglobulins expression and B cells differentiation
Aleksandra Bilska, Monika Kusio-Kobialka, Pawel Krawczyk, Olga Gewartowska, Bartosz Tarkowski, Kamil Kobylecki, Ewa Borsuk, Jakub Gruchota, Andrzej Dziembowski, Seweryn Mroczek

481 Effects of A-to-I editing on RNA structure, dynamics and molecular recognition
Christoph Müller-Hermes, Michael Sattler

482 ADAR1 regulates early T cell development via MDA5-dependent and -independent pathways
Taisuke Nakahama, Tuangtong Vogpipatana, Yuki Kato, Yukio Kawahara

483 Bacterial ribosomal RNA modification - functional orphans
Margus Leppik, Ermo Leuska, Triin Truu, Birgit Marjak, Aivar Liiv, Jaanus Remme

484 Loss of 5-methylcytosine (m5C) alters the biogenesis of non-coding Vault-derived small RNAs to coordinate human epidermal differentiation
Abdulrahim sajini, Michaela Frye, Gracjan Michlewski, Nila Roy Choudhury, Rebecca Wagner, Susanne Bornelöv, Tommaso Selmi, Sabine Dietmann

485 Multispecies conservation of tRNA modification mediated oxidative stress responses
Ulrike Abendroth, Pavlína Gregorová, Peter Sarin

486 Hierarchical modification of tRNA by TrmA, TrmB, and TruB
Sarah Schultz, Ute Kothe

487 Withdrawn

488 Translation and stability studies of mRNAs with enzymatically modified 3' ends
Miroslaw Smietanski, Dominika Strzelecka, Pawel Sikorski, Olga Perzanowska, Joanna Kowalska, Jacek Jemielity

489 mRNA cap 2'-*O*-ribose methylation regulates translation required for reward learning in *Drosophila*
Irmgard Haussmann, Yanying Wu, Nathan Archer, Mohanakarthik Nallasivan, Zsuzsana Bodi, Daniel Hebenstreit, Scott Waddell, Rupert Fray, Matthias Soller

490 NAD-RNA and its function in virus-infected cells
Barbora Svojanovska, Hana Macickova Cahova

491 Alkylation Repair And Demethylation Of RNA By The ALKBH3/ASCC Complex
Roopa Thapar, Andrew Arvai, Nima Mosammaparast, John Tainer

492 Phylogenetic reconstruction of human RNA methyltransferases
Georgia Tsagkogeorga, Keith Neil, Beth Thomas, Namshik Han, Oliver Rausch, Hendrik Weisser

493 Regulation of HIV-1 gene expression by post-transcriptional acetylation of cytidine in viral RNA transcripts
Kevin Tsai, Shalini Oberdoerffer, Bryan Cullen

494 Widespread Adenosine-to-Inosine editing of vascular microRNAs alters target gene selection and function during ischemia and angiogenesis
Reginal van der Kwast, Eveline Goossens, Karin Simons, Paul Quax, Anne Yael Nossent

495 Elucidation of ADAR1 functions in the Immune Response by using interactome data
Dragana Vukic, Leena Yadav, Markku Varjosalo, Liam Keegan, Mary O'Connell

496 Revealing where ribosomal RNA gets oxidized via Mass Spectrometry-generated maps
Jessica Willi, Peter Lobue, Patrick A. Limbach, Norbert Polacek

497 Identification and Characterization of Pseudouridine Synthases
Sebastian Gruenberg, Nan Dai, Brett Robb, Ivan Correa, Erbay Yigit

Poster Topic: RNA Structure and Folding

Abstracts 498 – 532

498 From retrotransposon genome structure to function: Probing the structure of Ty1 genomic RNA in distinct biological states using SHAPE
Angelika Andrzejewska, Malgorzata Zawadzka, Julita Gumna, David J. Garfinkel, Katarzyna Pachulska-Wieczorek

499 Nucleobase carbonyl groups are poor Mg^{2+} binders but excellent monovalent ion binders — Simple stereochemical rules stress the presence of K^+ at the rRNA decoding center
Filip Leonarski, Luigi D'Ascenzo, Pascal Auffinger

500 Investigating the structure, conformational landscape, and inhibition of the MALAT1 ENE triplex
Abeer Ageeli, Michael Yonkunas, Kayleigh McGovern-Gooch, Nathan Baird

501 The role of conformational dynamics in miRNA targeting
Lorenzo Baronti, Emilie Steiner, Parisa Ebrahimi, Sarah Friebe Sandoz, Ileana Guzzetti, Judith Schlagnitweit, Carolina Fontana, Luis Silva, Alan A. Chen, Katja Petzold

502 Bridging Solution Experiments and Molecular Simulations to Characterize RNA Folding Dynamics
Mattia Bernetti, Kathleen B. Hall, Giovanni Bussi

503 Sugar puckering resolves kinetic heterogeneity of exon recognition by a group II intron
Fabio D. Steffen, Mokrane Khier, Danny Kowerko, Roland K.O. Sigel, Richard Börner

504 On the role of metal cations in RNA stability and folding: The interplay of binding affinity and kinetics
Sergio Cruz-León, Nadine Schwierz

505 A structure-function analysis of FLT-3 RNA seeking therapeutic targets
Aaron D'Souza, Dhivya Jayaraman, Julia Kenyon, Andrew Lever

506 RNA Structural Characterization by Small Angle X-ray Scattering
Lixin Fan, Yun-Xing Wang

507 Visualizing the long non-coding subgenomic flavivirus RNAs in solution
Yupeng Zhang, Yikan Zhang, Xianyang Fang

508 Retrovirus-like transposons genome packaging: Gag function and structural determinants of Ty1 RNA dimerization.
Julita Gumna, Katarzyna J. Purzycka, Hyo Won Ahn, David J. Garfinkel, Katarzyna Pachulska-Wieczorek

509 Exploration of sharply-bent states in RNA two-way junctions using disulfide conformational capture
Ian Hamilton, Rick Russell

510 The large Stokes shift fluorogen-activating RNA aptamer *Chili*
Christian Steinmetzger, Irene Bessi, Claudia Höbartner

511 Structural insights into synthetic ligands targeting A-A pairs in disease-related CAG RNA repeats
Sanjukta Mukherjee, Leszek Blaszczyk, Wojciech Rypniewski, Christoph Falschlunger, Ronald Micura, Asako Murata, Chikara Dohno, Kazuhiko Nakatani, Agnieszka Kiliszek

512 Secondary structure of genomic RNA of influenza A virus as an aid in antisense oligonucleotide design.
Elzbieta Lenartowicz, Paula Michalak, Marta Soszynska-Jozwiak, Ewa Biala, Walter N. Moss, Julita Kesy, Barbara Szutkowska, Ryszard Kierzek, Elzbieta Kierzek

513 m^6A slows down RNA hybridization kinetics from site-specific measurements of NMR relaxation dispersion
Bei Liu, Honglue Shi, Christoph Kreutz, Hashim Al-Hashimi

514 Structure-based mutational analysis of the twister-sister ribozyme and implications on the cleavage mechanism
Elisabeth Mairhofer, Luqian Zheng, Aiming Ren, Ronald Micura

515 Nucleotide level resolution of RNA folding interactions within peptide based complex coacervates
McCauley Meyer, Saehyun Choi, Fatma Pir Cakmak, Christine Keating, Philip Bevilacqua

516 Structural investigation of RNA-cleaving deoxyribozymes
Mateusz Mieczkowski, Claudia Höbartner, Vladimir Pena

517 Investigating molecular determinants for *htrA* RNA thermometer behavior
Anna Chee, Edric Choi, Madeline Su, Yow Yong Tan, Rachel Mitton-Fry

518 RNA base pairing complexity in living cells visualized by correlated chemical probing
Anthony Mustoe, Nicole Lama, Patrick Irving, Samuel Olson, Kevin Weeks

519 Preselection for Monomers Increases the Phenotypic Potential of Random Pools: Application to *In Vitro* Selection of Nanoluciferase Aptamers
Mona Oumais, Fabio Chizzolini, Luiz F.M. Passalacqua, Andrej Luptak

520 Crystallization of human and chimp CPEB3 ribozymes
Anna Ilaria Przytula-Mally, Silke Johannsen, Eva Freisinger, Vincent Olieric, Roland K.O. Sigel

521 Network families of recurrent long-range interactions modules in RNA structures reveal embedded hierarchies
Vladimir Reinharz, Antoine Soulé, Eric Westhof, Jér@me Waldispühl, Alain Denise

522 Structural and functional analysis of SINEUPs antisense non-coding RNA for mRNA-specific translation up-regulation
Harshita Sharma, Hazuki Takahashi, Silvia Zucchelli, Stefano Gustincich, Piero Carninci

523 Structural variants of RNA Mango enable split aptamer designs for RNA-RNA interactions
Nicholas Shults, Steven Burden

524 Withdrawn

525 Let it go: Kinetics of exon unbinding in group II introns by single-molecule FRET and molecular dynamics
Fabio D. Steffen, Mokrane Khier, Danny Kowerko, Richard Börner, Roland K.O. Sigel

526 Single-molecule FRET imaging of riboswitch transcriptional complexes
Adrien Chauvier, Patrick St-Pierre, Jean-François Nadon, Mélanie Geffroy, Cibran Perez-Gonzalez, Sébastien H. Eschbach, Maxime Simoneau-Roy, Anne-Marie Lamontagne, Eric Masse, Juan Carlos Penedo, Daniel Lafontaine

527 A Workflow to Determine RNA Structures Using Cryo-EM
Zhaoming Su, Kalli Kappel, Kaiming Zhang, Grigore Pintilie, Rhiju Das, Wah Chiu

528 RNA structure maps across mammalian cellular compartments
Lei Sun, Furqan Fazal, Pan Li, Howard Chang, Qiangfeng Zhang

529 Exact distance measurement and spatial sampling in RNA
Beat Vögeli, Parker Nichols, Morkos Henen, Alexandra Born, Dean Strotz, Peter Güntert, Sandro Bottaro, Michele Parinnello, Kresten Lindorff-Larsen

530 Highly accurate RNA structure prediction adapted to design challenges in synthetic biology
Andrew Watkins, Do Soon Kim, Michael Jewett, Rhiju Das

531 GTP-binding RNA-aptamers display surprisingly intricate tertiary structures
Antje Wolter, Angela Pianu, Johannes Kremser, Elisabeth Strebitzer, Robbin Schnieders, Boris Fürtig, Kreutz Christoph, Duchardt-Ferner Elke, Wöhnert Jens

532 Defining the architecture of the influenza RNA genome by RNA-RNA-seq
Liqing Ye, Uddhav Ambi, Redmond Smyth

Poster Topic: RNA Synthetic Biology and Systems Biology

Abstracts 533 – 537

533 Design artificial protein factors to specifically edit RNAs
Wenjian Han, Meng Li, Wendi Huang, Tong Wei, Zefeng Wang

534 Splicing buffers suboptimal codon usage in human cells
Christine Mordstein, Rosina Savisaar, Robert Young, Jeanne Bazile, Lana Talmane, Juliet Luft, Michael Liss, Martin Taylor, Laurence Hurst, Grzegorz Kudla

535 The Search for RNA Aptamers that Selectively Bind Monosaccharides
Javier Portillo, Ronald Breaker

536 Extensive self-targeting by CRISPR-Cas systems in the plant pathogen *Xanthomonas albilineans*
Franziska Wimmer, Chase Beisel

537 Outcomes of genome targeting in bacteria vary between CRISPR nucleases and strains
Atul K. Singh, Elena Vialetto, Daphne Collias, Chase L. Beisel

Poster Topic: RNA Transport and Localization

Abstracts 538 – 551

538 A Deep Learning Assisted Screen for RNA Localization
Ethan Baker, Carl G. de Boer, Aviv Regev, Eliezer Calo

539 Identification of a localized RNA-based mechanism that regulates cell-matrix interactions
Liana Boraas, Charles Vejnar, Antonio Giraldez, Stefania Nicoli

540 mRNAs and Mitochondria co-transport to distant neuronal sites
Bar Cohen, Adi Golani, Topaz Altman, Eran Perlson, Yoav Arava

541 Cellular localisation of mRNA during angiogenesis
Guilherme Costa, Josh Bradbury, Shane Herbert

542 Novel bioconjugation system for mRNA therapy
Stefano Croce, Sascha Serdjukow, Birgit Graf, Jessica Sobotta, Thomas Frischmuth

543 Atlas of Subcellular RNA Localization Revealed by APEX‐seq
Furqan Fazal, Shuo Han, Kevin Parker, Pornchai Kaewsapsak, Jin Xu, Alistair Boettiger, Alice Ting, Howard Chang

544 Functional study of *Plasmodium* tRNA import machinery
Marta Cela, José Jaramillo, Delphine Kapps, Anne Théobalt-Dietrich, Renaud Geslain, Magali Frugier

545 A change in cell fixation protocol provides a clue on transferred mRNA biology
Gal Haimovich, Jeffrey Gerst

546 The function of the DEAD-box ATPase Dbp5 in the regulation of mRNA export
Stephanie Heinrich, Maria Hondele, Désirée Marchand, Carina Patrizia Derrer, Pascal Vallotton, Azra Lari, David Grunwald, Ben Montpetit, Karsten Weis

547 The mammalian nuclear basket and protein Tpr prevent nuclear exit of transcripts with retention elements but do not monitor the occurrence of splice sites
Haruki Iino, Philip Gunkel, Carina Elsner, Jens Bohne, Volker Cordes

548 A region overlapping exon 1-exon 2 junction facilitates nuclear export of singly-spliced MMTV env mRNA
Benedikt Hagen, Stanislav Indik

549 Understanding the role of RNA binding for Trim2 and Trim3 function
Inga Loedige, Marina Chekulaeva, Uwe Ohler

550 Development and Analysis of Mouse Models to Study the Function of the Constitutive Transport Element (CTE) in the NXF1 Gene
Magdalena Rutkowska, Aleksandra Stamper, Maria Michalak, Wenhao Xu, Jing Huang, David Rekosh, Marie-Louise Hammarskjold

551 Quantifying gene expression noise via single mRNA imaging in living cells
Franka Voigt, Johannes Wilbertz, Ivana Horvathova, Yinxiu Zhan, Gregory Roth, Jan Eglinger, Jeffrey A. Chao

Poster Topic: RNA Turnover

Abstracts 552 – 597

552 Activity studies of poxvirus decapping enzyme D9.
M. Bednarczyk, R. Kasprzyk, J.K. Peters, M. Magda, J. Kowalska, J.D. Gross, J. Jemielity

553 No-Go Decay substrates are uniquely cleaved upstream of the collided disome, resulting in 5'-OH ends phosphorylated prior to 5'-3' decay
Albertas Navickas, Sébastien Chamois, Rénette Saint-Fort, Julien Henri, Claire Torchet, Lionel Benard

554 Open reading frame controls mRNA stability in human pathogen and protist *Giardia lamblia*
Danielle Y. Bilodeau, Aaron R. Jex, Staffan G. Svärd, Olivia S. Rissland

555 mRNA decapping by an ApaH-like phosphatase in trypanosomes
Paula Andrea Castañeda-Londoño, Susanne kramer

556 NRDE2 negatively regulates exosome functions by inhibiting MTR4 recruitment and exosome interaction
Jianshu Wang, Jiyun Chen, Guifen Wu, Hongling Zhang, Xian Du, Suli Chen, Ke Wang, Shuaixin Gao, Shouxiang Zhang, Bin Kuai, Peng Zhao, Lantian Wang, Guohui Li, Catherine C.L. Wong, Yu Zhou, Jinsong Li, Caihong Yun, Hong Cheng

557 The dual role of mRNA decay factors in transcription and mRNA decay
Shiladitya Chattopadhyay, Jonathan Fischer, Sterling Churchman, Oren Barkai, Mordechai Choder

558 Biophysical studies of human NUDT16 stability and substrate specificity towards cap analogs
Magdalena Chrabaszczewska, Elzbieta Bojarska, Janusz Stepinski, Maciej Lukaszewicz, Jan Antosiewicz, Edward Darzynkiewicz

559 Identification of Tissue-specific RNA Exosome Cofactors as an Approach to Define Disease Mechanism
Julia de Amorim, Sara Leung, Ambro van Hoof, Anita Corbett

560 Substrate specificity of the TRAMP and exosome complexes *in vivo*
Clementine Delan-Forino, Christos Spanos, Juri Rapsilber, David Tollervey

561 The function of DIS3L2 in the mechanism of nonsense-mediated mRNA decay
Juan F. García-Moreno, Paulo J. da Costa, Juliane Menezes, Margarida Saramago, Sandra C. Viegas, Cecília Arraiano, Luísa Romão

562 The nuclear poly(A) RNA binding protein, Nab2, cooperates with the RNA exosome to alter the transcriptome for survival under stress
Katherine Mills-Lujan, Ayan Banerjee, Sara Leung, Milo Fasken, Anita Corbett

563 Effect of His-tag sequence location in Decapping Scavenger enzymes on their structure and hydrolytic activity towards dinucleotide cap analogs.
Aleksandra Ferenc-Mrozek, Maciej Lukaszewicz, Elzbieta Bojarska, Jan Antosiewicz, Edward Darzynkiewicz

564 The PERK mRNA: an unexpected non-NMD-target
Rafael Fernandes, Luísa Romão

565 Mineral nutrient-dependent translational regulation in *Arabidopsis thaliana*
Toru Fujiwara, Mayuki Tanaka, Izumi Aibara, Naoyuki Sotta, Yui Yamashita, Seidai Takamatsu, Yukako Chiba, Kyoko Miwa, Satoshi Naito

566 Nonsense-mediated mRNA decay regulates the exit from pluripotency
Elena Galimberti, Robert Sehlke, Veronika Herzog, Stefan Ameres, Andreas Beyer, Martin Leeb

567 Network of the cofactor complexes interacting with the human nuclear RNA exosome
Piotr Gerlach, Jan M. Schuller, Fabien Bonneau, Jér@me Basquin, Judith Ebert, Peter Reichelt, Sebastian Falk, Elena Conti

568 Oxidized cofactor NAD^+ promotes RNA 3' end decay
Xinfu Jiao, Jun Yang, Ewa Grudzien-Nogalska, Jacek Jemielity, Joanna Kowalska, Megerditch Kiledjian

569 LC-MS/MS-based analysis of mRNA 5' cap metabolism in cytoplasmic extracts of mammalian cells
Dominika Strzelecka, Karolina Kaczmarska, Paweł Sikorski, Jacek Jemielity, Joanna Kowalska

570 A hepatic post-transcriptional control of whole body metabolic homeostasis through CCR4-NOT deadenylase and FGF21
Sakie Katsumura, Tadashi Yamamoto, Nahum Sonenberg, Masahiro Morita

571 Regulation of metal homeastasis mRNAs by the *Saccharomyces cerevisiae* Nonsense-mediated mRNA decay pathway
Angelo Wong, Bessie Kebaara

572 Comparative RNA interactome capture as a tool to study RNP complex topology
Cornelia Kilchert, Svenja Hester, Shabaz Mohammed, Alfredo Castello, Lidia Vasiljeva

573 Quantitative proteomics revealed MTRES as a factor preventing stress-induced transcription deficiency in human mitochondria.
Anna V Kotrys, Dominik Cysewski, Sylwia D Czarnomska, Zbigniew Pietras, Lukasz S Borowski, Andrzej Dziembowski, Roman J Szczesny

574 Development of inhibitors and chemical tools to study cNIIIB nucleotidase
Dorota Kubacka, Mateusz Kozarski, Marek Baranowski, Dominika Strzelecka, Jacek Jemielity, Joanna Kowalska

575 YTHDF2-HRSP12-RNase P/MRP coordinate to destabilize m^{6}A-containing mRNAs
Yujin Lee, Ok Hyun Park, Hongseok Ha, Sung Ho Boo, Do Hoon Kwon, Hyun Kyu Song, Yoon Ki Kim

576 Disease-linked Amino Acid Substitutions in the EXOSC2 Cap Subunit Alter RNA Exosome Interactions
Sara W. Leung, Milo B. Fasken, Anita H. Corbett

577 Insight into the role of Nudt15 nudix hydrolase in RNA decay.
Anna Stefaniuk, Janusz Stepinski, Aleksandra Ferenc-Mrozek, Edward Darzynkiewicz, Maciej Lukaszewicz

578 Dynamic Profiling of Human RNA Decapping Protein hDcp2 Reveals the Regulation Of RNA Stability by a *cis*-Regulatory Motif
Yang Luo, Jeremy Schofield, Matthew Simon, Sarah Slavoff

579 rRNA degradation in Escherichia coli under the various nutritional conditions
Yasushi Maki, Shou Furuike, Hideji Yoshida, Hirotada Mori

580 Contribution of mRNA 3' UTRs in Substrate Recognition by the Nonsense-Mediated mRNA Decay Pathway
Savannah Mills, Kristian Baker

581 The RNA helicase UPF1 remodels histone mRNPs to facilitate 3'-5' decay of histone mRNA.
Vidhyadhar Nandana, Constanze Wild, Sutapa Chakrabarti

582 Strategies for studying the roles of NMD factors in human induced pluripotent stem cells
Sofia Nasif, Lena Grollmus, Oliver Mühlemann

583 Processive mode of action and substrate recognition are intertwined processes during the hydrolytic cycle of poly(A)-specific ribonuclease (PARN)
Mikael Nissbeck, Niklas Henriksson, Per Nilsson, Jens Berndtsson, Gergana Popova, Måns Ehrenberg, Anders Virtanen

584 Post-transcriptional regulation of maternal mRNAs by NMD factor, Upf1
Omar Omar, Arwa Abdelhamid, Emily Makowicz, Diana Bratu

585 Structural studies of the yeast mitochondrial RNA degradosome
Michal Razew, Zbigniew Warkocki, Michal Taube, Adam Kolondra, Mariusz Czarnocki-Cieciura, Elzbieta Nowak, Karolina Labedzka-Dmoch, Aleksandra Kawińska, Jakub Piatkowski, Pawel Golik, Maciej Kozak, Andrzej Dziembowski, Marcin Nowotny

586 Cell-to-cell variability in Nonsense-mediated mRNA decay
Hanae Sato, Robert Singer

587 Withdrawn

588 Hidden networks: probing the non-canonical functions of Arabidopsis SMG7
Neha Shukla, Claudio Capitao, Karel Riha

589 A macromolecular microRNA turnover complex from *Caenorhabditis elegans*
Mohini Singh, Pradipta Kundu, Saibal Chatterjee

590 Regnase-1 and Roquin - partners in an evolutionarily conserved RNA decay mechanism
Daria Sobanska, Bogna Juskowiak, Anca Neagu, Katarzyna Pachulska-Wieczorek, Katarzyna J Purzycka, Julita Gumna, Rafal Ciosk

591 Assessing in vivo consequences of disease-linked RNA exosome mutations using a budding yeast model.
Maria Sterrett, Liz Enyenihi, Derrick Morton, Sara Leung, Laurie Hess, Jillian Losh, Sergine Brutus, Brittany Avin, Jennifer Potter-Birriel, Taylor Craig, Jillian Vaught, Katherine Mills-Lujan, Ambro van Hoof, Anita Corbett, Milo Fasken

592 Exploring how the caspase-mediated cleavage of HuR alters its regulation of apoptosis
Bakhita Meqbel, Kholoud Ashour, Imed-Eddine Gallouzi, Christopher von Roretz

593 The Achilles heel of LINEs. A newly discovered regulatory mechanism of human mobile genetic elements.
Zbigniew Warkocki, Pawel Krawczyk, Dorota Adamska, Jose L. Garcia-Perez, Andrzej Dziembowski

594 The role of MCPIP2 in the human neuronal model cell line - SH-SY5Y.
Mateusz Wawro, Jakub Kochan, Karolina Wawro, Weronika Sowinska, Aleksandra Solecka, Przemek Krawczyk, Aneta Kasza

595 Down-regulation of different ribonuclease affect gene expression of multiple biological pathways and indicate functional diversity of ribonuclease
Hanjiang Fu, Kaiwen Shi, Kexin Ding, Yiran Zhong, Xiaofei Zheng

596 The effect of the Perlman syndrome DIS3L2 exoribonuclease in the regulation of gene expression
Dagmar Zigackova, Nandan Varadarajan, Tomas Skalicky, Zuzana Feketova, Stepanka Vanacova

597 ARF1 SBS - STAU1 complex structure uncovers target recognition by staufen1.
Deepak Kumar Yadav, Dagmar Zigackova, Tomáš Klumpler, Maria Zlobina, Monika Kubickova, Stepanka Vanacova, Peter Lukavsky

Poster Topic: RNA-Protein Interactions

Abstracts 598 – 638

598 Human cytomegalovirus harnesses multiple RNA-processing machineries for efficient viral replication
Jaewon Song, Sungwon Lee, Hyewon Kim, Kwangseog Ahn

599 Non-coding RNAs directly interact with key pluripotent transcription factors in human embryonic stem cells
Amir Argoetti, Shlomi Dvir, Yael Mandel-Gutfreund

600 Regulatory cascades underlying kidney development; A WT1 perspective
R Selvi Bharathavikru, Joan Slight, Stuart Aitken, Alex von Kriegsheim, Nicholas Hastie

601 Stress-induced changes to the RNA binding proteome in yeast
Stefan Bresson, Vadim Shchepachev, Christos Spanos, David Tollervey

602 RNA secondary structures bound in vivo by Staufen 2 across mammalian brain development
Anob Chakrabarti, Flora Lee, Sandra Fernández-Moya, Janina Ehses, Michael Kiebler, Nicholas Luscombe, Jernej Ule

603 Metaserver for the docking of RNA-protein complexes
Nithin Chandran, Sunandan Mukherjee, Pietro Boccaletto, Michal J. Boniecki, Janusz M. Bujnicki

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

605 U7 snRNP, FUS and hnRNP UL1 interact with each other out of the S phase of the cell cycle in human cells
Marlena Cichocka, Agata Stepien, Katarzyna Dorota Raczynska

606 iCRAC combined with NMR structures reveal the motif bound by Npl3 in vivo and an unexpected separation of the involvement of each RRM in different functions of Npl3
Antoine Cléry, Ahmed Moursy, Stefan Gerhardy, Katharina Hembach, Sanjana Rao, Mark D. Robinson, Vikram Panse, Frédéric Allain

607 Characterization of the novel RNA endonuclease, EndoU
Kristen Dias, Xueyan Xu, Fedor Karginov

608 The solution structure of Dead End bound to AU-rich RNA reveals an unprecedented mode of tandem RRM-RNA recognition required for mRNA repression
Malgorzata M. Duszczyk, Harry Wischnewski, Tamara Kazeeva, Fionna E. Loughlin, Christine von Schroetter, Ugo Pradère, Jonathan Hall, Constance Ciaudo, Frédéric H.-T. Allain

609 Activation of hypoxia-inducible factor signaling modulates the RNA protein interactome in Caenorhabditis elegans
R. Esmaillie, M. Ignarski, T. Krüger, D. Ahmad, K. Bohl, B. Schermer, T. Benzing, R.-U. Müller, F. Fabretti

610 Deciphering the function of FUS-dependent sdRNAs in human cells.
Kishor Gawade, P. Plewka, M.W. Szczesniak, K.D. Raczynska

611 Protein RsmC shows RNA annealing and chaperone activity during ribosome biogenesis
Keshav GC, Prabesh Gyawali, Hamza Balci, Sanjaya Abeysirigunawardena

612 Identification of RNA-RBP networks
Milan Gerovac, Jörg Vogel

613 Cap structure modifications influence stability of IFIT/mRNA complexes and determine specificity of IFIT1 and IFIT5.
Renata Grzela, Beata Miedziak, Anna Dobiezynska, Zbigniew M. Darzynkiewicz, Julia Bartkowska, Joanna Miszkiewicz, Michal Tyras, Joanna Trylska, Jacek Jemielity, Edward Darzynkiewicz

614 Structural insights into MLE-UNR-roX2 complex assembly during early steps of *Drosophila* dosage compensation
Nele Merret Hollmann, Pravin Kumar Ankush Jagtap, Tanit Guitart, Marisa Mueller, Pawel Masiewicz, Soeren von Buelow, Panagiotis Kastritis, Lara Jayne Sweetapple, Martin Beck, Peter B. Becker, Fatima Gebauer, Janosch Hennig

615 The RNA-Protein Interactome of Differentiated Kidney Tubular Epithelial Cells
Michael Ignarski, Constantin Rill, Rainer Kaiser, Madlen Kaldirim, Rene Neuhaus, Reza Esmaillie, Xinping Li, Corinna Klein, Katrin Bohl, Maike Petersen, Christian Frese, Martin Höhne, Illian Atanassov, Markus Rinschen, Katja Höpker, Bernhard Schermer, Thomas Benzing, Christoph Dieterich, Francesca Fabretti, Roman-Ulrich Müller

616 NMR solution structure of a *Legionella* ProQ-homolog and its interaction with RNA
Carina Immer, Carolin Hacker, Jens Wöhnert

617 NMR analysis reveals a structural and binding mechanism for SRSF3 and hnRNP A1 with hsa-pri-mir-30-1 RNA
Alisha N. Jones, Javier F. Caceres, Michael Sattler

618 The nuclear mRNA-binding protein Tho1 regulates TREX occupancy and nuclear mRNP assembly
Birte Keil, Dominik Meinel, Christoph Wierschem, Katja Sträßer

619 RNA-binding activity of Npl3 is required for mRNP assembly and nuclear mRNP export
Philipp Keil, Alexander Wulf, Nitin Kachariya, Michael Sattler, Henning Urlaub, Katja Straesser

620 Ferritin Iron Response Element (IRE)-mRNA Binding to Eukaryotic Translation Initiation Factor (eIF)4F
Mateen A Khan, Elizabeth C Theil, Dixie J Goss

621 Dynamic Recognition at Interfaces of Protein/RNA Complexes: What Can Computations Tell Us?
Miroslav Krepl, Jiri Sponer

622 Unconventional protein-RNA interactions in human embryonic stem cells
Amir Argoetti, Shlomi Dvir, Yael Mandel-Gutfreund

623 YB-1, an abundant core mRNA-binding protein, has the capacity to form an RNA nucleoprotein filament
DA Kretov, MJ Clément, G Lambert, D Durand, DN Lyabin, G Bollot, C Bauvais, A Samsonova, K Budkina, RC Maroun, L Hamon, A Bouhss, E Lescop, F Toma, PA Curmi, A Maucuer, L Ovchinnikov, D Pastré

624 RNA Binding Activity of Signal Transduction Proteins
Sabrina Mennour, Isabelle Girault, Hélène Malka-Mahieu, Virginie Quidville, Caroline Robert, Stéphan Vagner

625 Is there room for RNA during early meiosis?
Ana Rita Neves, Cristina Piñeiro, Simone Köhler

626 Application of RNA-binding proteins in mRNA capture method to analyze the transcriptome
Martyna Nowacka, Matylda Izert, Natalia Karolak, Maria Klimecka, Michal Koper, Maria W. Górna

627 Investigating the role of L18 in pre-5S rRNA maturation: structure and function.
Stephanie Oerum, Marjorie Catala, Clément Dégut, Pierre Barraud, Ciaran Condon, Carine Tisné

628 P23 acts as functional RBP in the macrophage inflammation response
Sebastian de Vries, Vladimir Benes, Alisandra Denton, Isabel Naarmann-de Vries, Yannic Schumacher, Reymond Sutandy, Björn Usadel, Kathi Zarnack, Julian König, Dirk Ostareck, Antje Ostareck-Lederer

629 Multidomain convergence of Argonaute during RISC assembly correlates with the formation of internal water clusters
Mi Seul Park, Araya-Secchi Raul, James A Brackbill, Hong-Duc Phan, Audrey C Kehling, Ekram W Abd El-Wahab, Daniel M Dayeh, Marcos Sotomayor, Kotaro Nakanishi

630 Global identification of RNA-binding proteins in *Arabidopsis* using mRNA interactome capture.
Marlene Reichel, Yalin Liao, Mandy Rettel, Chikako Ragan, Rastislav Horos, Matthias Hentze, Thomas Preiss, Anthony Millar

631 Role of m^{6}A in modulating hnRNP A2/B1-mediated RNA metabolism to promote breast cancer progression
Justin Roberts, Allison Porman, Eric Nguyen, Aaron Johnson

632 Structural characterization of Mei-P26 protein - a central regulator of RNA biosynthesis during stem cell fate decision
Anna Salerno-Kochan, Monika Gaik, Pritha Ghosh, Andreas Horn, Nithin Chandran, Rebecca Moschall, Daniela Strauss, Janusz M. Bujnicki, Jan Medenbach, Sebastian Glatt

633 RBPs TRAPPed – insights and challenges in characterizing the RNA associated proteome
Vadim Shchepachev, Stefan Bresson, Christos Spanos, Lutz Fischer, Juri Rappsilber, David Tollervey

634 RNA binding landscape and function of a molecular slave oscillator in circadian timekeeping and abiotic stress response
Tino Köster, Martin Lewinski, Katja Meyer, Dorothee Staiger

635 An improved RIP-seq technology to capture both direct and indirect protein-RNA interaction sites
Keijing Wu, Yaqiang Xue, Yue Sun, Shuang Chen, Yaxun Wei, Dong Chen, Yi Zhang

636 Regulation of the p53 expression profile by hnRNP K under stress conditions
Agata Swiatkowska, Mariola Dutkiewicz, Piotr Machtel, Martyna Kabacinska, Paulina Zydowicz-Machtel, Jerzy Ciesiolka

637 Identification of new *cis*-regulatory elements based on structural conservation
Johannes Braun, Sandra Fischer, Zhenjiang Z. Xu, Hongying Sun, Dalia H. Ghoneim, Anna T. Gimbel, Uwe Plessmann, Henning Urlaub, David H. Mathews, Julia E. Weigand

638 Structural insights into binding of N7-modified cap analogs by human eIF4E isoforms.
Joanna Zuberek, Mateusz Dobrowolski, Kaja Fac-Dabrowska, Janusz Stepinski, Dorota Kubacka, Jacek Jemielity, Edward Darzynkiewicz

Poster Topic: RNAs in Disease

Abstracts 639 – 685

639 Stabilization of dystrophin mRNA as a novel therapy for treating DMD.
Adi Amar-Schwartz, Yuval Cohen, Talya Dor, Rotem Karni

640 miRNAs in arthritis pathogenesis and therapy
Hiroshi Asahara, Yoshiaki Ito, Sho Mokuda, Ryo Nakamichi, Ryota Kurimoto, Tomoki Chiba

641 Towards understanding the role of aberrant splicing in prostate cancer disease progression
Anke Augspach, Salvatore Piscuoglio, Andre Kahles, Gunnar Rätsch, Mark Rubin

642 The onco-ribosome: A frontier of ribosome heterogeneity
Artem Babaian, Dylan Girodat, Hans-Joachim Wieden, Gregg Morin, Dixie Mager

643 Alternative mRNA polyadenylation modulates influenza A virus induced innate immune response
Valter Bergant, Philipp Hubel, Niklas de Andrade Krätzig, Lucie Dehau, Thomas Engleitner, Daniel Schnepf, Arno Meiler, Gregor Rot, Ronald Dijkman, Percy A. Knolle, Georg Kochs, Roland Rad, Peter Stäheli, Andreas Pichlmair

644 Splicing defect of the profilin gene alters actin dynamics in a *S. pombe* SMN mutant
Marie Antoine, Kristin Patrick, Johann Soret, Florence Rage, Pauline Duc, Rebecca Cacciottolo, Kelly Nissen, Ruben Cauchi, Newan Krogan, Christine Guthrie, Vladimir Sirotkin, Yannick Gachet, Remy Bordonne

645 CRISPR/Cas9 knockin modeling of a short tandem repeat disease
Jodi Bubenik, Curtis Nutter, Ruan Oliveira, Franjo Ivankovic, Lukasz Sznajder, Belinda Pinto, John Cleary, James Thomas, Emily Marr, Maury Swanson

646 Mechanism of Alcohol mediated tissue injury in Chronic Pancreatitis.
Bishnupriya Chhatriya, Piyali Sarkar Sarkar, Debasish Nath, Sukanta Ray, Kshaunish Das, Saroj K Mohapatra, Srikanta Goswami

647 Retinitis pigmentosa linked mutation of Prpf8
Zuzana Cvackova, Michaela Efenberkova, David Stanek

648 Splice-site changing oligonucleotides targeting the serotonin 2C may reduce spasticity after spinal cord injury
Samantha Danyi, Stefan Stamm, Alexander Rabchevsky, Peter Spielman, Samir Patel, David Cox

649 Molecular dissection of the cancer microRNA miR-888 cluster associated with aggressive prostate disease.
Tsuyoshi Hasegawa, Zachary Cadieux, Megan Golliher, Julius Nyalwidhe, Aurora Esquela-Kerscher

650 Effect of ALS-linked FUS mutations on U7snRNP, processing of histone mRNA's and genome stability
Ankur Gadgil, Agata Stepien, Agnieszka Walczak, Katarzyna Dorota Raczynska

651 Poly(A)-specific ribonuclease (*parn*) knockout zebrafish variants showed variable telomere lengths.
Sethu Madhava Rao Gunja, Varshni Rajgopal, Johan Ledin, Anders Virtanen

652 Improving fusion gene diagnosis in cancer using targeted RNA sequencing
Erin Heyer, Ira Deveson, Danson Wooi, David Thomas, Tim Mercer, James Blackburn

653 Transcriptomic studies provide insights into the tumor suppressive role of miR-146a-5p in non-small cell lung cancer (NSCLC) cells
Joseph Iacona, Nicholas Monteleone, Alexander Lemenze, Ashley Cornett, Carol Lutz

654 Repeat-associated non-AUG translation at CAG repeats in the ATXN3 gene context
Magdalena Jazurek-Ciesiolka, Alicja Komur, Martyna Urbanek-Trzeciak, Wlodzimierz Krzyzosiak, Agnieszka Fiszer

655 *RBFOX2 acts as a tumor suppressor in metastatic pancreatic cancer*
Amina Jbara, Chani Stossel, Miri Danan-Gotthold, Maria Raitses-Gurevich, Erez Y. Levanon, Talia Golan, Rotem Karni

656 Evidence for disrupted snRNP biogenesis links FUS-ALS to SMA
Daniel Jutzi, Sébastien Campagne, Ralf Schmidt, Jonas Mechtersheimer, Stefan Reber, Mihaela Zavolan, Frédéric Allain, Marc-David Ruepp

657 Specific inhibition of splicing factor activity by decoy RNA oligonucleotides
Polina Denichenko, Maxim Mogilevsky, Antoine Cléry, Thomas Welte, Jakob Biran, Odelia Shimshon, Georgina D. Barnabas, Miri Danan-Gotthold, Saran Kumar, Eylon Yavin, Erez Y. Levanon, Frédéric H. Allain, Tamar Geiger, Gil Levkowitz, Rotem Karni

658 Molecular pathogenesis of Diamond-Blackfan anemia and drug screening for the disease using zebrafish as a model animal
Tamayo Uechi, Maki Yoshihama, Yukari Nakajima, Mariko Nagatomo, Yutaka Suzuki, Etsuro Ito, Naoya Kenmochi

659 Plasma miR-320a as a liquid biopsy suppresses non-small cell lung cancer progression through AKT3 and its associated pathways
Akanksha Khandelwal, Rajeev Kumar Seam, Manish Gupta, Manjit Kaur Rana, Aklank Jain

660 A Potential Role of Extended Simple Sequence Repeats in Competing Endogenous RNA Crosstalk
Edyta Koscianska, Tomasz Witkos, Wlodzimierz Krzyzosiak, Agnieszka Fiszer

661 Splice modulators license C9orf72 ALS repeat RNA into nuclear export and RAN translation
Maartje J Luteijn, Hicham Mahboubi, Dominic Trojer, Cornelia Handl, Nicolas Pizzato, Martin Pfeifer, Daniela Gabriel, Elisa Giorgetti, Carole Manneville, Isabelle P. M. Garnier, Matthias Müller, Fanning Zeng, Kathrin Buntin, Roger Markwalder, Harald Schröder, Jan Weiler, Dora Khar, Jürg Hunziker, Mark Nash, Nicole Meisner-Kober

662 The impact of HAX-1 protein on regulation of transcripts involved in pro-inflammatory response of cancer cells.
Ewelina Macech-Klicka, Elzbieta Sarnowska, Nataliia Rusetska, Alicja Chrzan, Marcin Ligaj, Michal Szymanski, Tomasz Demkow, Ewa Grzybowska, Janusz A. Siedlecki

663 Disruption of IntS13 Interaction with Integrator Cleavage Module Contributes to Ciliopathy Disease
Lauren Mascibroda, Mohammad Shboul, Kai-Lieh Huang, Nathan Elrod, Todd Albrecht, William Russell, Bruno Reversade, Eric Wagner

664 Translational re-programming by the unfolded protein response drives resistance to anti-folates
Stefan Reich, Chi Nguyen, Canan Has, Sascha Steltgens, Himanshu Soni, Christiane Knobbe-Thomsen, Björn Tews, Grischa Tödt, Robert Ahrends, Jan Medenbach

665 Identifying RNA binding proteins relevant for melanoma progression
Neus Mestre-Farràs, Santiago Guerrero, Nadine Bley, Ezequiel Rivero, Aino Jarvelin, Alfredo Castello, Stefan Huttelmaier, Fátima Gebauer

666 miR-708-5p Suppresses Lung Cancer Cell Growth and Resistance Through Targeting of the Arachidonic Acid Signaling Pathway
Nicholas Monteleone, Joseph Iacona, Carol Lutz

667 Exploiting *Drosophila* to Examine RNA Exosome-linked Disease
Derrick Morton, Binta Jalloh, Maria Sterrett, Lily Kim, Thalia Le, Christorpher Rounds, Sara Leung, Milo Fasken, Kenneth Moberg, Anita Corbett

668 Molecular consequences of a U12 snRNA mutation causing cerebellar ataxia
Antto Norppa, Mikko Frilander

669 A novel zebrafish model system provides insight into the pathology of U8 snoRNA variants associated with Leukoencephalopathy with Calcifications and Cysts
Andrew Badrock, Carolina Uggenti, Siobhan Crilly, Paul Kasher, Yanick Crow, Raymond O'Keefe

670 Muscleblind mitigates FUS toxicity by modulating stress granule dynamics and restoring SMN levels
Ian Casci, Karthik Krishnamurthy, Sukhleen Kour, Vadreenath Tripathy, Nandini Ramesh, Eric Anderson, Lara Marrone, Jared Sterneckert, Amanda Gleixner, Christopher Donnelly, Marc-David Ruepp, Emanuela Zuccaro, Maria Pennuto, Piera Pasinelli, Udai Bhan Pandey

671 Small non-coding RNA transcriptome signatures of chondrocyte ageing
Mandy Peffers, Yongxiang Fang, Tim Welting

672 MBNL splicing regulators contribute to microtranscriptome composition in myotonic dystrophy
Agnieszka Piasecka, Michal Sekrecki, Michal Szczesniak, Arkadiusz Kajdasz, Krzysztof Sobczak

673 Protein interactors of antisense $(C_4G_2)_n$ RNA repeats from *C9orf72* repeat expansion mutation
Mirjana Malnar, Boris Rogelj

674 Brain organoids to study circRNA function in the pathogenesis of brain diseases
Agnieszka Rybak-Wolf, Nikos Karaiskos, Petar Glazar, Tancredi M. Pentimalli, Gizem Inak, Pawel Lisowski, Alessandro Prigione, Nikolaus Rajewsky

675 Identification and characterization of the biological roles of long non-coding RNAs in early stage breast cancer, ductal carcinoma in situ (DCIS)
Julia Samson, Sudipto Das, Conleth Murphy, Aoife O'Shea, Marie B Casey, Triona Hayes, Louise O'Callaghan, Xavier Miro, Dominic Rose, Kellie Dean

676 Inducing Protein Synthesis Errors through mutant tRNAs promotes tumor growth in mice
Mafalda Santos, Patrícia Pereira, A.Sofia Varanda, Joana Carvalho, Patrícia Oliveira, Fábio Trindade, Marta T.Pinto, Nuno Mendes, Fátima Carneiro, Rui Vitorino, Manuel Santos, Carla Oliveira

677 Noncoding RNAs in *M. tuberculosis*-infected macrophages
Ousman Tamgue, Sugata Roy, Lorna Gcanga, Sebastian Schmeier, Mumin Ozturk, Tanvir Alam, Bogumil Kaczkowski, Erik Arner, Lauren Whitehead, Shandre Pillay, Raygaana Jacobs, Malika Davids, Yulia Medvedeva, Keertan Dheda, Suraj Parihar, Piero Carninci, Vladimir Bajic, Frank Brombacher, Reto Guler, Harukazu Suzuki

678 EFTUD2/Snu114 missense variants associated with Mandibulofacial dysostosis Guion-Almeida type disrupt both protein function and splicing of EFTUD2/Snu114 pre-mRNA
Huw Thomas, Katherine Wood, Weronika Buczek, Kathryn Hentges, William Newman, Raymond O'Keefe

679 An ALS-causing mutation in FUS leads to deficits in translation in vivo
Agnieszka M. Ule, Nicol Birsa, Jack Humphrey, Seth Jarvis, Francesca Mattedi, Anny Devoy, Gabriella Viero, Giampietro Schiavo, Vincent Plagnol, Elizabeth Fisher, Pietro Fratta

680 Single-color spatially multiplexed RNA in situ hybridization to reveal tumor heterogeneity
Lena Voith von Voithenberg, Deborah Huber, Govind V. Kaigala

681 Targeting RNA Pol II Pausing to Alter Human Gene Expression
Jason Watts, Joshua Burdick, Vivian Cheung

682 Do redox-regulated microRNAs play a role in age-related muscle wasting?
Katarzyna Whysall, Ana Soriano, Claire Stewart, Brian McDonagh

683 Disease modelling of core pre-mRNA splicing factor haploinsufficiency
Katherine A. Wood, Charles F. Rowlands, Huw B. Thomas, Weronika A. Buczek, Tracy A. Briggs, Simon J. Hubbard, Kathryn Hentges, William G. Newman, Raymond T. O'Keefe

684 Deciphering translation dysregulation mechanisms across multiple models of ALS
Erik Lehmkuhl, Alyssa Coyne, Ileana Lorenzini, Eric Alsop, Kendall Jensen, Rita Sattler, Daniela Zarnescu

685 Transcriptome analysis based on RNA sequencing in understanding pediatric myelodysplastic syndrome
Lorena Zubovic, Silvano Piazza, Paolo Macchi

Poster Topic: RNPs: Biogenesis, Structure & Function

Abstracts 686 – 698

686 Molecular and functional characterization of snoRNP protein partners
Laeya Baldini, Anne Robert, Yoann Abel, Céline Verheggen, Athanase Visvikis, Bruno Charpentier, Stéphane Labialle

687 Biophysical characterization of FUS liquid droplets.
Leonidas Emmanouilidis, Laura Esteban Hofer, Maria Theresia Hondele, Karsten Weis, Gunnar Jeschke, Frédéric Allain

688 The enigmatic role of the exon junction complex component CASC3
Jennifer V. Gerbracht, Volker Boehm, Thiago Britto-Borges, Sebastian Kallabis, Christian K. Frese, Janine Altmüller, Christoph Dieterich, Niels H. Gehring

689 SART3 binding to post-splicing snRNPs suggests a molecular mechanism for spliceosome recycling
Klara Klimesova, David Stanek

690 Investigation the role in mRNA export of the actin binding protein, Moesin
Ildikó Kristó, Csaba Bajusz, Péter Borkúti, Zoltán Kovács, Aladár Pettkó-Szandtner, Péter Vilmos

691 Characterization of new assembly intermediates during box H/ACA snoRNP biogenesis.
Florence Schlotter, Franck Vandermoere, Stéphane Labialle, Céline Verheggen, Séverine Massenet

692 Single mRNP analysis by super-resolution microscopy and fluorescence correlation spectroscopy reveals that small mRNP granules represent mRNA singletons
Àngels Mateu-Regué, Jan Christiansen, Frederik Otzen Bagger, Christian Hellriegel, Finn Cilius Nielsen

693 Structural dynamics in the essential human telomerase three-way junction
Christina Palka, Nick Forino, Rhiju Das, Michael Stone

694 Modeling the oscillating cellular stress response to hepatitis C virus infection
Philipp Klein, Stefan Kallenberger, Alessia Ruggieri

695 Functional interactions of the metalloprotein YbeY, involved in ribosomal metabolism, with the putative metal efflux protein YbeX
Ismail Sarigül, Tanel Tenson, Ülo Maiväli

696 Ataxin-2 RNA granules: assembly and clearance of intracellular foci implicated in ALS and SCA2
Amanjot Singh, Baskar Bakthavachalu, Rashi Singh, Devam Purohit, Devasena Thiagarajan, Sai Shruti, Joern Huelsmeier, Jens Hillebrand, Arnas Petrauskas, Roy Parker, K VijayRaghavan, Mani Ramaswami

697 TSSC4 is a novel U5 snRNP-specific protein important for snRNP biogenesis
Jitka Vojáčková, Celine Verheggen, Edouard Bertrand, David Staněk

698 Disease-causing point mutations in FMRP's RNA binding domains affect RNP-granule stability in vivo.
Emily Starke, Scott Barbee

Poster Topic: Splicing Mechanism

Abstracts 699 – 713 + 870

699 A Complete Structural View of Pre-mRNA Splicing by the Spliceosome
Ruixue Wan, Rui Bai, Chuangye Yan, Yigong Shi

700 Combinatorial control of *Spo11* alternative splicing by modulation of RNA polymerase II dynamics and splicing factor recruitment during meiosis
Eleonora Cesari, Maria Loiarro, Chiara Naro, Livia Pellegrini, Vittoria Pagliarini, Donatella Farini, Pamela Bielli, Claudio Sette

701 Development of a *S. cerevisiae*-based system to study the mechanism of backsplicing
Maja K Cieplak-Rotowska, Magda Konarska

702 Cwc15 stabilizes the spliceosomal catalytic interactions
Marcin Magnus, Katarzyna Eysmont, Maja Cieplak-Rotowska, Magda Konarska

703 Combinatorial recognition of 3' splice sites by UHM splicing factors
Manel Tari, Valerie Manceau, Jean de Matha Salone, Asaki Kobayashi, David Pastre, Alexandre Maucuer

704 Withdrawn

705 Exonic UACUAC motif affects splicing of defective introns at early steps of spliceosome assembly
Jadwiga Meissner, Magda Konarska

706 A missense DDX38 mutation linked with retinitis pigmentosa
Mina Obuca, David Stanek

707 DDD01004659- a novel modulator of pre-mRNA Splicing-->
Andrea Pawellek, Andrew Woodland, David Gray, Angus Lamond

708 Role for pre-mRNA secondary structure in efficient splicing
Ramya Rangan, Rhiju Das

709 Regulation of miR-17-92 cluster splicing by hnRNP A1
Maria Gabriela Santos, Patricia Coltri, Guilherme Henrique Silva

710 The tRNA Splicing Endonuclease of *Haloferax volcanii* - Transcription Repression with CRISPRi and Potential Substrates
Thandi S. Schwarz, Charles J. Daniels, Sarah J. Berkemer, Peter F. Stadler, Anita Marchfelder

711 Probing the functional role of *S. pombe* splicing factor SpPrp16, an ATP dependent RNA helicase in splice-site recognition
Amit Kumar Sharma, Drisya Vijaykumari, Rakesh Kumar, Pushpinder Singh Bawa, Subhashini Srinivasan, Usha Vijayraghavan

712 Fluctuations in inositol polyphosphates alter substrate selection for the first step of splicing
Elizabeth Steidle, Varun Gupta, Charles Query

713 Two conserved A-C mismatches in yeast U6 snRNA's internal stem-loop have multiple and opposing functions
Allyson D. Yake, Sean T. Hinds, Erik D. Anderson, Janina Görnemann, Ian S. Norden, Christine N. Kujak, Benjamin A. Rackovan, David A. Brow

Poster Topic: Splicing Regulation including Alternative Splicing

Abstracts 714 – 759 + 871

714 Disruption of autoregulatory feedback mechanisms of the minor spliceosome core di-snRNP proteins 65K and 48K, leads to cell cycle defects.
Maureen Akinyi, Mariia Shcherbii, Mikko Frilander

715 Alternative splicing of Gephyrin, a code for the diversity of inhibitory synapses
Raphael Dos Reis, Judith Carrasco Sala, Etienne Kornobis, Alyssa Pereira, Celine Jahannault-Talignani, Christian Muchardt, Hans Maric, Fabrice Ango, Eric Allemand

716 Regulation of splicing integrin α6 during development and differentiation
Mohammed Alshehri, David Elliott

717 A novel approach to detect the influence of RNA-binding proteins on pre-mRNA processing
Maciej Bak, Andreas Gruber, Mihaela Zavolan

718 KDM3A regulates alternative splicing of cell-cycle genes
Mai Baker, Mercedes Bentata, Gillian Kay, Eden Engal, Yuval Nevo, Ahmad Siam, Sara Dahan, Maayan Salton

719 Missense variations in *RBM10* cause a new syndrome with intellectual disability
Jeanne Mari Vejen Bang, Jens Michael Hertz, Klaus Brusgaard, Christina Fagerberg, Brage Storstein Andresen

720 Changes of phosphorylation drive alternative splicing modulation by mild heat shock in human cells
Christelle Aigueperse, Valentin Vautrot, Luisa Vigevani, Panagiotis Papasaikas, Nathalie Nicot, Tony Kaoma, Pierre de la Grange, Laurent Vallar, Juan Valcarcel, Christiane Branlant, Isabelle Behm-Ansmant

721 Exon Junction Complexes Suppress Spurious Splice Sites to Safeguard Transcriptome Integrity
Volker Boehm, Thiago Britto-Borges, Anna-Lena Steckelberg, Kusum K. Singh, Jennifer V. Gerbracht, Elif Gueney, Lorea Blazquez, Janine Altmüller, Christoph Dieterich, Niels H. Gehring

722 *Arabidopsis thaliana* alternative splicing regulation trought light induced changes in transcriptional elongation
Grzegorz Brzyzek, Micaela A. Godoy Herz, M. Guillermina Kubaczka, Lucas Servi, Michal Krzyszton, Craig Simpson, John Brown, Szymon Swiezewski, Ezequiel Petrillo, Alberto R. Kornblihtt

723 Structural basis for 5'-splice site splicing correction induced by a small molecule
Sebastien Campagne, Sarah Boigner, Simon Ruedisser, Ahmed Moursy, Laurent Gillioz, Anna Knoerlin, Johnatan Hall, Hasane Ratni, Antoine Clery, Frederic Allain

724 Development of Splice Switching Small Molecules as Inducers of Apoptosis
Emma Campbell, Olivia Rutherford, Andrea Taladriz-Sender, Carika Weldon, Cyril Dominquez, Laurence H. Hurley, Ian C. Eperon, Glenn A. Burley

725 SRSF2 regulation of MDM2 reveals splicing as a therapeutic vulnerability of the p53 pathway
Matias Montes, Daniel Comiskey, Safiya Khurshid, Dawn Chandler

726 SUVA: splicing site usage variation analysis from RNA-seq data reveals the functionality of low-frequency cancer-associated alternative splicing
Lei Liu, Ran Sun, Chao Cheng, Xiaoguang Yang, Luguo Sun, Xianling Cong, Yi Zhang, Yuxin Li

727 Alternative RNA splicing controls cancer cell plasticity
Chonghui Cheng

728 The patterns of *Alu* exonisation in human cancers
Mariela Cortés-López, Laura Schulz, Julian König

729 RBM4 is essential for brain development via its role in alternative splicing regulation
Dhananjaya D, Woan-Yuh Tarn

730 Pre-mRNA bound U2 snRNP contains novel protein components
Andrey Damianov, Jeffrey Huang, William Barshop, James Wohlschlegel, Douglas Black

731 Characterization of the protein-RNA network leading to the different splicing outcome of SMN1 and SMN2 exon 7 using CLIR-MS/MS
Laurent Gillioz, Antoine Cléry, Alexander Leitner, Ruedi Aebersold, Frédéric Allain

732 Weak exons are hotspots for disruptive splicing perturbations
David Glidden, Luke Buerer, William Fairbrother

733 SmD3-b splicing factor modulates plant immunity response
Anna Golisz, Michal Krzyszton, Monika Stepien, Jakub Dolata, Justyna Piotrowska, Joanna Kufel

734 Formation of cryptic last exons by the splicing factor SFPQ
Pat Gordon, Corinne Houart

735 Regulation of tandem acceptor splice site usage in the context of proximate exons
Pavla Hujová, Lucie Grodecká, Premysl Soucek, Tomáš Freiberger

736 Genome-wide siRNA screening revealed an interplay between the exosome and first steps of pre-spliceosome assembly
Anna Hojka-Osinska, Aleksander Chlebowski, Roman Szczesny, Andrzej Dziembowski

737 Allotopic expression of *nad7* rescues the Arabidopsis *slow growth3* mutant
Wei-Yu Hsieh, Sang-Chu Lin, Jo-Chien Liao, Chiung-Yun Chang, Ming-Hsiun Hsieh

738 When alternative splicing meets Wnt signaling
Jiancheng Yu, Ni Li, Huairui Yuan, Sirui Zhang, Hong Zhu, Zefeng Wang, Qun Qin, Jingyi Hui

739 Dynamic effects on co-transcriptional splicing regulation and transcriptional changes induced by fast depletion of Ptbp1 in mES cells
Camilla Iannone, Fursham M. Hamid, Takayuki Nojima, Nicholas J. Proudfoot, Eugene V. Makeyev

740 Npl3-mediated alterations of nuclear RNA export affect splicing fidelity
Agata Jaskulska, Magda Konarska

741 SRRM3 regulates a functional program of microexons in endocrine pancreas
Jonàs Juan-Mateu, Simon Bajew, Fanny Rubio-Moscardo, Miguel A. Valverde, Manuel Irimia, Juan Valcárcel

742 Obesity-related alternative splicing in adipose tissue
Dorota Kaminska, Marcus Alvarez, Elina Nikkola, Arthur Ko, Chelsea K. Raulerson, Karen L. Mohlke, Markku Laakso, Päivi Pajukanta, Jussi Pihlajamäki

743 LNAs in the sequencing space
Lars Jønson, Lukasz Kielpinski, Jonas Vikeså, Mads Aaboe Jensen, Peter Hagedorn

744 The Kreb's cycle enzyme fumarase regulates pre-mRNA splicing through ubiquitin-like protein Hub1
Kiran Kumar Kolathur, Shravan Kumar Mishra

745 Genome-Wide Kinetic Analysis of pre-mRNA Processing in *C. elegans*
Eichi Watabe, Hidehito Kuroyanagi

746 The U2AF1 splicing factor controls cell-fate determination in a dose-dependent manner via transcription regulation
Abdelhamid Mahdi Laaref, Yacine Bareche, Laurent Manchon, Laure Lapasset, Jamal Tazi

747 Dual roles of XAB2 in the regulation of gene expression
Shuai Hou, Dajun Qu, Yue Li, Baohui Zhu, Dapeng Liang, Xinyue Wei, Wei Tang, Qian Zhang, Weijie Wang, Qi Wang, Sikandar Azam, Misbah Khan, Liye Zhang, Haixin Lei

748 Withdrawn

749 Withdrawn

750 Identification of RBPMS as a smooth muscle master splicing regulator via proximity of its gene with super-enhancers
Erick Nakagaki-Silva, Clare Gooding, Miriam Llorian, Aishwarya Jacob, Frederick Richards, Adrian Buckroyd, Chris Smith

751 Comparative transcriptomics analysis reveals a conserved alternative splicing program during primate neurodifferentiation
Andrew Wallace, Julia Philipp, Jeremy Sanford

752 Regulated Splicing in a Dramatically Reduced Splicing System
Viktor Slat, Martha Stark, Stephen Rader

753 The role of the U12-dependent spliceosome in cellular differentiation.
Mariia V Shcherbii, Maureen V Akinyi, Mikko J Frilander

754 Alternative Splicing Modulation and Growth Inhibition of Colorectal Cancer Cells by a Novel Class of Compounds
Muhammad Sohail, Lulzim Shkreta, Johanne Toutant, Jean-Philippe Babeu, François Boudreau, David S. Grierson, Benoit Chabot

755 SAM68 interaction with U1snRNP regulates alternative splicing
Suryasree Subramania, Laurence M. Gagné, Sébastien Campagne, Victoire Fort, Julia O'Sullivan, Karel Mocaer, Miki Feldmüller, Jean-Yves Masson, Frédéric H.T. Allain, Samer Hussein, Marc-Étienne Huot

756 Structural basis of alternative splicing regulation by MBNL
Katarzyna Taylor, Lukasz Sznajder, Piotr Cywoniuk, James Thomas, Maurice Swanson, Krzysztof Sobczak

757 SRSF1 modulates PTPMT1 alternative splicing to regulate lung cancer cell radioresistance
Wenjing Zhang, Jinyao Zhao, Yang Wang

758 Biochemical Characterisation of RNA Binding by ZFR – An Essential Zinc Finger Protein Associated with Splicing
Alexander Will, Nazmul Haque, J. Robert Hogg, Atlanta Cook

759 RBM10 functions as a tumor suppressor in lung cancers by mediating splicing of eukaryotic translation initiation factor 4H
Sirui Zhang, Yongbo Wang, Zefeng Wang

Poster Topic: Targeting RNA for Therapy

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760 The role of TCF7L2 alternative splicing in tumor progression and its modulation by splice-switching antisense oligonucleotides (SSOs)
Ariel Bashari, Pushkar Malakar, Regina Golan-Gerstl, Rotem Karni

761 From pathogenesis to therapy of triplet repeat expansion diseases
Adam Ciesiolka, Pawel Joachimiak, Agata Luzna, Emilia Kozlowska, Michal Michalak, Wlodzimierz Krzyzosiak, Agnieszka Fiszer

762 SINEUPs: a functional class of lncRNAs that activates translation as a novel strategy for gene therapy of neurological disorders
Stefano Espinoza, Carlotta Bon, Riccardo Luffarelli, Diego Cotella, Claudio Santoro, Ivano Condo, Piero Carninci, Silvia Zucchelli, Stefano Gustincich

763 Insights into the structural basis of nonspecific binding of RNA by small molecules
Megan Kelly, Honglue Shi, Laura Ganser, Chia-Chieh Chu, Kelly Huynh, Yuze Hou, Hal Bogerd, Bryan Cullen, Hashim Al-Hashimi

764 RNA *trans*-splicing for the treatment of HIV infection
Amanda Buckingham, Sophia Ho, Freja Ekman, Hoi Ping Mok, Carin Ingemarsdotter, Andrew Lever

765 Universal RNAi triggers for specific inhibition of mutant huntingtin, ataxin-3, ataxin-7 and atrophin-1 expression.
Anna Kotowska-Zimmer, Julia Ostrowska, Krzysztof Grochowina, Adam Ciesiolka, Pawel Joachimiak, Agnieszka Fiszer, Marta Olejniczak

766 Transcriptional activation of HIV by structure-switching the 7SK RNA
Samuel Olson, Chase Weidmann, Anthony Mustoe, Nancie Archin, Anne-Marie Turner, David Margolis, Kevin Weeks

767 Identification of RNA binding proteins involved in the acquisition of resistance to chemotherapy treatment in Pancreatic Ductal Adenocarcinoma (PDAC).
Valentina Panzeri, Isabella Manni, Giulia Piaggio, Emanuela Pilozzi, Gabriele Capurso, Claudio Sette

768 Designing and investigating the action mechanism of PNA antimicrobials against *Salmonella enterica* serovar Typhimurium
Kristina Popova, Anant Preet, Verena Herbst, Jörg Vogel

769 Prophylactic and therapeutic potential of NS1 shRNA against influenza infection
Priya Ranjan, Neetu Singh, Jenish Patel, Weiping Cao, Shivaprakash Gangappa, Paras Prasad, Suryaprakash Sambhara, Paul Knight

770 Optimization of cell-penetrating peptide based microRNA-146a delivery for targeting of skin inflammation
Gemma Carreras Badosa, Julia Maslovskaja, Helen Vaher, Egon Urgard, Liina Tserel, Kai Kisand, Piret Arukuusk, Ülo Langel, Margus Pooga, Ana Rebane

771 Structural basis of thioflavin T binding between G-quadruplexes at the homodimer interface of the fluorogenic RNA aptamer Corn
Ljiljana Sjekloca, Adrian Ferre D'Amare

772 High-throughput Identification of Effective Peptide Nucleic Acid Targets through a Synthetic sRNA Library
Sarah Wilkins, Kristina Popova, Jörg Vogel

773 High-temperature transcription generates synthetic RNA with reduced immunogenicity.
Monica Z. Wu, Gregory Rougellis, Haru Asahara, Vladimir Potapov, George Tzertzinis, G. Brett Robb, Jennifer L. Ong, Bijoyita Roy

Poster Topic: Translational Mechanism and Regulation

Abstracts 774 – 817

774 eIF3-mediated ribosome recruitment by histone H4 mRNA during translation initiation
Hassan Hayek, Laure Schaeffer, Aurélie Janvier, Franck Martin, Gilbert Eriani, Christine Allmang

775 *Structure of the human ribosome in the classical PRE-state reveals the role of uS19 in translation fidelity*
Varun Bhaskar, Alexandra Graff-Meyer, Andreas Schenk, Simone Cavadini, Ottilie von Loeffelholz, Kundhavai Natchiar, Caroline Artus-Revel, Hans-Rudolf Hotz, Bruno Klaholz, Jeffrey Chao

776 Thermodynamic determinants of translational frameshifting
Neva Caliskan, Lars Bock, Natalia Korniy, Frank Peske, Marina Rodnina, Helmut Grubmüller

777 hnRMPM is a novel hypoxic IRES trans-acting factor
Tsung-Ming Chen, Ming-Chih Lai, Ying-Fan Chen, Ming-Chang Hong, Tzu-Yung Lin, H. Sunny Sun

778 The X-linked DDX3X RNA helicase dictates translation re-programming and metastasis in melanoma
Maciej Ciesla, Bengt Phung, Adriana Sanna, Nicola Guzzi, Giulia Beneventi, Phuong Cao Thi Ngoc, Martin Lauss, Rita Cabrita, Eugenia Cordero, Ana Bosch, Frida Rosengren, Jari Häkkinen, Klaus Griewank, Annette Paschen, Katja Harbst, Dirk Schadendorf, Kristian Pietras, Cristian Bellodi, Göran Jönsson

779 Translation of the *psbA* is regulated by mRNA secondary structure changes
Piotr Gawronski, Christel Enroth, Lars Scharff

780 Footprints in bacteria - Ribosome profiling combined with RNA-Seq of *Escherichia coli* LF82 and *Enterococcus faecalis* OG1RF in a mixed culture under aerobic and anaerobic growth-conditions
Franziska Giehren, Michaela Kreitmeier, Zeno Sewald, Klaus Neuhaus

781 Genome-wide Survey of Queued Ribosomes
Peixun Han, Yuichi Shichino, Mari Mito, Shungo Adachi, Satoshi Hashimoto, Tsuyoshi Udagawa, Kenji Kohno, Yuichiro Mishima, Toshifumi Inada, Shintaro Iwasaki

782 eIF3, an important factor for histone mRNAs translation
Hassan Hayek, Laure Schaeffer, Aurélie Janvier, Franck Martin, Gilbert Eriani, Christine Allmang

783 Nonsense-mediated mRNA decay factor affects aggresome formation
Hyun Jung Hwang, Yeonkyoung Park, Joori Park, Byungju Kim, Kwon Jeong, Jong-Bong Lee, Yoon Ki Kim

784 Crucial roles of ribosome ubiquitination in quality controls and gene regulations
Toshifumi Inada

785 Ribosomal incorporation of consecutive D- and β-amino acids
Takayuki Katoh, Hiroaki Suga

786 Oxidative stress suppresses the effect of codon choice on the efficiency of protein synthesis.
Lorenzo E Leiva, Sara Elgamal, Omar Orellana, Michael Ibba, Assaf Katz

787 Single molecule and ensemble analysis of 2A protein-mediated frameshifting in EMCV RNA
Anuja Kibe, Matthias Zimmer, Neva Caliskan

788 Mitochondrial translation repression in absence of gravity
Yusuke Kimura, Shintaro Iwasaki

789 Cell permeable inhibitors of cap dependent translation
Natalia Kleczewska, Pawel J. Sikorski, Joanna Kowalska, Jacek Jemielity

790 Multi-omics approaches to study translational regulation in aggressive B-cell lymphomas
Joanna A. Krupka, Jie Gao, Fengyuan Hu, Shamith Samarajiwa, Daniel Hodson

791 DDX3 participates in translational control of inflammation induced by infections and injuries
Ming-Chih Lai, Yu-Chang Ku, Min-Hua Lai, Chen-Chia Lo

792 Study of interaction between frameshift-stimulating mRNA pseudoknots and the ribosome by single-molecule FRET
Yi-Ting Lee, Jin-Der Wen

793 Understanding the role of a dual ribosomal system in zebrafish development
Katrin Friederike Leesch, Susanne Kandolf, Katarina Belacic, Irina Grishkovskaya, David Haselbach, Andrea Pauli

794 Sequential ubiquitination of ribosomal protein uS3 triggers the subunit dissociation leading to degradation of nonfunctional 40S subunit
Sihan Li, Takato Sugiyama, Misaki Kato, Ken Ikeuchi, Atsushi Ichimura, Yoshitaka Matsuo, Toshifumi Inada

795 Single-molecule imaging of mRNA translation during cellular stress
Daniel Mateju, Jeffrey Chao

796 Hypusine-modified eIF5A enhances nonsense-mediated mRNA decay
Michael Mathews, Mainul Hoque

797 Ribosome ubiquitination is required for translational control during the UPR in yeast
Yasuko Matsuki, Yoshitaka Matsuo, Shintaro Iwasaki, Yasushi Saeki, Hideyuki Yoko, Yu Nakano, Tsuyoshi Udagawa, Tohru Yoshihisa, Keiji Tanaka, Nicholas Ingolia, Toshifumi Inada

798 Regulated hnRNP K - rpS19 interaction in erythroid cell maturation
Isabel Naarmann-de Vries, Bodo Moritz, Henning Urlaub, Roberta Senatore, Dierk Niessing, Antje Ostareck-Lederer, Dirk Ostareck

799 Unveiling the molecular mechanism of function of natural anti-cancer drugs that target the eukaryotic 80S ribosome
Simone Pellegrino, Mélanie Meyer, Evidente Antonio, Veronique Mathieu, Alexander Kornienko, Denis LJ Lafontaine, David L Mobley, Christopher D Vanderwal, Scott C Blanchard, Gulnara Yusupova, Marat Yusupov

800 Self-association regulates translation repression activity of eIF4G-binding RGG-motif protein, Scd6
Gopalkrishna Poornima, Mythili Ravishankar, Priyabrata Nag, Sabnam Parbin, Praveen Kumar Verma, Tanweer Hussain, Purusharth I Rajyaguru

801 Translation of the human ABCE1 transcript is regulated by upstream open reading frames
Joana Silva, Luísa Romão

802 Consequences of pathogenic *Secisbp2*missense mutations (R543Q) probed by ribosome profiling
Ulrich Schweizer, Noelia Fradejas Villar, Simon Bohleber, Wenchao Zhao

803 Withdrawn

804 RNA surveillance targets in protein translation therapeutics
Yoni Sheinberger, Aurora Paola Borroni, Iris Alroy

805 5' -phosphorothiolate dinucleotide cap analogues: reagents for messenger RNA modification and potent small-molecular inhibitors of decapping enzymes
Pawel Sikorski, Blazej Wojtczak, Kaja Fac-Dabrowska, Anna Wojtczak, Marcin Warminski, Dorota Kubacka, Elzbieta Nowak, Marcin Nowotny, Joanna Kowalska, Jacek Jemielity

806 ncRNA in mammalian oocyte and early embryo development
Andrej Susor, Daria Aleshkina, Edgar Llano, Rajan Iyyappan

807 Detection and quantification of protein variants with N-terminal extensions containing mitochondrial targeting signals
Michal Swirski, Krzysztof Starecki, Joanna Kufel

808 Noncoding RNA "SINEs" up-regulate protein translation of target protein coding mRNA
Hazuki Takahashi, Naoko Toki, Harshita Sharma, Piero Carninci

809 sxRNA: Modulating mRNA Regulatory Motifs Through the Binding of Structurally Interacting, microRNA
Francis Doyle, Zachary Wurz, Scott Tenenbaum

810 New Insights into the Translational Landscape of the Heat Shock Response
Phaedra R. Theodoridis, J. J. David Ho, Nathan C. Balukoff, Miling Wang, Valentina Rivadeneira, Jonathan R. Krieger, Stephen Lee

811 Withdrawn

812 RiboTRIBE: Using RNA editing to monitor translation
Weijin Xu, Michael Rosbash

813 The 30S subunit searching mechanism in translation initiation
Cheng-Han Yang, Jin-Der Wen

814 Translation Regulation by CITI Sites
Yun Yang, Xiaojuan Fan, Sirui Zhang, Zefeng Wang

815 Antisense oligonucleotides that mimic pseudoknot structures are highly efficient in stimulating -1 ribosomal frameshifting
Chien-Hung Yu, René C.L. Olsthoorn

816 Structures of the antibiotic viomycin bound to the ribosome in rotated state
Lin Zhang, Yinhui Wang, Xiaoxue Zhou, Biyu Ren, Jie Zhou

817 Epistatic Translational Control of mtDNA-Encoded Cytochrome C Oxidase Subunits
Kuanxing Gao, Man Cheng, XIANG-DONG Fu, Xiaorong Zhang

Poster Topic: tRNA: Processing and Function

Abstracts 818 – 849

818 Cytoplasmic pre-tRNA maturation in human cells
Yasutoshi Akiyama, Shawn Lyons, Anaïs Aulas, Takaaki Abe, Paul Anderson, Pavel Ivanov

819 Roles of tRNAs and tRNA-derived small RNAs in Neurological Function and Development
Alex Bagi, Jonathan Howard, Todd Lowe

820 A tRNA half modulates translation as stress response in *Trypanosoma brucei*
Rebecca Brogli, Roger Fricker, Marek Zywicki, Marina Cristodero, André Schneider, Norbert Polacek

821 Significance of multiple, parallel primary tRNA nuclear exporters in budding yeast
Kunal Chatterjee, Shubhra Majumder, Anita K. Hopper

822 Unexpected effect on selenoprotein expression by lack of i^6A modification in $tRNA^{[Ser]Sec}$
Noelia Fradejas Villar, Simon Bohleber, Wenchao Zhao, Ulrich Schweizer

823 Structural and functional insights into multimeric enzymes modifying tRNAs
Nhan van TRAN, Can WANG, Leslie MULLER, Nathalie ULRYCK, Roxane LESTINI, Juliette LETOQUART, Gabrielle BOURGEOIS, Sarah CIANFERANI, Marc GRAILLE

824 Targeting modes of Argonaute-loaded tRNA fragments
Lingyu Guan, Spyros Karaiskos, Andrey Grigoriev

825 Intron removal from $tRNA^{Leu}_{CAA}$ genes in *S. cerevisiae*
Sachiko Hayashi, Yuichi Shichino, Shintaro Iwasaki, Tohru Yoshihisa

826 tRNA introns: turnover mechanisms and possible functions
Alicia Bao, Lauren Peltier, Anita Hopper

827 Diverse mechanisms of translation inhibition by tRNA-derived stress-induced RNAs
Shawn Lyons, Yasutoshi Akiyama, Vivek Advani, Nancy Kedersha, Paul Anderson, Pavel Ivanov

828 A perspective on noncanonical tRNA gene evolution: tRNAs for translation and tRNAs for other functions
Akio Kanai

829 Kti12, a PSTK-like tRNA dependent ATPase essential for tRNA modification by Elongator
Rościsław Krutyhołowa, Alexander Hammermeister, Rene Zabel, Wael Abdel-Fattah, Annekathrin Reinhardt-Tews, Mark Helm, Michael J. R. Stark, Karin D. Breunig, Raffael Schaffrath, Sebastian Glatt

830 Physiological role of queuosine tRNA modification in parasitic protist *Trypanosoma brucei*
Sneha Kulkarni, Helmut Stanzl, Alan Kessler, Eva Hegedusova, Juan D Alfonzo, Zdenek Paris

831 Exploration of Tissue-Specific Expression and Modification Patterns for tRNAs and tRNA-derived Small RNAs
Aidan Manning, Jonathan Howard, Andrew Holmes, Todd Lowe

832 Uncovering how conserved tRNA isopentenyltransferases generate variable subsets of i6A37-modified tRNAs; and a system for mitochondrial targeting and screening of point mutations in the human population for potential pathogenicity.
Abdul Khalique, Sandy Mattijssen, Alexander Haddad, Richard Maraia

833 Overexpression of tRNA^{Gly} affects the solubility of a cyclin, altering growth of *Schizosaccharomyces pombe*
Loreto Arias, Sandra Moreira, Assaf Katz, Omar Orellana

834 Intricate subcellular trafficking of queuosine modified tRNAs in *Trypanosoma brucei*
Eva Hegedusova, Sneha Kulkarni, Alan Kessler, Brandon Burgman, Juan Alfonzo, Zdenek Paris

835 Investigating the role of tRNA-modifying enzymes in human proteostasis
Marisa Pereira, Diana Ribeiro, André Maia, Hugo Osório, Miguel Pinheiro, Miguel Mano, Manuel Santos, Ana Soares

836 A stable tRNA-like molecule is generated from the long noncoding RNA *GUT15* in *Arabidopsis*
Patrycja Plewka, Agnieszka Thompson, Maciej Szymanski, Przemyslaw Nuc, Katarzyna Knop, Agnieszka Rasinska, Aleksandra Bialkowska, Zofia Szweykowska-Kulinska, Wojciech M. Karlowski, Artur Jarmolowski

837 Codon-specific translation reprogramming promotes resistance to targeted therapy
Francesca Rapino, Sylvain Delaunay, Florian Rambow, Zhaoli Zhou, Lars Tharun, Pascal De Tullio, Olga Sin, Kateryna Shostak, Sebastian Schmitz, Jolanda Piepers, Bart Ghesquière, Latifa Karim, Benoit Charloteaux, Marc Vooijs, Sebastian A Leidel, Michel Georges, Rehinard Büttner, Jean-Christophe Marine, Alain Chariot, Pierre Close

838 Organization of transfer RNA genes in plant genomes
Agnieszka Rasinska, Maciej Szymanski, Wojciech Karlowski

839 Modulation of translation efficiency: a new player in dendritic cell function and T cell priming
Marisa Reverendo, Rafael J. Argüello, Christine Polte, Voahirana Camosseto, Nathalie Auphan-Anezin, Zoya Ignatova, Evelina Gatti, Philippe Pierre

840 A large-scale molecular evolutionary analysis uncovers a variety of polynucleotide kinase Clp1 family proteins in the three domains of life
Motofumi Saito, Asako Sato, Shohei Nagata, Satoshi Tamaki, Masaru Tomita, Haruo Suzuki, Akio Kanai

841 An archaeal *in vitro* reconstitution system for pre-tRNA splicing and characterization of a putative RNA-regulating protein PF1614
Ayano Sakai, Motofumi Saito, Asako Sato, Masaru Tomita, Satoshi Tamaki, Akio Kanai

842 Dual pathways of tRNA hydroxylation ensure efficient translation by expanding decoding capability
Yusuke Sakai, Satoshi Kimura, Tsutomu Suzuki

843 Production, Isolation, and Use of Endogenously Modified tRNA-Derived Small RNAs from Mammalian Cells.
Aleksej Drino, Vera Oberbauer, Conor Troger, Eva Janisiw, Matthias Schaefer

844 Oxidative Stress Triggers Selective tRNA Retrograde Transport in Human Cells during the Integrated Stress Response
Hagen Schwenzer, Frank Jühling, Alexander Chu, Laura J. Pallett, Thomas F Baumert, Mala Maini, Ariberto Fassati

845 Species-specific T-box:tRNA-mediated synchronization of cell wall and protein synthesis in staphylococci
Vassiliki Stamatopoulou, Nikoleta Giarimoglou, Shuang Li, Maria Apostolidi, Jinwei Zhang, Constantinos Stathopoulos

846 High-throughput analysis of tRNA expression in Arabidopsis thaliana
Maciej Szymanski, Patrycja Plewka, Przemyslaw Nuc, Agnieszka Rasinska, Artur Jarmolowski, Wojciech Karlowski

847 Computational analysis of tRNA gene clusters in bacterial evolution
Yuka Takahashi, Shohei Nagata, Masahiro C. Miura, Masaru Tomita, Satoshi Tamaki, Akio Kanai

848 Predicting tRNA gene expression using comparative genomics and DNA sequence features
Bryan Thornlow, Joel Armstrong, Russell Corbett-Detig, Todd Lowe

849 Meta-analysis of small RNA sequencing data using missRNA reveals induction of 5'-capped tRNA halves in human cell lines.
Agnieszka Chelkowska, Jan Kosinski, Tomasz Andree, Marianna Plucinska, Marek Zywicki

Poster Topic: Viral RNAs

Abstracts 850 – 865

850 Defining the tick-borne encephalitis virus (TBEV) miRNA interactome in human neurons
Nicolas Fossat, Nkerorema Djodji Damas, Louise Nielsen, Jens Bukh, Agnete Kirkeby, Troels Scheel

851 Highly efficacious antiviral protection of plants by small interfering RNAs identified *in vitro*.
Selma Gago Zachert, Jana Schuck, Claus Weinholdt, Marie Knoblich, Vitantonio Pantaleo, Ivo Grosse, Torsten Gursinsky, Sven-Erik Behrens

852 Withdrawn

853 In-gel SHAPE probing reveals structures of dengue virus UTRs
Kieran Toms, Michelle Law, Xiaoyu Chen, Dhivya Jayaraman, Aaron D'Souza, Andrew Lever, Julia Kenyon

854 The number of 5'terminal guanosines modulates HIV-1 RNA destiny.
Siarhei Kharytonchyk, Jackie M. Esquiaqui, Alice Telesnitsky

855 Nonreplicative RNA recombination between genomic fragments of positive stranded RNA viruses
Alice Mac Kain, Marie-Line Joffret, Francis Delpeyroux, Marco Vignuzzi, Maël Bessaud

856 Movement and accumulation of potato virus Y in infected plants.
Mateusz Mielczarek, Anna Pawlowska, Krzysztof Treder

857 Molecular evolutionary analysis of hepatitis C virus non-structural protein NS5A and interferon sensitivity
Akane Nishigata, Shohei Nagata, Masaru Tomita, Akio Kanai

858 Transcription apparatus and 5' poly(A) mRNA leaders of the yeast cytoplasmic linear plasmids suggest their close relationship to poxviruses
Michal Sykora, Vaclav Vopalensky, Josef Novak, Silvia Mrvova, Libor Krasny, Tomas Masek, Martin Pospisek

859 RNA Export of Unspliced RNA as a Modulator of HIV Replication
Patrick Jackson, Marie-Louise Hammarskjold, David Rekosh

860 Structural Basis for Translation Termination-Reinitiation at Overlapping Open Reading Frames in Viruses
Madeline Sherlock, Jeffrey Kieft

861 Exoribonuclease-Resistant RNAs Exist within both Coding and Noncoding Subgenomic RNA
Anna-Lena Steckelberg, Quentin Vicens, Jeffrey Kieft

862 Structure of segment 8 vRNA influenza virus type A in cell lysates
Barbara Szutkowska, Ewa Biala, Marta Soszynska-Jozwiak, Elzbieta Lenartowicz, Julita Kesy, Pawel Zmora, Klaudia Wieczorek, Ryszard Kierzek, Elzbieta Kierzek

863 A model for the activation of antiviral RNA interference in *Drosophila melanogaster*
Loïc Talide, Simona Paro, Eric Aguiar, Laurent Troxler, Fatima Alghoul, Eric Westhof, Joao Marques, Jean-Luc Imler, Carine Meignin

864 Ion exchange membrane chromatography and gel filtration as a tool to investigate plant RNA viruses
Krzysztof Treder, Anna Pawlowska, Agata Kaczmarek, Mateusz Mielczarek

865 Regulation of RNA virus infection decoded by RNA structurome and interactome studies
Qiangfeng Cliff Zhang, Shaojun Zhang, Pan Li, Yifan Wei, Miao Mei, Lei Tang, Wenze Huang, Lei Sun, Xu Tan, Jianfeng Dai

Late Poster Abstracts

866 Toward 'RNA-Free' CRISPR-Cas9 Through Chemical Modification
Adrian Pater, Sunit K. Jana, Eman A. Ageely, Christopher L. Barkau, Elise Malek-Adamian, Masad J. Damha, and Keith T. Gagnon

867 Category: Biology and Mechanism of Transcription Leveraging the repetitive part of our genome
Stefan Oberlin and Michael T. McManus

868 RNAcentral: a hub of information for non-coding RNA sequences
Blake A. Sweeney, Boris Burkov, Anton I. Petrov, Rob Finn, Alex Bateman, and The RNAcentral Consortium

869 Rfam: the database of 3,000+ non-coding RNA families
Ioanna Kalvari, Joanna Argasinska, Eric Nawrocki, Rob Finn, Alex Bateman, and Anton I. Petrov

870 Cellular hypoxia and Fas pre-mRNA alternative splicing
Arvydas Kanopka, Inga Peciuliene, Gintare Januleviciute, Egle Jakubauskiene, Laurynas Vilys

871 Endogenous genes exhibit different intron dispersal patterns that demonstrate independent post-transcriptional splicing
Allison Cote, Sareh Bayetpour, Arjun Raj

NOTES

1 tRNA Biology in Gene Regulation

Phillip Sharp
Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA, USA

Regulation of gene expression is central to normal development and physiology as well as to many disease processes. RNA as the intermediate between DNA—the source of information—and protein synthesis, is frequently regulated at the stage of synthesis-degradation or the object of regulation by factors such as in modulation of translation. RNA can also be a regulatory factor, for example in the case of small nuclear RNAs, microRNAs, and nuclear non-coding RNAs. The advent of relatively inexpensive and massively parallel DNA sequencing has revealed both the extent of changes in RNA populations as well as in large populations of mostly transient, low-abundance RNAs that seem to be important in gene regulation. A conceptual framework for considering functions of low-abundance nuclear RNAs emerged from the recognition that many membrane-less bodies in cells had the properties of liquids and are likely condensates formed through phase transitions mediated by weak cooperative multivalent interactions. RNA is a major constituent of many condensates in cells and its polymeric nature provides sites for the binding of proteins that frequently have intrinsic disorder domains that can associate through weak cooperative interactions; short tracts of RNA itself can transiently associate. Beyond the stable macro-condensates such as the nucleolus, there are micro-condensates that are more transient in stability, reflecting the variation of gene expression at the level of transcription and RNA splicing. These condensates are important in the activity of super-enhancers and perhaps typical enhancers. Enhancer-associated RNA may be an important element of condensate formation for super-enhancers. In this case, transcription of RNA would further control transcription. It is likely that related processes are critical in RNA splicing through interactions with SR proteins and other RNA binding proteins. These condensates are dynamic in exchange of components within a matter of seconds and transient in stability. This character can be seen in the bursting of transcription with pulses of several polymerases elongating across the gene. Such dynamic and transient properties challenge static models of cell state and force the consideration of more interlocking feedback systems. Results from a system-level characterization of interchangeable embryonic cell states will be discussed from this perspective.

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2 Snapshots of the catalytic spliceosomes

Max Wilkinson[1] [Scaringe Award], Sebastian Fica[1], Wojciech Galej[2], Clément Charenton[1], Kiyoshi Nagai[1]
[1]MRC Laboratory of Molecular Biology, Cambridge, UK; [2]EMBL, Grenoble, France

The spliceosome performs two chemical reactions to remove introns from pre-mRNA. In the first reaction, branching, the branchpoint adenosine attacks the 5'-splice site, forming a branched lariat intermediate. In the second, exon ligation, the 5'-splice site attacks the 3'-splice site, releasing the lariat-intron and forming mature messenger RNA. Electron cryomicroscopy has provided vivid snapshots of these reactions, showing how the snRNAs fold to form the active site, how the splice sites are brought together, and how the spliceosome is remodelled between the two reactions. The structures show how splice sites in the intron are recognised by base pairing to snRNAs, and in the exceptional case of the 3'-splice site by intramolecular pairing to the branched lariat intermediate. This talk will describe these structures, focusing on the yeast P complex, a revised model for the yeast C-complex spliceosome, and new insights into the dynamic interplay between the two steps of splicing.

3 Structures of the Catalytically Activated Yeast Spliceosome Reveal the Mechanism of Branching

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Pre-mRNA splicing is executed by the spliceosome. Structural characterization of the catalytically activated complex (B^*) is pivotal for understanding the branching reaction. In this study, we assembled the B^* complexes on two different pre-mRNAs from *Saccharomyces cerevisiae* and determined the cryo-EM structures of four distinct B^* complexes at overall resolutions of 2.9-3.8 Å. The duplex between U2 snRNA and the branch point sequence (BPS) is discretely away from the 5'-splice site (5'SS) in the three B^* complexes that are devoid of the step I splicing factors Yju2 and Cwc25. Recruitment of Yju2 into the active site brings the U2/BPS duplex into the vicinity of 5'SS, with the BPS nucleophile positioned 4 Å away from the catalytic metal M2. This analysis reveals the functional mechanism of Yju2 and Cwc25 in branching. These structures on different pre-mRNAs reveal substrate-specific conformations of the spliceosome in a major functional state.

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4 Rearrangements within the U6 snRNA core during the transition between the two catalytic steps of splicing

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Although the spliceosome undergoes dynamic changes during the splicing cycle, its RNA catalytic core, composed of the U6 catalytic triplex and the adjacent intramolecular stem-loop (U6-ISL), remains unchanged in all current structures visualized by cryo-EM. Both steps of splicing take place at the same spliceosomal catalytic center. Rearrangements of the catalytic core during the transition between these two steps of splicing are facilitated by an ATPase Prp16, promoting substrate repositioning. Using defective alleles of *prp16* as stage-specific markers, we genetically mapped mutations within the core of yeast U6 snRNA that modulate conformational changes between the two catalytic steps. On the basis of genetic interactions, we propose that not only the catalytic triplex but also U6-ISL are necessary components of the spliceosomal catalytic center. Our analysis indicates that U6-ISL exists in two competing states, changing between default, non-catalytic and transient, catalytic conformations, which differ by a degree of flexibility within the lower ISL segment. Whereas stable interactions in the upper ISL and the catalytic triplex promote catalysis and their disruptions favor exit from the catalytic conformation, destabilization of the lower ISL stem promotes catalysis and its stabilization supports exit from the catalytic conformation.

The proposed mechanism of U6-ISL function appears to be general, as the relative flexibility of the lower U6-ISL stem is conserved across all eukaryotes and similar structural features are found even in U6atac and domain V of group II introns. We propose that the identified U6-ISL mutations affect the relative stabilities of spliceosomal conformations; such genetic manipulations can be used to prepare spliceosomal complexes suitable for visualization of the catalytic structure by cryo-EM.

5 Functional Analysis of Cwc24 ZF-domain in 5′ Splice Site Selection

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The essential splicing factor Cwc24 contains a Zn-finger (ZF) domain required for its function in splicing. Cwc24 binds over the 5' splice site after the spliceosome is activated, and its binding prior to Prp2-mediated spliceosome remodeling is important for proper interactions of U5 and U6 with the 5' splice site sequence and selection of the 5' splice site. We show here that Cwc24 transiently displaces Prp8 to interact with the 5' splice site in formation of functional RNA catalytic core during spliceosome remodeling. The ZF motif is required for specific interactions of Cwc24 with the 5' splice site. Deletion of the ZF domain or mutation in the conserved ZF residues greatly weakened the association of Cwc24 with the spliceosome, and lowered the affinity and specificity for its interaction with the 5' splice site, resulting in aberrant interactions of U5 and U6 with the 5' splice site. Cwc24 ZF mutants also display dominant-negative property, sharing the same consequence of Cwc24 depletion in causing aberrant cleavage at the 5' splice site. Our results suggest a crucial role for the ZF motif of Cwc24 in recognizing the 5' splice site to specify the position for pre-mRNA cleavage at the 5' splice site.

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6 A Distinct Subset of Human Short Introns with Weak Pyrimidine Tract: U2AF Heterodimer Is Replaced by SPF45/RBM17 as General Splicing Factor

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The length distribution of human pre-mRNA introns is very extensive (from shorter than 50 nt through much longer than 1,000,000 nt). To search for a general splicing factor that is specifically involved in human short introns, we screened siRNA library targeting 155 kinds of human nuclear proteins based on splicing activity of a model pre-mRNA with 56-nt intron (intron 7 of the *HNRNPH1* gene).

We identified a known alternative splicing regulator SPF45 (also known as RBM17) as a constitutive splicing factor that is essential to splice out this 56-nt intron. Our whole transcriptome sequence (RNA-Seq) analysis in SPF45 deficient cells revealed that SPF45 is required for the efficient splicing of the vast majority of short introns. We show that U2AF heterodimer cannot recognize the short pyrimidine tract and SPF45 replaces it for interacting with the U2 snRNP. This interaction is mediated through binding between the U2AF-homology motif (UHM) of SPF45 and the UHM-ligand motif (ULM) of SF3b155 component in U2 snRNP.

We conclude that SPF45 is substituted for U2AF heterodimer to splice out a distinct set of human short introns.

7 A human post-catalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation

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During exon ligation, the *S. cerevisiae* spliceosome recognizes the 3´-splice site (3´SS) of precursor mRNA through non-Watson-Crick pairing with the 5´SS and the branch adenosine, in a conformation stabilised by Prp18 and Prp8. Here we present the 3.3 Å cryoEM structure of a human post-catalytic spliceosome just after exon ligation. The 3´SS docks at the active site through conserved RNA interactions in the absence of Prp18. Unexpectedly, the metazoan-specific FAM32A directly bridges the 5´-exon and intron 3´SS of pre-mRNA and promotes exon-ligation, as shown by functional assays. CACTIN, SDE2, and NKAP – factors implicated in alternative splicing – further stabilize the catalytic conformation of the spliceosome during exon ligation. Together these four proteins act as exon ligation factors. Our study reveals how the human spliceosome has co-opted additional proteins to modulate a conserved RNA-based mechanism for 3´-splice site selection and to potentially fine-tune alternative splicing at the exon ligation stage.

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8 CWC27, a new player in EJC assembly by the spliceosome

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The Exon Junction Complex (EJC) tags mRNA exon junctions following intron removal by spliceosomes. It plays a central role in post-transcriptional gene expression control. Organized around the RNA helicase eIF4A3, EJCs serve as platforms for multiple peripheral factors and contribute to alternative splicing, mRNA localization, translation efficiency and mRNA stability control by nonsense-mediated mRNA decay (NMD). At a physiological level, developmental defects and human pathological disorders are associated with altered expression of EJC proteins. To dissect mechanisms regulating EJC deposition during splicing, we isolated the splicing factor CWC27 which function remains unknown. We recently showed that protein-truncating mutations in CWC27 human gene are associated to retinal degeneration, brachydactyly, craniofacial and neurological abnormalities (Xu et al. 2017).

We used RNA-seq to question the impact of CWC27 knock-down on human cell transcriptome. Independently, CWC27 partners were characterized by immunoprecipitation coupled to quantitative mass spectrometry. Both the splicing factor CWC22 and the EJC factor eIF4A3 were found as major partners. CWC22 has previously been shown to escort eIF4A3 to active spliceosome (Barbosa et al. 2012). Protein domains involved in the association of CWC27, CWC22 and eIF4A3 were confirmed by expressing full-length and truncated versions of these three proteins. Interestingly, patients' CWC27 truncations lead to the loss of interactions with both CWC22 and eIF4A3. Using recombinant human proteins we reconstituted a trimeric CWC27/CWC22/eIF4A3 complex and solved its 3D structure by X-ray crystallography. This structure illuminates the early contacts of eIF4A3 with central splicing factors.

Our findings reveal that CWC27 is involved in eIF4A3 recruitment by spliceosome shedding new light on the early stages of the stepwise assembly of EJCs and its potential link to related genetic disorders.

9 CryoEM structure of spliceosomal E complex and biochemical analyses reveal a unified mechanism for intron definition, exon definition, and back-splicing

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The molecular mechanisms of exon definition and back-splicing are fundamental unanswered questions in pre-mRNA splicing. Here we report cryoEM structures of the yeast E complex, providing the first view of the earliest event in the splicing cycle that commits pre-mRNAs to splicing. The E complex structure suggests a unified model for intron definition, exon definition, and back-splicing mediated circRNA biogenesis that are supported by our biochemical analyses. This model should inspire experiments in many other systems to understand the mechanism and regulation of these processes.

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10 Cryo-EM Structures of a Group II Intron Reverse Splicing into DNA

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Group II introns are a class of mobile genetic elements with evolutionary ties to both the eukaryotic spliceosome and mammalian LINE elements. An active group II intron retroelement forms when a highly conserved self-splicing ribozyme binds to an intron-encoded maturase protein. The maturase protein contains several domains, allowing the intron to proliferate within the host cell genome through a copy-and-paste mechanism known as retrotransposition. The first step of retrotransposition begins with reverse splicing of the intron ribozyme into genomic DNA. Using single particle cryo-EM, we have solved two distinct structures at 3.6 Å resolution of a group II intron in the process of invading a double-stranded DNA (dsDNA). These represent the first structures of a retroelement invading a dsDNA. We have visualized the group II intron bound to dsDNA immediately preceding the first step of reverse splicing. We have also captured the state immediately after the first step of strand invasion but before the second step of reverse splicing. When comparing these two states, several key conformational changes are observed that explain the mechanism for substrate shuffling between the first and second steps of reverse splicing. A conserved structure within the maturase protein is essential for modulating these large-scale conformational rearrangements required for swapping the 3′ and 5′ splice site substrates during catalysis. Targeted mutational studies of this RNA-protein contact completely inhibit forward splicing, which suggests this interaction is required for both forward and reverse splicing. These conformational changes are coupled to newly visualized dynamics occurring within the catalytic domain V. The analogous U2/U6 snRNA in the spliceosome likely undergoes similar dynamics during catalysis. The large-scale conformational dynamics observed in the group II intron have strong parallels to those seen in cryo-EM structures of the spliceosome. We hypothesize that these conformational dynamics first evolved in a group II intron ancestor and were conserved in spliceosomal descendants. Therefore, these structures describe a molecular mechanism of DNA integration that supports a retroelement origin for the spliceosome.

11 Self-regulatory network of the spliceosome

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Regulation of pre-mRNA splicing greatly contributes to eukaryotic gene control. The spliceosome 5 snRNPs and over 200 associated proteins carry out intron removal and can display distinct regulatory effects. To systematically explore the functions of core and auxiliary splicing factors, RNA-seq analyses were carried out upon the knockdown of over 300 splicing-related components. First insight emerging from these analyses is that expression and/or splicing of preponderance of the splicing-related factors is affected by the knockdown of least one of the other components of the splicing machinery, with most of the changes leading to the generation of unproductive mRNA isoforms. While cross-modulation of splicing regulatory factors was reported previously, our data and gene ontology analyses reveal an extensive regulatory network involving the components of the core spliceosome. Hubs of this network include early factors like U2 snRNP SF3B1, which is target of anti-tumor drugs and is frequently mutated in cancer, and impacts on the expression/splicing of 60% of the other spliceosomal factors, as well as late-acting components, like recruited at activation of the spliceosome CWC22, which links pre-mRNA splicing with Exon Junction Complex assembly. Remarkably, analysis of The Cancer Genome Atlas (TCGA) data indicate that some of the auto-regulatory circuits operate in various cancer types and can be rewired from those observed in normal tissues. Our data imply that interpretation and prediction of the effects of genetic alterations or other perturbations of the splicing machinery, including those induced by drugs targeting the spliceosome, requires understanding the complexity of the splicing self-regulatory network.

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12 RNA binding to a chromatin modifier: structure and function

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Many DNA-binding proteins (RNA pol II, transcription factors, CTCF and epigenetic modifiers) also bind RNA, but only in a few cases is there a detailed understanding of the role, if any, of RNA binding. For example, Polycomb Repressive Complex 2 (PRC2) binds RNA with high affinity and limited specificity in vitro and in vivo (Wang et al., Mol. Cell 2017), but it has been highly controversial whether these interactions are functionally important. We have identified RNA-binding amino acids on the surface of the EZH2 subunit of PRC2 (Long et al., eLife 2017). We have used this information to engineer a separation-of-function mutant of PRC2 that has normal histone methyltransferase and chromatin-binding capabilities but is defective in RNA binding. We have introduced the mutant into human induced pluripotent stem cells (iPSCs) using CRISPR-Cas9 genome editing, which does not affect expression of pluripotency markers. However, the mutant has a striking defect in iPSC-cardiomyocyte differentiation. Genome-wide and transcriptome-wide experiments reveal the molecular details of the role of PRC2-RNA interaction in stem cell differentiation, showing that this interaction is essential for reinforcing the epigenetic repression of differentiation-related transcription factors.

13 Rhythmic gene expression is controlled by alternative splicing triggering nonsense mediated decay

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Mammals are homoeothermic organisms and it is a common assumption that their core body temperature is constant. However, body temperature oscillates with the time of the day, is altered in diverse pathological conditions and - especially in skin - is influenced by the environmental temperature. We have recently identified a thermometer-like kinase, which alters SR protein phosphorylation and thereby globally controls alternative splicing (AS). Alternative splicing can generate mRNA variants containing premature termination codons, which are then degraded by the nonsense mediated decay (NMD) pathway. Here we investigate if AS coupled to NMD is regulated by temperature and how this mechanism globally regulates temperature dependent gene expression. RNA-seq on temperature entrained primary hepatocytes reveals that temperature-controlled NMD-inducing splicing events are very frequent, as almost 50% of the strongest NMD events respond to temperature. Temperature-controlled AS-NMD is evolutionarily conserved and pervasively found within RNA binding proteins - including most SR proteins. Within SR proteins, generation of the NMD isoform is under autoregulatory control and anti-correlates with gene expression. This results in rhythmic protein expression under circadian body temperature cycles *in vivo*. Crispr-Cas9 mediated deletion of NMD-inducing exons shows that AS-NMD is necessary to generate 24-hour rhythms in SR-protein gene expression. Furthermore, temperature-dependent differences in gene expression are reduced after inhibition of the NMD pathway, supporting a global role of AS-NMD in establishing temperature-dependent gene-expression profiles. This includes diminished temperature-dependent expression of two important cold-induced RNA binding proteins *Rbm3* and *Cirbp*. In *Rbm3* and *Cirbp* we *de novo* identify heat-induced NMD isoforms, which likely account for their temperature-controlled differences in gene expression. As high expression levels of *Cirbp* and *Rbm3* are considered beneficial in different diseases - such as cancer or neurodegeneration - we are currently establishing methods to alter their expression levels by interfering with the NMD inducing splicing isoform.

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14 A high-throughput chemical screen to identify splicing modulators of nSR100/Srrm4-dependent microexons.

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A major challenge in developing effective treatments for complex diseases, such as autism spectrum disorder (ASD), is to identify common underlying mechanisms that can be pharmacologically modulated. Alternative splicing (AS) represents a key layer of gene regulation, yet its function and potential as a therapeutic target in many diseases remains largely unexplored. We recently discovered a large programme of highly conserved, nSR100/SRRM4-regulated neuronal microexons that is frequently disrupted and causally linked to over one-third of analyzed autism cases. Notably, mutant mice with nSR100 deficiency display multiple hallmark features of ASD, such as impaired synaptic transmission, as well as social and behavioural deficits. Modulating splicing inclusion of the nSR100-regulated microexon programme therefore represents a novel therapeutic strategy for the treatment of ASD. Through the development and subsequent application of highly sensitive, dual-luciferase splicing reporters and the 'Systematic Parallel Analysis of endogenous RNA regulation coupled to barcode Sequencing' (SPAR-Seq) system, we have recently assayed ~100,000 compounds for their efficacy in selectively modulating nSR100-dependent microexons. Further integrated analysis of our results from chemical and genetic screens, as well as RNA-Seq profiling, has elucidated the global effects of these small molecules on splicing regulation. Consequently, this screen has revealed several compounds of interest for the pharmacomodulation of the nSR100 regulatory hub, and we are currently assessing their potential for rescuing splicing inclusion of nSR100-dependent microexon inclusion in the brain, as well as autism-associated behavioural phenotypes. In summary, our current study has identified a selection of small molecules that can modulate splicing inclusion of nSR100-dependent microexons that may hold future therapeutic potential for the treatment of a substantial fraction of autism patients.

15 Modeling and Predicting the Activities of *Trans*-Acting Splicing Factors with Machine Learning

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Alternative splicing (AS) is generally regulated by *trans*-splicing factors that specifically bind to *cis*-elements in pre-mRNAs. The human genome encodes ~1,500 RNA binding proteins (RBPs) that potentially regulate AS, yet their functions remain largely unknown. To explore their potential activities, we fused the putative functional domains of RBPs to a sequence-specific RNA-binding domain and systemically analyzed how these engineered factors affect splicing. We discovered that ~80% of low-complexity domains in endogenous RBPs displayed distinct context-dependent activities in regulating splicing, indicating that AS is under more extensive regulation than previously expected. We developed a machine learning approach to classify and predict the activities of RBPs based on their sequence compositions and further validated this model using endogenous RBPs and synthetic polypeptides. These results represent a systematic inspection, modeling, prediction, and validation of how RBP sequences affect their activities in controlling splicing, paving the way for *de novo* engineering of artificial splicing factors.

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16 The Alazami Syndrome-associated protein LARP7 guides U6 small nuclear RNA modification and contributes to splicing robustness

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The La-related protein 7 (LARP7) forms a complex with the 7SK small nuclear RNA (snRNA) to regulate RNA polymerase II transcription. Aberrant expression of LARP7 has been implicated in several cancers and mutations in the LARP7 gene have been linked to the Alazami syndrome, a severe developmental disorder characterized by primordial dwarfism and intellectual disability. Here, we report a so far unknown role of this protein in RNA modification. We show that LARP7 physically connects the spliceosomal U6 snRNA with a distinct subset of box C/D small nucleolar RNAs (snoRNAs) guiding the 2'-O-methylation of U6. Consistently, these modifications are severely compromised in the absence of LARP7. Although general splicing remains largely unaffected, transcriptome-wide analysis revealed perturbations in alternative splice site usage in LARP7-depleted cells. Importantly, we identified defects in 2'-O-methylation of the U6 snRNA in Alazami syndrome siblings which express a mutated LARP7 variant, suggesting that alterations in splicing fidelity might contribute to the etiology of the Alazami syndrome. Futhermore, our data identify LARP7 as a paradigm for a double-sided RNA binding protein serving as a bridging factor for snoRNA-guided modification of target RNAs.

17 Knockout of box H/ACA RNAs that guide the two U1 snRNA pseudouridylations affects specific subsets of human splicing events

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The U1 small nuclear RNA/RNP (snRNA/snRNP) recognizes the 5' splice site (5'ss) early in pre-messenger RNA splicing. The 5'ss/U1 interaction is mainly driven by base-pairing between 5'ss and the 5' end of U1 with up to 11 base pairs. However, 5'ss sequences in the human transcriptome exhibit extreme diversity, forming distinct 5'ss/U1 helices often involving mismatches, bulges, asymmetric loops and other registers. During U1 snRNP maturation, the uridines at positions 5 and 6 are modified to pseudouridines. They both share the hydrogen-bonding potential, yet pseudouridines usually base-pair with enhanced stability. As the cellular role of U1 pseudouridines was unknown, we used CRISPR/cas9 to knock out the box H/ACA RNAs that guide specific pseudouridylation at either position. We analysed the global splicing patterns of K562 cells with these knockouts by RNA sequencing, and only considered the hundreds of splicing changes that were reversed by transient reintroduction of the respective box H/ACA RNA. Each knockout altered specific splicing subsets: knockout of ACA47 for pseudouridine 5 tends to affect splicing events with nonconsensus nucleotides at 5'ss position +4, complementary to pseudouridine 5. Knockout of U109 for pseudouridine 6 changes splicing events with weak overall splicing signals. Both knockouts affect expression of many splicing factors, probably indirectly via splicing. Overall, K562 cells tolerate well the absence of either U1 box H/ACA RNAs, and the splicing effects in individual knockouts are subtle. Hence, each pseudouridine appears largely dispensable for splicing in human culture cells, which is not obvious given their conservation across eukaryotic kingdoms.

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18 Secondary motifs of moderate affinity enable concentration-dependent regulation by Rbfox in development

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Rbfox1, 2, and 3 constitute a family of splicing factors that mediate alternative splicing and mRNA stability over the course of animal development, regulating numerous exons of genes expressed in the maturing brain, heart, and muscle. Indeed, mutations in human Rbfox proteins are associated with autism spectrum disorders, epilepsy, and cardiac pathologies. It has long been appreciated that Rbfox proteins primarily mediate their regulatory functions through binding to GCAUG motifs in introns and 3' UTRs. However, only about half of binding peaks identified by crosslinking and immunoprecipitation-sequencing (CLIP-seq) are explained by GCAUG or the related primary motif, GCACG. We conducted high-throughput biochemical assays in which human RBFOX2 was incubated with oligonucleotide libraries containing >60,000 natural RNA sequences of ~100 nt to reveal that Rbfox proteins bind to six additional, previously unappreciated 5mers with moderate affinity. Binding to these novel "secondary" motifs in vivo was detected in three different CLIP datasets. These secondary motifs occur more frequently than canonical Rbfox motifs in the transcriptome and can account for more than half of Rbfox CLIP peaks that lack presence of a canonical motif. These Rbfox secondary motifs regulate exon inclusion in a splicing reporter in a manner dependent on the expression level of Rbfox proteins. Furthermore, conserved secondary motifs are significantly enriched in the 3' UTRs of transcripts demonstrating Rbfox1-dependent stability, as demonstrated by knockdown and re-expression of the protein. Finally, secondary motifs regulate RNA splicing in neuronal development, when cellular Rbfox concentrations are particularly high. We demonstrate here that binding to moderate affinity motifs with high transcriptomic frequencies allows variation in cellular Rbfox levels to activate distinct regulatory regimes in RNA splicing and stability.

19 Contribution of alternative splicing dynamics to deterministic cell reprogramming: a comparison between B cell and MEFs reprogramming

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Alternative pre-mRNA splicing (AS) plays important roles during development and in the maintenance of stem cell pluripotency. Although reprogramming of somatic cells into induced pluripotent stem cells (iPS) is a highly inefficient and stochastic process, transient activation of the transcription factor C/EBPα prior to induction of OSKM (Yamanaka) factors poises mouse B cells for rapid and deterministic reprogramming into iPS[1,2]. Our RNA-seq analyses during reprogramming in this system identified distinct clusters of AS events with characteristic patterns of changes and highlighted commonalties and differences with those associated with reprogramming of MEFs[3]. Parallel gene expression profiling of splicing factors, and enrichment of their predicted binding sites in the corresponding AS regions, suggested regulatory mechanisms coordinating key AS changes. Using MEFs reprogramming as a test system, we observed that alteration of the pronounced switch observed in the isoforms of the transcription factor LEF1 led to a change in the induction of pluripotency markers, which were greatly enhanced upon overexpression of the iPS-associated LEF1 isoform. Knockdown of predicted regulators of early AS events, including CPSF3 and hnRNP UL1 repressed reprogramming. Conversely, MEFs reprogramming was restrained by the overexpression of predicted repressors of early AS events such as TIA1 and CELF2. Our data reveal the functional relevance of candidate AS events and regulators of somatic cell reprogramming and provide datasets to infer and test key regulatory circuits relevant for the induction and maintenance of cell pluripotency.

1. Di Stefano, B. et al. C/EBPα poises B cells for rapid reprogramming into induced pluripotent stem cells. Nature 2014.
2. Di Stefano, B. et al. C/EBPα creates elite cells for iPSC reprogramming by upregulating Klf4 and increasing the levels of Lsd1 and Brd4. Nat Cell Biol 2016.
3. Cieply, B. et al. Multiphasic and Dynamic Changes in Alternative Splicing during Induction of Pluripotency Are Coordinated by Numerous RNA-Binding Proteins. Cell Rep 2016.

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20 Mechanisms and targets of introns dependent regulation of cell survival and adaptation to starvation

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Most eukaryotic genes are interrupted by introns that are removed by splicing. Introns are found in the smallest of the eukaryotic genomes, and in the human genome their sequences make up 90% of primary transcripts. While introns are important to permit alternative splicing in higher eukaryotes, it is not clear why they were preserved in small eukaryotic genomes like the budding yeast where alternative splicing is virtually absent. Although a few introns help streamline expression of their host genes, our study reveals that introns do not only affect gene expression but may also function independent of their host genes. This striking discovery was obtained by deleting each of the 295 single introns from the yeast genome and analyzing the impact of each deletion on cell growth and gene expression (~ 40 000 growth curves and 20 000 expression data points). We report that a majority ofintrons promote resistance to starvation by mediating nutrient sensing dependent repression of ribosome biogenesis and present new unpublished data clarifying the intron mechanism of function.

21 The RNA helicase DHX34 has a dual role in NMD and in pre-mRNA splicing and is mutated in MDS/AML patients

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We previously identified the DExD/H-box RNA helicase DHX34 as a novel Nonsense-mediated decay (NMD) factor in a genome-wide RNAi screen in the nematode *C. elegans*. We showed that DHX34 acts in concert with core NMD factors to co-regulate a large number of RNA targets in human cells, zebrafish embryos and in *C. elegans* (1, 2). We also demonstrated that DHX34 promotes mRNP remodeling in order to activate NMD (2). Interestingly, unlike other core NMD (smg*)* genes in nematodes, this gene is essential in nematodes, strongly suggesting that it has additional cellular functions.

Here, we report that DHX34 is involved in the regulation of pre-mRNA splicing in mammalian cells. An interactome of endogenously epitope-tagged DHX34 cell lines revealed preferential interactions with components of the spliceosome with a clear enrichment for the spliceosomal complex C. This suggests a function for DHX34 in the late steps of the splicing reaction. In agreement with a function of DHX34 in RNA splicing we find that depletion of DHX34 results in a large number of splicing changes, affecting different types of alternative splicing. We searched for endogenous RNA targets and binding sites of DHX34 using the Cross-Linking Immunoprecipitation protocol (CLIP). We found that DHX34 is mostly associated with pre-mRNAs and can be found at exon-intron boundaries. A function of DHX34 in pre-mRNA splicing underlies the functional role of several DExD/H-box RNA helicases in mediating structural changes of the spliceosome and also to control the fidelity of the splicing process. Only recently, several mutations in DHX34 have been linked to familial Myelodysplasia (MDS)/Acute Myeloid Leukemia (AML) (Rio-Machin et al., under review). We showed that these reported DHX34 mutations compromise its activity in the NMD pathway. Given that novel pathway mutations in MDS/AML involve multiple components of the splicing machinery, we will discuss the impact of these novel DHX34 mutations in its function in constitutive and alternative splicing. Altogether, these data show the cellular functions of the RNA helicase DHX34 and how loss-of-function mutations compromise different RNA processing activities.

1. Longman et al. (2013) *Nucleic Acids Res.,* **41**, 8319-8331.
2. Hug,N. and Cáceres,J.F. (2014) *Cell Rep.,***8**, 1845-1856.

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22 *Drosophila* Sister of Sex-lethal reinforces a male-specific gene expression pattern by controlling Sxl-lethal-dependent alternative splicing

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In *Drosophila melanogaster*, female development is governed by a single RNA-binding protein, Sex-lethal (Sxl), that controls the expression of key factors involved in dosage compensation, germline homeostasis and the establishment of female morphology and behaviour. Sxl protein expression in female flies is maintained by an auto-regulatory, positive feedback loop with Sxl controlling splicing of its own mRNA. Until now, it remained unclear how males prevent accidental triggering of the Sxl expression cascade and protect themselves against runaway protein production.

We have identified the protein Sister-of-Sex-lethal (Ssx) as an inhibitor of Sxl auto-regulatory splicing. Sxl and Ssx have a comparable RNA-binding specificity and compete for binding to RNA regulatory elements present in the *Sxl* transcript. In cultured *Drosophila* cells, Sxl-induced changes to alternative splicing can be reverted by the expression of Ssx. Moreover, in adult male flies ablation of the *ssx* gene results in a low level of productive *Sxl* mRNA splicing and Sxl protein production in isolated, clonal cell populations in various organs and tissues. In sum, this demonstrates that through direct competition Ssx safeguards male animals against Sxl protein production to reinforce a stable, male-specific gene expression pattern.

23 Spatiotemporal organization of the *E. coli* transcriptome: Translation-independence and engagement in regulation

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Until recently bacterial transcripts were not assumed to have distinct localization patterns. We have previously demonstrated that *E. coli* mRNAs may localize to where their products localize in a translation-independent manner (Nevo-Dinur et al., *Science*, 2011). These findings challenged the transcription-translation coupling dogma, although the scope of RNA localization in bacteria remained unknown.

We developed a protocol that assigns sub-cellular localization data to each detectable transcript in E. coli between the membrane, cytoplasm and poles (Rloc-seq). Our results reveal asymmetric distribution of RNAs on a transcriptome-wide scale, which significantly correlates with the localization of the resulting proteome. The results further demonstrate that translation-independent RNA localization is prevalent in E. coli. The poles are uniquely enriched with stress-related mRNAs and small RNAs (sRNAs). Surprisingly almost all sRNAs became dramatically enriched in the poles after osmotic stress, although only few are known to be involved in response to this stress, suggesting a polygenic model for sRNA-mediated regulation. The accumulation of sRNAs at the poles upon stress is due to relocation of their chaperone Hfq to this compartment.

Our results show unexpected level of intricacy in bacterial transcriptome organization and highlight the poles as hubs for regulation.

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24 High-throughput dissection of function and binding preferences of a nuclear localization element common in long RNAs

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The subcellular distribution of RNA molecules is an important aspect of their biology. Long RNA molecules such as mRNAs and long noncoding RNAs (lncRNAs) are produced in the nucleus and have varying efficiencies of export to the cytoplasm, and those have important functional implications. The nuclear retention of mRNAs was shown to be important for buffering protein levels from transcriptional noise and for rapid response to varying stimuli. lncRNAs are as a group more nuclear than mRNAs and many of the known lncRNA functions take place in the nucleus and so require retention on chromatin or in the nucleoplasm.

We recently used a massively parallel RNA assay (MPRNA) to identify SIRLOIN, a Alu repeat-derived sequence element that can drive the nuclear localization of mRNAs and lncRNAs via interaction with HNRNPK (Lubelsky & Ulitsky, Nature 2018). The SIRLOIN element serves as a paradigm for functional modules within long RNAs. We now expanded our MPRNA approach to analyze a library of sequences with extensive mutagenesis of the SIRLOIN element and to evaluate how individual bases in this element affect its functionality in determining localization and its binding to HNRNPK. Through insertions and deletions in the sequence we find a stretch of 20 nt that is essential for SIRLOIN function. By single and double mutagenesis and insertion of HNRNPK binding sites or control sequences in different positions we find that HNRNPK must bind in a specific sequence context, with loss of binding at the WT position compensated by binding in only a single alternaive site, and no evidence for importance of secondary structure in element functionality.

We also performed RNA antisense purification followed by mass spectrometry (RAP-MS) to identify additional protein binding partners of SIRLIOIN that now help us place HNRNPK in the context of a larger pathway that we propose affects the export effiiciencies of hundreds of mRNAs and lncRNAs.

These studies explain how sequence-specific binding of an RNA binding protein can influence the post-transcriptional fate of long RNA molecules, and how to design sequences with desired localization properties.

25 Trypanosomes can initiate nuclear export co-transcriptionally

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The nuclear envelope serves as important mRNA surveillance system. In yeast and human, several control systems act in parallel to prevent nuclear export of unprocessed mRNAs. Trypanosomes lack homologues to most of the involved proteins and their nuclear mRNA metabolism is non-conventional exemplified by polycistronic transcription and mRNA processing by trans-splicing. We here visualised nuclear export in trypanosomes by intra- and intermolecular multi-colour single molecule FISH. We found that, in striking contrast to other eukaryotes, the initiation of nuclear export requires neither the completion of transcription nor splicing. Nevertheless, we show that unspliced mRNAs are mostly prevented from reaching the nucleus-distant cytoplasm and instead accumulate at the nuclear periphery in cytoplasmic nuclear periphery granules (NPGs). Further characterisation of NPGs by electron microscopy and proteomics revealed that the granules are located at the cytoplasmic site of the nuclear pores and contain most cytoplasmic RNA binding proteins but none of the major translation initiation factors, consistent with a function in preventing faulty mRNAs from reaching translation. Our data indicate that trypanosomes regulate the completion of nuclear export, rather then the initiation. Nuclear export control remains poorly understood, in any organism, and the described way of control may not be restricted to trypanosomes.

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26 A Deletion in the Mouse Nxf1 Intron 10 CTE Results in Downregulation of Genes Involved in Learning and Synaptic Plasticity

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The Nxf1 protein is an mRNA export receptor for many viral and host cell mRNAs. It has been well established that Nxf1 can interact directly with Constitutive Transport Elements (CTEs) in mRNAs that retain introns to overcome cellular restrictions and allow export and translation of these mRNAs.

We have previously shown that the NXF1 gene itself contains a CTE in a retained internal 1.8 kB intron (intron 10) and that this leads to the expression of an alternative short Nxf1 protein isoform from this mRNA. The NXF1 CTE is similar in structure and sequence to the CTE in Mason-Pfizer Monkey Virus (MPMV) and is present in the NXF1 gene in most mammals as well as teleost fish. The small Nxf1 protein is highly expressed in rodent hippocampal and some cortical neurons and traffics in neuronal granules, suggesting a potential role for CTE regulation in dendritic mRNA trafficking. To further study this, we used Cas9 nickase and two guide RNAs to generate deletions in the mouse Nxf1 CTE. Mice with a 19 nt deletion in the Nxf1 binding region of the CTE were generated. These mice develop normally and are fertile. However, mice with homozygous deletions show aggressive and hyperactive behaviors (including circling).

RNA Seq on total mRNA from the hippocampus of 16 week mice with homozygous CTE deletions compared to mRNA from wildtype mice, showed a significant downregulation of several "Immediate Early Genes" (IEG) mRNAs, as well as other mRNAs implicated in learning and synaptic plasticity. These include c-Fos, Fosb, Junb, Egr1 and Arc, a gene of known retrotransposon origin. Significant upregulation of intron retention was observed in c-Fos. Upregulated mRNAs include several mitochondrial oxidative phosphorylation genes, suggesting a neuronal stress response. We are currently sequencing total and cytoplasmic mRNA in different organs and establishing neuronal and fibroblast cells in vitro for further molecular characterization. Taken together, our results support the hypothesis that the NXF1 gene plays an important role in synaptic plasticity and learning. This is likely to be related to the fate of specific mRNAs with retained introns in the brain.

27 Integration of protein, RNA and RBP localisation maps to understand subcellular organisation

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In order to fully comprehend the complexity of cellular function, it is crucial to understand the subcellular distribution of its components. It has become increasingly evident that not only proteins, but also RNAs, are differentially distributed amongst cellular components and that the correct subcellular localisation of RNA is crucial for cell physiology. To date, RNA localisation studies have been focused on specific RNA species within highly polarised cells. In order to more comprehensively explore RNA localisation, we have modified Localization of Organelle Proteins by Isotope Tagging (LOPIT) which enables interrogation of the spatial proteome on a cell-wide scale (Christoforou *et al*2016, Geladaki *et al*2019), to generate the first complete RNA localisation map of the cell (LoRNA - localisation of RNA). We recover the proteome and transcriptome from the same sample, thus allowing RNA subcellular localisation to be inferred from protein data via transfer learning. We identify membrane-localised RNAs encoding proteins that are enriched in cell organelles, including several that lack signals for ER-localised translation via the signal recognition particle, suggesting that RNA localisation may be a general driver of protein sub-compartmentalisation. Furthermore, we also detect unexpected localisation for some lncRNA which are distributed beyond the usual nucleus-cytoplasm axis. Finally, we have also employed OOPS, a recent method we have developed to purify RNA-protein complexes (Queiroz *et al*, 2019) to identify the subcellular localisation of RNA binding proteins (RBPs). We integrate this RBP map with corresponding protein (LOPIT) and RNA (LoRNA) maps to interrogate the role of RBPs in RNA localisation. Taken together, application of these spatial workflows allows us to comprehensively explore how RNA and proteins are organised at the subcellular level, and opens new opportunities to explore the dynamics of spatial reorganisation of cellular components.

References:

Christoforou, A. *et al.* A draft map of the mouse pluripotent stem cell spatial proteome. *Nat. Commun.* **7,**8992 (2016).

Geladaki, A. *et al.* Combining LOPIT with differential ultracentrifugation for high-resolution spatial proteomics. *Nat. Commun.* **10,**331 (2019).

Queiroz, R. M. L. *et al.* Comprehensive identification of RNA–protein interactions in any organism using orthogonal organic phase separation (OOPS). *Nat. Biotechnol.* **37,**169–178 (2019).

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28 Alternative 3' UTRs directlocalization of functionally diverse protein isoforms in neuronal compartments

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The proper subcellular localization of RNAs and local translational regulation is crucial in highly compartmentalized cells, such as neurons. RNA localization is mediated by specific *cis*-regulatory elements usually found in mRNA 3'UTRs. Therefore, processes that generate alternative 3'UTRs – alternative splicing and polyadenylation – have the potential to diversify mRNA localization patterns in neurons. Here, we performed mapping of alternative 3'UTRs in neurites and soma isolated from mESC-derived neurons. Our analysis identified 593 genes with differentially localized 3'UTR isoforms. In particular, we have shown that two isoforms of *Cdc42* gene with distinct functions in neuronal polarity are differentially localized between neurites and soma of mESC-derived and mouse primary cortical neurons, at both mRNA and protein level. Using reporter assays and 3'UTR swapping experiments, we have identifiedthe role of alternative 3'UTRs and mRNA transport in differential localization of alternative CDC42 protein isoforms. Moreover, we used SILAC to identify isoform-specific *Cdc42* 3'UTR-bound proteome with potential role in *Cdc42* localization and translation. Our analysis points to usage of alternative 3'UTR isoforms as a novel mechanism to provide for differential localization of functionally diverse alternative protein isoforms.

29 Makorin 1 controls embryonic patterning by alleviating Bruno-mediated repression of *oskar* translation

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Makorins are evolutionary conserved proteins that contain C_3H-type zinc finger modules and a RING E3 ubiquitin ligase domain. In *Drosophila* maternal Makorin 1 (Mkrn1) has been linked to embryonic patterning but the mechanism remains unsolved. Here, we show that Mkrn1 is required for translational activation of *oskar*, whose product is critical for axis specification and germ plasm assembly. We demonstrate that Mkrn1 interacts with poly(A) binding protein (pAbp) and binds *osk* 3' UTR in a region adjacent to A-rich sequences. This binding site overlaps with Bruno (Bru) responsive elements (BREs), which regulate *osk* translation. We observe increased association of the translational repressor Bru with *osk* mRNA upon depletion of Mkrn1, indicating that both proteins compete for *osk* binding. Consistently, reducing Bru dosage rescues viability and Osk protein level in ovaries from *Mkrn1* females. We conclude that Mkrn1 controls embryonic patterning and germ cell formation by specifically activating *osk* translation via displacing Bru from its 3' UTR.

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30 Structural basis of RNA transport: Tropomyosin 1 – Kinesin high-resolution structure and its interaction with RNA

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mRNA localization, especially when coupled with translational control, is central to diverse processes such as cell differentiation, cell migration, and embryonic axis specification. In *Drosophila*, localization of *oskar* mRNA to the posterior pole of the oocyte is essential for the formation of the germline and abdomen in the future embryo. *oskar* mRNA localization is achieved through a fine balance of kinesin- and dynein-mediated transport along the oocyte microtubule cytoskeleton. Recent studies have shown that an atypical isoform of Tropomyosin 1 (Tm1-I/C) acts in *oskar* mRNA localization, and that it does so by recruiting kinesin heavy chain (Khc) to the mRNA (*Veeranan-Karmegam et al.,*2016; *Gaspar et al*., 2017). This finding was surprising because, to date, classical tropomyosins were found to associate with the actin cytoskeleton. To study the molecular mechanism whereby Tm1 acts in recruitment of kinesin to *oskar*, we tested if Tm1-I/C makes direct contacts with RNA and kinesin. EMSA and NMR titration experiments showed that the Tm1-I/C N domain interacts directly with RNA. Furthermore, *in vitro* binding assays between GST-tagged full-length and truncated Khc showed that Tm1-I/C and kinesin also interact directly. We further narrowed down the Tm1/Khc interaction surface and ultimately obtained a crystal structure of the Tm1-I/C coiled-coil region alone and in complex with Khc. The Tm1-I/C-Khc complex is formed of two parallel kinesin chains and a Tm1-I/C chain antiparallel to the Khc homodimer, forming together a positively charged surface, which might accommodate RNA together with the N domain of Tm1. Moreover, in contrast to classical Tm1, which forms a parallel homodimer, Tm1-I/C crystallized as an antiparallel coiled-coil. This alternative conformation suggests a neat mechanism that might prevent heterodimerization of the Tm1-I/C isoform with classical muscular Tm1 isoforms, and might additionally provide specificity for the interaction of Tm1-I/C with Khc. Our study provides unprecedented insight into cargo-adaptor-motor assembly and regulation in RNA transport.

Veeranan-Karmegam, R. *et al*. "A new isoform of Drosophila non-muscle Tropomyosin 1 interacts with Kinesin-1 and functions in oskar mRNA localization." *J. Cell Sci.*2016

Gaspar, I. *et al*. "An RNA-binding atypical tropomyosin recruits kinesin-1 dynamically to oskar mRNPs." *EMBO J.*2017

31 Staufen1 reads out structure and sequence features in ARF1 dsRNA for target recognition.

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Most posttranscriptional regulation of gene expression is based on RNA elements in mRNAs recognized by RNA-binding proteins (RBPs). Besides primary sequence elements, a second layer of information is embedded in 3'UTRs of mRNAs in the form of RNA structure. Double-stranded RBPs (dsRBPs) can bind structures in 3'UTRs and then exert their function based on dsRNA target recognition through a combination of structure and sequence. Staufen1 (STAU1) is a dsRBP involved in mRNA transport and localization, translational control and mRNA decay by a STAU1-mediated mRNA decay (SMD) pathway. The STAU1 binding site (SBS) within human ADP-ribosylation factor1 (ARF1) 3'UTR is one such target and STAU1 binding to the SBS regulates ARF1 cytoplasmic mRNA levels by the SMD pathway. However, how STAU1 recognizes specific mRNA targets is still unknown.

Our structure of the ARF1 SBS - STAU1 complex uncovers target recognition by STAU1. STAU1 dsRNA binding domain (dsRBD) 4 interacts with two pyrimidines and one purine from the minor groove side *via* helix α1, β1-β2 loop anchors the dsRBD at the end of the dsRNA and lysines in helix α2 bind to the phosphodiester backbone from the major groove side. STAU1 dsRBD3 displays the same binding mode with specific recognition of one guanine base. Mutants disrupting minor groove recognition of ARF1 SBS reduce SMD *in vivo* but have minor effect on *in vitro* binding. Our data suggest how dsRNA recognition by STAU1 mediates diverse functions in gene expression pathways.

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32 Cyclin *CLB2* mRNA localization regulates the mitotic progression

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The cell cycle is the sequence of events controlling the growth, the duplication of the genome and its partition in two daughter cells. These events rely on robust transcriptional, translational and post-translational circuits controlling the expression of cell cycle genes. While the expression of many cell cycle regulators has been characterized, how their mRNA and protein abundance are coordinated in single cells during cell cycle phases transition remains to be elucidated.

Here, we characterized the expression of the conserved B-type cyclin *CLB2* in S. cerevisiae. By using a combination of single molecule RNA FISH (smFISH) in fixed cells and mRNA measurements in living cells using the MS2 system, we followed *CLB2* from transcription to degradation in single cells and throughout the cell cycle. Our data showed that the *CLB2* mRNA is transcribed during the S-G2 phase and it is rapidly destroyed before mitosis end, in a process coordinated with Clb2 protein destruction. Interestingly, *CLB2* mRNAs localized to the bud in a She2/She3 complex-dependent manner. Despite the mRNA localization in the bud, the Clb2 protein was recruited in the mother nucleus, suggesting that this mechanism does not control the asymmetric distribution in the bud. To understand the role of *CLB2* mRNA localization, we developed an mRNA translation reporter to simultaneously visualize single mRNA and nascent proteins in individual yeast cells. This approach revealed that *CLB2* mRNAs are preferentially translated in the bud, as it was previously shown for the *ASH1* mRNA. Additionally, lack of *CLB2* mRNA localization did not affect the mRNA stability, but it led to a decrease in Clb2 protein levels and changes in mitotic progression, suggesting that regulation of mRNA translation timing is important for progression. Altogether, our results indicate that the localization of the *CLB2* mRNA and its local translation precisely modulate Clb2 protein levels, generating a growth dependent timer to control the progression into mitosis.

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33 RNA-mediated Gene Regulation and Immunity: Structure-function of Riboswitches; Mechanism and Applications of Type I CRISPR.

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I will present work from my lab studying two of the main themes in RNA biology. (1) **RNA serving as a guide** to direct the action of proteins on nucleic acid targets. Such systems carry great potential for therapeutics and biotechnology applications, as exemplified by RNAi and CRISPR-Cas. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and the nearby *cas* (CRISPR-associated) operon establish an RNA-based adaptive immunity system in prokaryotes. They are further classified into multiple-effector (Class I) and single-effector (Class II) systems. Type I CRISPR-Cas, the most prevalent CRISPR system, belongs to Class 1 and can be further categorized into six subtypes. It features a sequential target-searching and degradation process. First, a multi-subunit surveillance complex called Cascade (CRISPR associated complex for antiviral defense) recognizes the matching dsDNA target flanked by an optimal protospacer adjacent motif (PAM), promotes the heteroduplex formation between CRISPR RNA (crRNA) and the target strand (TS) DNA and displaces the non-target strand (NTS) DNA, resulting in R-loop formation at the target site. Subsequently, the helicase-nuclease fusion enzyme Cas3 specifically binds to the Cascade/R-loop complex, nicks the NTS, and processively degrades DNA over long distances. I will summarize work from my lab aimed at understanding the mechanisms of PAM recognition, directional R-lop formation, and nuclease recruitment/activation. I will further present recent data from my lab demonstrating the genome editing activities of Type I CRISPR in human cells. (2) **Structured RNAs regulating gene expression *in cis***. I will summary work from my lab aimed at elucidating the structure, function, and conformation dynamics of many riboswitches.

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34 Primary sequence relevance in plant microRNAs processing

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MicroRNAs (miRNAs) are small RNAs of 20-22 nucleotides present in plants and animals. They are coded within the genome and play a crucial role as post-transcriptional gene regulators. For the model plant, Arabidopsis thaliana (Arabidopsis), are 326 precursors and 428 mature sequences. These miRNAs regulate genes involved in development, hormone signaling and stress response.

These small RNAs are generated from longer precursors with fold-back structures with the miRNA located in one of their arms. In plants, miRNA precursors are processed in the nuclei by a complex harboring the protein DICER-LIKE1 (DCL1). This complex recognizes structural determinants in the precursors to produce all the cuts and release the mature miRNA.

To gain insights into MIRNA processing in plants, we performed a random mutagenesis of the MIR172a precursor of Arabidopsis with an emphasis in finding mutants with higher activity. miR172 regulates flowering time and flower patterning. Overexpression of miR172 produces early flowering plants with flower defects, resembling the ap2 phenotype. The strength of these phenotypes can therefore be used to assess processing efficiency of the mutant precursor.

Through this approach, we identified hot spot positions for MIRNA processing. Interestingly, most of them were located at DCL1 cleavage sites. Small changes in these regions had a strong effect on the biogenesis of the small RNA. This allows us to define the cleavage sites architecture of Arabidopsis MIRNA precursors. Moreover, we show that the spatial configuration is usually conserved between angiosperms.

Furthermore, we recovered a mutant precursor with a single change that enhance the accumulation of the miRNA by several folds. This demonstrates that even simple changes in the DCL1 cleavage site architecture can strongly modulate the biogenesis of miRNAs in plants. The results obtained provide new insights into the molecular basis of miRNA biogenesis in plants.

35 Arabidopsis thaliana mRNA Adenosine Methylase (MTA) is a new player in miRNA biogenesis regulatory pathway.

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Methylation of adenosine at N6 position (m^6A) is one of the most abundant mRNA modifications. In this study we uncovered the role of MTA and m^6A methylation in plant (Arabidopsis thaliana) miRNA biogenesis. We used NGS to show that miRNA levels are downregulated in *mta* hypomorphic mutant whereas pri-miRNA levels are upregulated in such plants. We then identified a set of 11 pri-miRNAs that are m6A methylated using m6A-IP seq. Furthermore, RNA-IP using MTA-GFP tagged Arabidopsis plants showed enrichment of pri-miRNAs in the MTA-GFP line (including 8 pri-miRNAs found in m6A IP), further proving that pri-miRNAs are also substrates for m6A methylation by MTA. We also report that MTA interacts with RNA Pol II and TGH (known miRNA biogenesis related player) indicating that MTA acts in early stages of miRNA biogenesis. Lastly, we show that MTA modulates auxin response in plants via methylation of pri-miR393b. Our data indicate that MTA is an important player in the biogenesis of a set of Arabidopsis miRNAs.

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36 MicroRNA arm switching regulated by uridylation

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MicroRNAs (miRNAs) are processed from hairpins by sequential cleavage events by Drosha and Dicer, which release a short RNA duplex consisting of 5p and 3p strands. Strand selection is a pivotal step that decides which strand from the duplex remains in the Argonaute protein to direct gene silencing. Alternative strand selection (or "arm switching") was observed in a few miRNAs and some cell types. However, the molecular mechanism and physiological significance of arm switching remain unknown. Here we show that mir-324 undergoes dynamic arm switching in a cell type-specific manner and that terminal uridylyltransferases TUT4 and TUT7 mediate the arm switching. Uridylation of pre-mir-324 by TUT4/7 causes a shift in the Dicer cleavage site by 3 nt. Due to the change in the cleavage site, a shorter duplex is produced, from which the 3p is preferentially selected at the expense of the 5p miRNA. We further find that, in high-grade glioma, TUT4/7 are upregulated and consequently miR-324-3p dominates over miR-324-5p. Disruption of the alternative maturation by depleting TUT4/7 was sufficient to suppress epithelial-to-mesenchymal transition markers and to impair the viability of glioblastoma. This study offers the first example of regulated arm switching and uncovers a novel role of TUT4/7 as a molecular switch in alternative miRNA maturation and in glioblastoma maintenance.

37 Deciphering target-directed miRNA degradation

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microRNAs (miRNAs) are small non-coding RNAs (ncRNAs) that post-transcriptionally regulate expression of more than half of human messenger RNAs (mRNAs). miRNAs serve as guides for Argonaute (Ago) proteins to target mRNAs with partially complementary sequences for decay/translational repression. Certain target RNAs (mRNAs or ncRNAs) selectively bind miRNAs with more extensive complementarity and induce miRNA decay in a poorly understood process called target-directed miRNA degradation (TDMD).

Here, we used HSUR1 – a small non-coding RNA from the oncogenic Herpesvirus saimiri that induces degradation of host miR-27 – as a model to study TDMD. We performed systematic mutagenesis of HSUR1 to define the sequence complementarity requirements for TDMD. Interestingly, for the HSUR1 mutants exhibiting complementarity to the extreme 3' end of miR-27, extended miR-27 isoforms (isomiRs) appeared. These isomiRs likely represent failed products of TDMD and could mean that features of the pairing between the TDMD target and miRNA dictate which enzymes are recruited to modify the miRNA 3′ end. Small RNA sequencing revealed that a mixture of adenylates and uridylates is added to the 3′ end of miR-27 during TDMD.

We then determined X-ray structures of human Ago2 bound to miRNA and TDMD targets. The RNAs form a bipartite duplex composed of both 5' (seed) and 3' paired miRNA regions connected by an unpaired flexible linker. Ago2 is physically too small to accommodate the 3' paired duplex, which juts out into the bulk solvent with the miRNA 3' end exposed. The Ago-released miRNA 3' end is subjected to intracellular tailing and trimming, which is also observed when 3'-end binding by Ago is mutationally compromised. Importantly, the isomiRs generated in the presence of HSUR1 mutants are associated with all four human Agos, indicating that all Ago proteins can adopt the TDMD conformation. Our results reveal the structural mechanism of TDMD and could facilitate discovery of the enzymes responsible for decay, as well as *in silico* predictions of novel TDMD targets.

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38 AGO2 localizes to mitochondria and regulates mitochondrial function and gene expression

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Mitochondria are central to many cellular functions, and are semi-autonomous organelles that require the import of more than 1500 nuclear encoded proteins for proper function. Recently, the nuclear encoded Argonaute 2 (AGO2) and nuclear-encoded miRNAs have been found localized to the mitochondrial matrix, and in muscle cells AGO2 upregulates mitochondrial protein translation in a miRNA-dependent manner (Zhang et al. 2015). However, the mechanism by which AGO2 is imported into mitochondria and its role in regulating mitochondrial function are not well understood. Human AGO2 does not contain a predictable mitochondrial targeting sequence. Using a series of genetic constructs, we find that import of AGO2 into mitochondria is accompanied by a cleavage event at the N-terminus. We also find that the N-terminal domain is necessary for targeting AGO2 to mitochondria, whereas disruption of the C-terminal domain also interferes with AGO2 import. In order to identify the mitochondria-specific functions of AGO2 and control its subcellular localization, a series of cell lines and constructs were generated. CRISPR-Cas technology was used to generate AGO2 null cell lines, and AGO2 constructs with a mitochondrial targeting sequence were developed. Loss of AGO2 results in altered mitochondrial functional parameters including respiration rate, mitochondrial morphology, and mitochondria DNA replication dynamics. In addition, AGO2 ablation leads to altered mitochondrial gene expression, with an overall increase in mitochondria transcripts. Examining small RNA expression within mitochondria reveals that AGO2 physically interacts with a subset of mitochondria-derived tRNA fragments. This study identifies required domains for the import of AGO2 into mitochondria, and suggests a non-canonical import mechanism. Our data further reveal the important roles of AGO2 in controlling mitochondrial functions and offers novel insight at the crossroads of small RNA and mitochondrial biology.

39 Qin: A novel RNA endonuclease of the piRNA biogenesis pathway

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The discovery of PIWI-interacting RNAs (piRNAs) has been a major contribution to the ever-growing class of non-coding RNAs, owing to their fundamental role in silencing transposons in animal germline cells. Transposons are parasitic DNA elements that move within the genome and may disrupt gene integrity. piRNAs act by guiding the PIWI-clade argonaute proteins (Aub and Ago3) in their cleavage of transposon transcripts. Failure to do so can lead to defects in gametogenesis and infertility. The biogenesis of piRNAs takes place in the perinuclear structure known as the nuage, where precursor piRNA-transcripts are converted to functional piRNAs via the ping-pong cycle.

During the ping-pong cycle the generation of piRNAs and the silencing of transposons are tightly coupled, generating a transposon-sequence-based piRNA amplification loop. Qin is an indispensable protein of ping-pong that has multiple tudor domains. Qin knockout results in such developmental disorders as fused/missing dorsal appendages in Drosophila embryos, which fail to develop into fully functioning adults and remain as sterile female flies. The hallmark of Qin deletion is the presence of a futile homotypic (Aub-Aub) ping-pong cycle - as opposed to the physiological heterotypic (Aub-Ago3) one - resulting in an increase in sense piRNAs and a concomitant decrease in antisense piRNAs, thereby resulting in transposon de-repression. Despite the fact that Qin has been isolated in many co-immunoprecipitates of ping-pong cycle proteins and the effects of its knockout are known, its precise role in piRNA biogenesis has remained elusive so far.

Nucleases are required in the ping-pong pathway to cleave the 3'-end of the piRNA intermediates, but no such proteins have been discovered so far. We discovered that the tudor domains of Qin carry an RNA endonuclease activity with preference for single-stranded RNA, therefore proposing Qin as the first example of an RNase in the ping-pong cycle. We also put forward a model for why Qin activity favors the functional heterotypic ping-pong pathway. Finally, this work sheds light on the probable function and the unusual abundance of other tudor-domain-containing proteins involved in piRNA biogenesis pathways, by proposing that RNase activity in the ping-pong cycle might be more abundant than previously thought.

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40 Planarians recruit piRNAs for mRNA turnover in adult stem cells

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PIWI proteins utilize small RNAs called piRNAs to silence transposable elements, thereby protecting germline integrity. In planarian flatworms, PIWI proteins are essential for regeneration, which requires adult stem cells termed neoblasts. Here, we characterize planarian piRNAs and examine the roles of PIWI proteins in neoblast biology. We find that planarian PIWI proteins SMEDWI-2 and SMEDWI-3 cooperate to degrade active transposons in the ping-pong cycle. Unexpectedly, we also discover a dichotomy of PIWI function in neoblast mRNA surveillance. While SMEDWI-2 and SMEDWI-3 degrade numerous neoblast mRNAs via the ping-pong cycle, SMEDWI-3 also protects a subset of neoblast mRNAs from degradation. Protected mRNAs are thereby safeguarded by SMEDWI-3 to be eventually deployed during stem cell differentiation. Mechanistically, the distinct activities of SMEDWI-3 are dictated by its targets' secondary structures and the degree of complementarity between mRNAs and antisense piRNAs. Planarian PIWI proteins thus act as both nucleases and protective RNAbinding proteins, enabling planarians to repurpose piRNAs for critical roles in neoblast mRNA turnover.

41 The dynamics of bacterial small RNA-guided mRNA targeting through Hfq

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The mechanism of RNA-guided targeting is a huge part of immunity and stress response in bacteria and eukaryotes. RNA target recognition is usually mediated by proteins and protein complexes. One example of such a system is the chaperone Hfq that facilitates the interactions of small noncoding RNAs (sRNAs) with their target mRNAs, which is the main layer of posttranscriptional regulation in many bacterial species. Hfq has distinct binding surfaces that can recognize ARN, 3' U-rich or AU-rich sequence motifs. Therefore, RNAs can be arranged on the Hfq chaperone in various orientations. It is still not fully understood how Hfq's multiple binding surfaces accelerate sRNA-mRNA annealing and how the annealing process depends on the RNA orientation.

We developed a TIRF-based single-molecule fluorescence platform to visualize Hfq-mediated sRNA-mRNA annealing process in real time. Using this platform, distinct events which take place before and during the formation of the Hfq-mRNA-sRNA ternary complex can be observed. These events include recruitment of the sRNA-Hfq complex to the mRNA, reversible annealing and stable sRNA-mRNA annealing. Moreover, our assay allows for a direct observation of the helix formation initiation and shows that sRNA-mRNA annealing can be achieved via multiple pathways. Hfq mutants that disrupt mRNA or sRNA recruitment showed how each surface of Hfq contributes to its chaperone activity. Substitution of alanine for arginines on the rim of Hfq resulted in a higher number of unsuccessful annealing attempts, showing that rim of Hfq is needed for complete RNA base pairing. Surprisingly, the lifetimes of the annealed complexes strongly depend on the orientation of the sRNA on Hfq, explaining why certain classes of sRNAs regulate their targets more efficiently than others. Using our single-molecule platform, we are currently testing how different sRNAs interfere with ribosome binding and translation initiation. Together, these data provide the first comprehensive model of bacterial sRNA-mRNA annealing measured on time scales which are relevant in vivo and further explain how the conformation of sRNA-Hfq complexes drive efficient regulation of target mRNAs.

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42 Defining the "licence to cut" : structural and functional insights from deconstructing the eukaryotic mRNA 3' end processing machinery

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Cleavage and polyadenylation factor (CPF/CPSF) is a large, multi-protein complex essential for formation of eukaryotic mRNA 3′ ends. CPF cleaves each pre-mRNAs at a specific site before adding a poly(A) tail. The cleavage event not only defines the 3′ end of the mature mRNA, but also triggers termination of transcription by RNA polymerase II. It therefore represents an irreversible commitment to 3′ end formation, and the activity of the Ysh1/CPSF-73 endonuclease subunit is highly regulated.

Here, using a fully recombinant approach, we present a systematic structural and functional dissection of the *Saccharomyces cerevisiae* pre-mRNA processing machinery, thus defining a minimal machinery for 3′ end formation *in vitro*. We show that reconstitution of specific pre-mRNA cleavage requires incorporation of the Ysh1 endonuclease into an eight-subunit "CPF_{core}" complex. In this primed state, Ysh1 can be fully activated by accessory cleavage factors IA and IB, which bind substrate pre-mRNAs and CPF, likely facilitating assembly of an active complex. Using X-ray crystallography, electron microscopy and mass spectrometry, we determine a high-resolution structure of Ysh1 bound to Mpe1 and reveal the arrangement of subunits within CPF_{core}. By conducting activity assays and RNA binding experiments with CPF_{core}, we also define a minimal RNA substrate and determine likely binding sites of key proteins on this substrate. Together, our data suggest that the active mRNA 3′ end processing machinery is a highly dynamic assembly that is licensed to cleave only when all protein factors come together at the polyadenylation site. We propose a model for 3′ end formation on our minimal substrate.

43 Cryo-EM studies of the human pre-mRNA 3'-end processing machinery

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Nearly all eukaryotic messenger RNA precursors must undergo cleavage and polyadenylation at their 3¢-end for maturation, which is carried out by a large machinery with many protein factors. The cleavage and polyadenylation specificity factor (CPSF) has a central role in this processing. It recognizes the AAUAAA polyadenylation signal (PAS) and its CPSF73 subunit catalyzes the cleavage of the pre-mRNA. CPSF contains two sub-complexes, CPSF160-WDR33-CPSF30-Fip1 (also known as mPSF, mammalian polyadenylation specificity factor) and CPSF73-CPSF100-symplekin (mCF, mammalian cleavage factor). We reported earlier the cryo-EM structure of human CPSF160-WDR33-CPSF30 in complex with an AAUAAA PAS RNA, providing the first molecular insight into the specific recognition of this hexanucleotide for 3¢-end processing. We have extended our studies to the entire CPSF, and our cryo-EM reconstructions have provided the first structural information on the organization of this central factor of the machinery, revealing the molecular mechanism for direct contacts between mPSF and mCF. The presentation will describe the latest results from these new studies.

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44 U1snRNP protects transcripts from incorrect poly(A) site usage via modulation of the C-terminal domain of RNA polymerase II

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The maturation of mRNAs is a complex process that involves multiple steps and proteins. One of them is U1snRNP, which is essential for splicing, but also inhibits premature cleavage and polyadenylation (PCPA). Though, the exact mechanism remains elusive. Our group studies disease causing mutations that create new 5' splice sites in 3'UTRs, leading to binding of U1snRNP and therefore to the suppression of the authentic polyadenylation signal (PAS). Not correctly processed mRNAs are degraded resulting in decreased mRNA levels. To this end we created stable inducible cell lines harboring these mutations. Using chromatin immunoprecipitation (ChIP) we discovered that in the context of the mutation within a short gene the phosphorylation pattern of the C-terminal domain (CTD) of RNA polymerase II is altered. Here, binding of U1snRNP induces higher Ser5 and Ser2 phosphorylation levels. This seems to be unfavorable for 3' end processing where usually a low Ser5-P/Ser2-P ratio is observed. However, a longer gene where the full extent of CTD phosphorylation unfolds until transcription reaches the mutant site did not show alterations in the Ser5-P/Ser2-P ratio. Still in both genes, we observed delayed unloading of Pcf11, which is a part of cleavage factor IIm. Thus, suppression of PCPA functions via a transient modulation of the CTD or associated factors transmitting a signal to RNA polymerase II not to deposit 3' end processing factors. We hypothesize that U1snRNP interacts with one or more of the cyclin-dependent kinases that are known to phosphorylate the CTD. One promising candidate is CDK12, which is involved in suppression of intronic polyadenylation (Dubbury et al., 2018, Nature). We used a specific CDK12/ CDK13 inhibitor, which surprisingly did not restore the wildtype phenotype but rather induced a complete suppression of 3' end processing and RNA decay in a U1-dependent manner. Importantly, the density of engaged RNA pol II complexes did not change. Possibly the simultaneous activation of CDK12 by U1snRNP and inhibition of the kinase activity poisoned the processing complex. To further investigate this, we are currently using the inhibitor in our ChIP experiments and in addition performing CDK12/13 knock down/out experiments.

45 CDK11 is required for transcription of replication-dependent histone genes

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Expression of canonical, replication-dependent histones (RDH) is highly upregulated during S-phase when they package the newly synthesized DNA. We used iCLIP, which identifies protein-RNA interactions at nucleotide resolution, to study the function of the cyclin-dependent kinase 11 (CDK11), an essential gene in several cancers. CDK11 directly binds RNA via its N-terminal region, which lacks any canonical RNA binding domain. It predominantly binds the RDH mRNAs, and is required for their efficient expression. ChIP-seq revealed strong enrichment of CDK11 on RDH genes, with strongest binding in S phase, which depended on RNA and active transcription. CDK11 phosphorylates serine 2 (Ser2) of the C-terminal domain (CTD) of RNA polymerase II (RNAPII) and its depletion reduced RNAPII association with RDH genes. Altogether, CDK11 acts as a Ser2 CTD kinase specifically at RDH genes to control their optimal transcription.

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46 Structural Basis of Transcription: RNA Polymerase backtracking and its reactivation

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Regulatory DNA sequences or erroneous incorporations into the RNA during DNA transcription cause RNA polymerase backtracking, and inactivation in all kingdoms of life. Reactivation requires RNA transcript cleavage to realign the newly generated 3'-end with the active site. Essential transcription factors (bacterial GreA/GreB, or eukaryotic TFIIS) accelerate this reaction. We report four cryo-EM reconstructions of *Escherichia coli* RNA polymerase representing the entire reaction pathway: A backtracked complex (i); a backtracked complex with GreB before (ii), and after (iii) RNA cleavage; and a reactivated, substrate bound complex with GreB before RNA extension (iv).

Compared with eukaryotes, the backtracked RNA adopts a different conformation. RNA polymerase conformational changes cause distinct GreB states: i) a fully engaged GreB before cleavage, which is stabilized in the RNA polymerase active site by the backtracked RNA; ii) a disengaged GreB after cleavage; and iii) a dislodged, loosely bound GreB removed from the active site to allow RNA extension. These reconstructions give insights on the catalytic mechanism and dynamics of RNA cleavage and extension, and suggest how GreB targets backtracked complexes without interfering with canonical transcription. In addition, the backtracked RNA may play a role in cleavage by coordinating the nucleophile (in the backtracked complex), and stabilizing GreB for cleavage (in the GreB bound pre-cleavage complex).

47 Transcription initiation defines both mRNA termini in mitochondria of trypanosomes

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Digenetic hemoflagellate *Trypanosoma brucei*, a causative agent of African human trypanosomiasis, belongs to *Kinetoplastea*, a taxonomic class defined by possession of a kinetoplast. This nucleoprotein body contains mitochondrial DNA (kDNA) of two kinds: maxicircles encoding rRNAs, ribosomal proteins and subunits of respiratory complexes, and minicircles bearing guide RNAs required for pre-mRNA editing. Maxicircles and minicircles are interlinked and packed into a dense disc-shaped network by association with histone-like proteins. Decades of kDNA studies unraveled fascinating phenomena of general biological significance, such as DNA bending and U-insertion/ deletion mRNA editing, and revealed exquisite details of genome replication, and mRNA editing and translation. However, the mechanisms of transcription initiation and generation of monocistronic mRNAs remain virtually unexplored. Contrary to the enduring view of polycistronic transcription as a prevailing RNA synthesis mode, we present evidence that individual maxicircle protein-coding genes are independently transcribed into 3′ extended precursors. The transcription-defined 5′ terminus is converted into monophosphorylated state by the novel pyrophosphohydrolase complex, termed the PPsome. Composed of MERS1 NUDIX enzyme and MERS2 pentatricopeptide repeat RNA binding subunit, the PPsome binds to specific sequences near mRNA 5′ termini. Most guide RNAs lack PPsome recognition sites and remain triphosphorylated. RNA editing substrate binding complex (RESC) stimulates MERS1 hydrolase activity and enables an interaction between the PPsome and the polyadenylation machinery. We provide evidence that both 5′ pyrophosphate removal and 3′ adenylation are essential for mRNA circularization, a molecular basis of mitochondrial mRNA stability. Furthermore, we uncover a mechanism by which antisense RNA-controlled 3′-5′ exonucleolytic trimming defines the mRNA 3′ end prior to adenylation. We conclude that mitochondrial mRNAs are transcribed and processed as insulated units irrespective of their genomic location. Importantly, both 5′ and 3′ ends of mature mRNAs are defined by transcription initiation on sense and antisense strands, respectively. These findings introduce a concept of mitochondrial gene-specific transcriptional control with broad implications in developmental transitions and pathogenesis.

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48 The transcription and splicing modulator HP1γ tethers pre-mRNA to chromatin via intronic repeated sequences

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HP1 proteins are readers of the H3K9me3 histone modification associated with repression. As such, they are best known for their role in gene silencing and structuring of heterochromatin. Yet, HP1 proteins are also RNA-binding proteins and the HP1γ/Cbx3 family member is largely present both at promoters and inside the coding region of transcribed genes, together with RNA polymerase II, where it regulates transcription initiation and alternative splicing. To gain insight in the role of the RNA binding activity of HP1γ in transcriptionally active chromatin, we have captured and analyzed RNAs associated with this protein. We find that a prerequisite for RNAs to be bound by HP1γ is the presence of sequences derived from certain families of transposable elements and that HP1γ is part of a mechanism that tethers the nascent RNA to the chromatin template via these repeated sequences. As introns contain repeats while exons are repeat-free, this tethering via HP1 ceases as soon as introns are excised, allowing for mRNA release from the chromatin template. Along the same logic, because enhancers are located in repeat-free regions, enhancer RNAs are not retained on chromatin, a mechanism that in part may allow them to escape maturation. Altogether, our data unveils an unsuspected shaping of the distribution of repeats by the constrains of transcription. They also re-unite the heterochromatic and euchromatic functions of HP1s by documenting how these proteins, in association with repeat sequences, can participate in the structuring of heterochromatin while also playing a role in discriminating introns from exons.

49 Distinct roles for the polyA+ and polyA- H2a.X mRNA isoforms throughout the cell cycle

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The H2a.X gene (H2AFX) gives rise to both polyA- and polyA+ mRNA. The former is S-phase-specific ending in the 3' stem-loop structure typical of replication-dependent histone mRNA, whereas the latter is generated by cleavage and polyadenylation, like most eukaryotic mRNA. Despite being translated from polyA+ and polyA- mRNA isoforms, H2a.X is generally considered a replication-independent histone. Using RNAi to deplete the polyA+ isoform and CRISPR-Cas9 to delete the histone 3' processing signals, we demonstrate that both H2a.X mRNA isoforms play important roles. Depletion of H2a.X polyA+ mRNA in HeLa cells or RPE cells arrested cells in G1 results in decreased phospho (γ)-H2a.X signalling, and cell cycle arrest due to accumulation of unrepaired DNA damage that delays S-phase entry. Removal of polyA- H2a.X mRNA from exponentially growing cells using CRISPR, results in a drastic decrease in γ-H2a.X signalling, both in HCT116 cells and HeLa cells, likely due to a decrease of H2a.X protein in chromatin. H2a.X is less stable than the canonical H2a protein, and during repair of DNA damage newly synthesized H2a.X protein must be incorporated into chromatin. The polyA+ isoform provides the only de novo H2a.X source outside of S-phase but it is likely that it also generates an important pool of H2a.X protein that is incorporated into chromatin during S-phase. Preliminary data suggest that H2a.X polyA+ and polyA- mRNA isoforms are translated by distinct mechanisms. Translation of polyA- mRNA is inhibited under DNA damage, whereas polyA+ mRNA is still translated. Thus polyA+ mRNA is the major source of de novo H2a.X protein synthesis under DNA damage conditions, independent of the cell cycle phase. In conclusion, deletion of either H2a.X polyA+ or polyA- mRNA has clear phenotypic consequences. Thus H2a.X plays a key role both in and outside of the S-phase. This suggests that H2a.X must be available for incorporation into nucleosomes during DNA replication in S-phase and also when DNA damage repair requires de novo nucleosomal H2a.X deposition.

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50 HnRNP Proteins Organise Human pre-mRNAs into 40S Ribonucleosome Units

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The concept of the eukaryotic ribonucleosomes that were proposed to condense pre-mRNA analogously to nucleosomes condensing DNA emerged in the 1980s (1). Initially discovered more than 50 years ago (2), 40S ribonucleosomes were later shown to be composed of heterogenous nuclear ribonucleoproteins (hnRNPs) (3). Due to the lack of sensitive high throughput "-omics" techniques, the structural and molecular analysis of these large particles was limited at that time and subsequent research focused on characterisation of single hnRNPs. Equipped with today's state-of-the-art technology, we decided to revisit and comprehensively characterise the 40S ribonucleosome and its functions. Affinity purification combined with rate zonal centrifugation yielded highly enriched 40S ribonucleosomes of which we measured the relative abundance of its constituents using label-free quantitative mass spectrometry. This analysis identified abundant hnRNP ‘core proteins', as well as several uncharacterised protein components. In addition, we extracted and sequenced ribonucleosome-protected RNA fragments, which revealed that ribonucleosomes are preferentially bound in a non-random fashion to intronic sequences of transcripts emerging from protein coding genes. This corroborated the original hypothesis that ribonucleosomes function in pre-mRNA condensation by potentially bringing exons closer together for splicing. Furthermore, we are applying cryo- and negative stain electron microscopy to decipher the structural organisation of these particles at high resolution. Taken together, we provide new insights into the 40S ribonucleosome composition, architecture and RNA binding spectrum. In addition, we provide evidence for its role in intron definition prior to splicing.

References

1. Chung SY, Wooley J. Set of novel, conserved proteins fold pre-messenger RNA into ribonucleosomes. Proteins. 1986 Nov; 1(3): 195-210.

2. Samarina OP, Krichevskaya AA, Georgiev GP. Nuclear ribonucleoprotein particles containing messenger ribonucleic acid. Nature. 1966 Jun; 210(5043): 1319-22.

3. Beyer AL, Christensen ME, Walker BW, LeStourgeon WM. Identification and characterization of the packaging proteins of core 40S hnRNP Particles. Cell. 1977 May; 11(1): 127-38.

51 RNA-mediated regulation of transcription: epigenetic marks and RNA regulate the histone methyltransferase PRC2 through the same functional centre

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Polycomb repressive complex 2 (PRC2) is a histone methyltransferase that maintains the repressed state of thousands of genes during development in all metazoans. PRC2 binds to thousands of transcripts, while interactions with RNA inhibit the enzymatic activity of PRC2. We previously showed that PRC2 binds to RNA promiscuously [1], with variations of affinities between target transcripts [2] that can be attributed to short repeats of consecutive guanines and G-quadruplex-forming sequences [3]. Observations of high-affinity and inhibitory interactions between PRC2 to RNA opened a mechanistic conundrum: how can PRC2 methylate histones within the RNA-rich environment of the nucleus?

Using the RBDmap approach - for mapping protein-RNA interactions using crosslinking with mass-spectrometry - complemented with mutagenesis, we recently identified a major RNA-interaction region within the regulatory centre of PRC2 [4]. This observation was reinforced by crosslinking experiments using 4-thiouridine-labelled RNA. Importantly, the RNA-binding site of PRC2 was previously shown to interact with methylated histone-tail peptides that lead to allosteric activation of PRC2 at repressed genes. Accordingly, we show that upon binding of methylated histone tails to the regulatory centre of PRC2, RNA-mediated inhibition is alleviated, allowing PRC2 to modify nucleosomes. Enzymatic and binding assays combined with additional crosslinking with mass spectrometry - to map protein-protein interactions within different types of PRC2 complexes - reveal that the regulatory centre of PRC2 remains exposed and available to bind RNA while PRC2 forms complexes with most of its accessory subunit proteins. Our ongoing work reveals how RNA regulates PRC2 at the molecular level, within the context of transcriptional regulation and development.

References

[1] C. Davidovich, et al., *Nat Struct Mol Biol.* (**2013**); "*Promiscuous RNA binding by Polycomb repressive complex 2*."

[2] C. Davidovich, et al., *Mol Cell.* (**2015**); "*Toward a consensus on the binding specificity and promiscuity of PRC2 for RNA*."

[3] X. Wang, et al., T.R. Cech & C. Davidovich, *Mol Cell.* (**2017**); "*Targeting of Polycomb Repressive Complex 2 to RNA by Short Repeats of Consecutive Guanines*."

[4] Q. Zhang, et al., R. Bonasio & C. Davidovich. *Nat Struct Mol Biol.* (**2019**); "*RNA exploits an exposed regulatory site to inhibit the enzymatic activity of PRC2*."

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52 Precise small molecule cleavage of a r(CUG) repeat expansion in a myotonic dystrophy mouse model

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Designing small molecules that selectively target RNA structure and modulate RNA function remains challenging. Small molecules that selectively bind to the 1 x 1 nucleotide U/U internal loop found in the RNA repeat expansion ((rCUG)^{exp}) that is causative of myotonic dystrophy type 1 (DM1) improve DM1-associated defects in cells and in animals. DM1 is untreatable and the most common adult on-set form of muscular dystrophy. Herein we illustrate a novel approach in which an r(CUG)^{exp}-targeting small molecule is conjugated to the natural product bleomycin a5, a DNA-cleaving anticancer therapeutic, resulting in the selective cleavage of r(CUG)^{exp} over DNA in vitro. In DM1 patient-derived myotubes, the small molecule cleaves the mutant *DMPK* transcript containing r(CUG)^{exp}, resulting in significant improvement of DM1-associated splicing defects and a reduction of r(CUG)^{exp}-MBNL1 nuclear foci. Importantly, the compound can discriminate between the structure of the long r(CUG)^{exp} repeat and other mRNAs containing shorter r(CUG) repeats, whereas an antisense oligonucleotide targeting the sequence of r(CUG)^{exp} affects the abundance of all r(CUG)-containing transcripts regardless of secondary structure. Further, the small molecule is an RNA selective cleaver as it cleaves r(CUG)^{exp} without affecting DNA or activating the DNA damage response pathway. In a mouse model of DM1, small molecule cleavage results in significant improvement of myotonia and DM1-associated defects. RNA-seq analysis from mouse muscle demonstrates that the small molecule broadly improves DM1-associated splicing defects and gene expression changes without off-target effects. With these studies, we demonstrate that small molecules can selectively target an RNA structural element and the cleavage of such structures offers a promising approach to modulate RNA repeat expansion biology from cells to pre-clinical animal models of human disease.

53 Inhibition of Nonsense-Mediated mRNA Decay in Cystic Fibrosis

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The W1282X nonsense mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene causes a severe form of cystic fibrosis (CF), but current CF treatments are not adequate for patients with this mutation. The truncated CFTR-W1282X protein has residual activity, but it is expressed at a very low level, due to nonsense-mediated mRNA decay (NMD). Thus, a gene-specific NMD inhibition strategy may lead to an effective allele-specific therapy for CF. NMD requires the binding of protein complexes called exon junction complexes (EJCs) on spliced mRNA. An EJC bound downstream of a premature-termination codon (PTC) strongly enhances NMD of the target mRNA. Other studies and our unpublished data suggest that the CFTR-W1282X mRNA harbors multiple NMD-inducing EJCs. Previously, we showed that synthetic antisense oligonucleotides (ASOs) designed to prevent binding of multiple EJCs downstream of PTCs attenuate NMD in a gene-specific manner. These results suggested that a cocktail of ASOs could be used for stabilizing mRNA harboring certain disease-causing nonsense mutations. Using CFTR minigene NMD reporters, we identified lead ASOs that efficiently target individual EJCs downstream of the W1282X mutation. Combining the three lead ASOs specifically increases the expression of endogenous CFTR W1282X mRNA and CFTR protein in transfected human bronchial epithelial cells. All three EJCs >50 nt downstream of the nonsense mutation have to be targeted for effective NMD inhibition by ASOs. These results set the stage for the development of an allele-specific therapy for CF caused by the W1282X mutation.

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54 Reversal of pleotropic effect of toxic RNA with expansion of CGG repeats by short antisense oligonucleotides

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Fragile X-associated Tremor-Ataxia Syndrome (FXTAS) is a dominant, progressive neurodegenerative disorder, which is caused by RNA gain of function of expanded CGG repeats (CGG^{exp}). Expandable trinucleotide repeat tract is localized within the 5'-untranslated region (5'UTR) of the *FMR1* gene encoding FMRP protein. FXTAS patients show specific manifestation of clinical symptoms that include intention tremor, gait ataxia cognitive deficits and brain atrophy. As of now, the CGG^{exp}-induced sequestration of the important component of microprocessor (DGCR8) and alternative splicing regulator (SAM68) as well as accumulation of the aggregated form of polyglycine protein (FMRpolyG), which is a product of repeat-associated non-ATG codon (RAN) translation of long CGG repeats are considered to be main triggering factors of neurodegenerative processes in FXTAS. Since the causative molecular targets are well defined, FXTAS is highly amenable to the development of RNA targeting therapy. We examined the capacity of antisense oligonucleotides (ASOs) targeting nuclear and cytoplasmic fraction of CGG^{exp} RNA as potential therapeutic agents. Short ASOs composed of only nine or eleven LNA units (locked nucleic acids) bind to the CGG^{exp} with very high affinity. These ASOs were delivered to FXTAS patients derived cells *via* unassisted uptake or directly into brain of mouse model expressing the transgene with 90 CGG repeats exclusively in neural cells *via* intracerebroventricular administration. ASOs dispersed nuclear foci of CGG^{exp} RNA and reduced the overall burden of this toxic RNA. As DGCR8 and SAM68 were released from sequestration, the total level of miRNAs increased significantly and defect of alternative splicing regulation was corrected. The treatment decreased biosynthesis of soluble form of FMRpolyG and accumulation of its insoluble form within nuclei, but also interfered translation of native FMRP protein. Importantly, reduction of FMRpolyG intranuclear inclusions in the cerebellum and amygdala positively correlates with behavioral and molecular manifestations of clinically relevant features of FXTAS. Our data demonstrate that short ASOs rescue both nuclear and cytoplasmic effects of toxic CGG^{exp} and can be consider as therapeutic strategy in FXTAS.

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55 Ribosome meets RISC at expanded CAG repeat tract - allele-selective RNAi approach for therapy of polyglutamine diseases

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Polyglutamine (polyQ) diseases are neurological disorders caused by CAG repeat expansion in ORFs of specific genes. This group includes Huntington's disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA) and several spinocerebellar ataxias (SCA). RNAi-based targeting of mutation site is an attractive therapeutic option for polyQ diseases. We designed CAG repeat-targeting siRNAs, with specific base substitutions that make them similar to miRNAs, and showed preferential decrease of mutant protein level in cellular models of HD, SCA3, SCA7 and DRPLA. Now we have investigated mechanism of activity of these allele-selective siRNAs in HEK293-based cell lines and HD iPSC-derived human neural progenitors.

In one type of cellular constructs we placed CAG repeat tract within specific sequence context at different locations of luciferase sequence. Silencing preference of mutant transcripts was obtained for repeats located within ORF but not for 3'UTR region, which is typical target location for microRNAs. Also, we found that specific huntingtin gene (HTT) sequence surrounding the repeat tract positively affects allele-selectivity of silencing.

Moreover, we developed Flp-In T-REx 293 models with inducible expression of the first exon of huntingtin with normal or mutant repeat tract fused with Nano-luciferase. These cell lines were used for precise investigation of kinetics of the silencing process as well as to study translation inhibition initiated by CAG repeat-targeting siRNAs, also by polysome fractionation. We found that during the inhibition of mutant HTT these siRNAs cause translational repression, which precedes slight mRNA decay.

Additionally, to characterize targeted transcripts we performed their quantification in fibroblast cells using digital droplet PCR (ddPCR) and single-molecule fluorescent in situ hybridization (smFISH). HTT transcripts abundance and location was also investigated in HD neural progenitors after treatment of cells with selected siRNAs.

We show that activity of atypical CAG repeat-targeting siRNAs is more similar to the activity of miRNAs, especially those acting in a cooperative manner and targeting ORF regions. Moreover, these siRNAs offer allele-selective therapeutic strategy for several polyQ diseases.

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56 A specialized post-transcriptional program in chemoresistant, quiescent cancer cells

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Quiescent (G0) cells are a clinically relevant fraction in cancers, which include dormant cancer stem cells, and resist clinical therapy. G0 cells reveal extensive changes in gene expression at the protein and translation levels. We previously identified that the translation mechanism is altered in G0 cancer cells. We find that G0 leukemic cells show similar proteome and translatome to cells isolated post-chemotherapy. These data suggest that specialized post-transcriptional mechanisms in G0 leukemic cells regulate a distinct translatome to mediate chemoresistance.

To understand the role of post-transcriptional regulation in chemoresistance, we compared global transcriptome, translatome and proteome profiling in chemoresistant G0 acute monocytic leukemic (AML) cells. We find that chemotherapy or G0 induction leads to DNA damage responsive ATM and stress signaling, which alter post-transcriptional and translational mechanisms. ATM and stress activated p38 MAPK/MK2 increase AU-rich-element (ARE) bearing pro-inflammatory cytokine and immune gene mRNAs, by regulating a key ARE RNA binding protein and modifying canonical translation. AREs are present on 3'UTRs of tightly regulated oncogenes and cytokines, to post-transcriptionally control their expression. Both rate limiting steps-mRNA cap recognition and tRNA recruitment-in canonical translation are altered. These signaling pathways lead to low mTOR activity in G0, which activates the cap complex inhibitor, eIF4EBP to impair canonical translation, leading to non-canonical translation of specific mRNAs with specialized cap binding and ribosome recruitment factors. In addition, stress and interferon signaling are activated to reduce the canonical tRNA recruitment mechanism, enabling non-canonical translation of specific mRNAs.

These changes permit translation of ARE bearing pro-inflammatory cytokine TNFα, and immune and cell-migration modulators that promote survival. Co-inhibiting p38 MAPK and TNFα that promote anti-apoptosis-prior to or along with chemotherapy-decreases chemoresistance in AML cells, in vivo, and in patient samples, without affecting normal cells. Our studies reveal a pro-inflammatory subpopulation in AML that mediates resistance, enabled by DNA damage- and stress-regulated post-transcriptional and translational mechanisms that are mediated by AREs and a critical ARE RNA binding protein. Disrupting ARE regulation reduces TNFα and chemoresistance. These studies reveal the significance of post-transcriptional regulation of pro-inflammatory and immune gene-mediated chemoresistance.

57 RNAi screen revealed mitochondrial RNA degrading and processing enzymes as factors controlling human mitochondrial double-stranded RNA, a potent trigger of inappropriate interferon response

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Recently we have discovered that transcription of the mitochondrial genome is a major source of double-stranded RNA in humans. These RNA species can be released from mitochondria to cytoplasm resulting in activation of the innate immune system (Dhir et al., Nature, 2018). Importantly, we found that cells from patients with mutations in PNPase encoding gene accumulate mitochondrial dsRNA (mt-dsRNA), which is accompanied with activation of antiviral interferon response, partially explaining the pathogeneses of patients' diseases. To identify other factors involved in mt-dsRNA metabolism, we performed an siRNA screening. We screened 270 genes encoding proteins involved in different aspects of mitochondrial biology and observed several cases of up- and down-regulation of mt-dsRNA. In addition to the mitochondrial degradosome core components (PNPase and RNA helicase SUV3), one of the most prominent hits was a mitochondrial poly(A) polymerase, MTPAP, an enzyme mutated in spastic ataxia 4 autosomal recessive disorder. Silencing of MTPAP led to the accumulation of dsRNA comparable to inactivation of degradosome subunits. In vitro reconstitution of dsRNA decay reactions showed that polyadenylation is required for degradosome-mediated degradation of blunt mt-dsRNAs, which was further supported by in vivo experiments. Interestingly, our in vitro biochemical assays also revealed that disease-causing mutation in MTPAP not only inhibits polyadenylation of the 3' end of RNA substrate but also blocks its processing by other proteins, indicating that occupation of RNA's end by mutated MTPAP can contribute to pathogenesis of spastic ataxia. In conclusion, our study revealed the decay pathway of dsRNA in mitochondria and allowed us to identify several other factors involved in mt-dsRNA metabolism. Their further investigation, together with ones identified in the genome-wide screen which we completed recently, may reveal new aspects of mtRNA metabolism and extend the repertoire of mechanisms by which mitochondria control the fate of the cell and contribute to human health.

Dhir A, Dhir S, Borowski LS, Jimenez L, Teitell M, Rötig A, Crow YJ, Rice GI, Duffy D, Tamby C, Nojima T, Munnich A, Schiff M, de Almeida CR, Rehwinkel J, Dziembowski A, Szczesny RJ, Proudfoot NJ. Mitochondrial double-stranded RNA triggers antiviral signalling in humans. Nature, 2018, PMID: 30046113.

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58 Manipulating RNA processing to enhance or inhibit HIV-1 gene expression/replication

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Control of RNA processing plays a central role in the expression and replication of HIV-1. From a single transcript, over 69 viral mRNAs are generated through the process of alternative RNA splicing. Disrupting the balance of spliced viral mRNAs results in dramatic alterations in virus replication. Control is mediated in part through the action of host SR proteins whose activity, in turn, is regulated by SR kinases (CLK1-4, SRPKs, DYRK1a). Our recent studies have demonstrated disparate roles for the CLK class of SR kinases in the modulation of HIV-1 gene expression: depletion of CLK1 enhancing HIV-1 gene expression, loss of CLK2 decreasing it and reduction of CLK3 having no effect. Altered viral protein expression was correlated with changes in abundance of the corresponding HIV-1 RNAs. To probe the basis for the differences in observed responses, we examined the impact of individual CLK depletion on SR protein abundance and modification. Consistent with their distinct roles in regulating HIV-1 gene expression, depletion of individual CLKs resulted in selective alteration of SR protein expression: loss of CLK1 reducing SRSF9 levels while depletion of CLK2 altered SRSF4&6 expression. Parallel analyses determined that changes in expression of these SR proteins results in marked changes in HIV-1 gene expression and confirmed their interaction with viral RNA. To determine whether similar modulation of SR kinase function with small molecules could be used to control HIV-1 replication, we screened inhibitors of the CMGC kinases and identified several able to suppress HIV-1 gene expression and replication with IC_{50}<50 nM. The most active of these compounds were shown to dramatically decrease HIV-1 protein and RNA accumulation with minimal impact on cell viability. The most active compounds were also potent inhibitors of CLK2 but not CLK3 activity *in cellulo*, consistent with the depletion studies described above. Together, these findings demonstrate the unique role individual SR kinases play in the regulation of HIV-1 RNA processing and the utility of selective CLK inhibitors as therapeutics for the treatment of viral infections.

59 System-wide identification of the cellular RNA-binding proteins that control the initial steps of HIV-1 infection

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After entry into the host cell, human immunodeficiency virus type 1 (HIV-1) RNA is reverse transcribed into DNA, imported into the nucleus and integrated into the cellular chromosome. It is well established that these processes are regulated by cellular RNA-binding proteins (RBPs). However, it remains unknown which RBPs are required and why. As reverse transcription occurs inside the capsid core, we hypothesise that RBPs involved in the early stages of HIV-1 infection must be loaded inside the viral particles together with the viral RNA. To identify the cellular RBPs that interact with HIV-1 RNA within particles, we have developed a new method, named 'in virion' interactome capture (ivRIC). It employs biochemical purification of the HIV-1 particles from the supernatant of infected CD4+ lymphocytes, followed by UV protein-RNA crosslinking, capture of the viral RNA and quantitative proteomics. Using ivRIC we identified 61 cellular RBPs that interact with HIV-1 RNA inside the capsid core. These proteins include classical and unconventional RBPs, one third of which has known although poorly understood links with HIV-1 biology. Moreover, 20% of the identified proteins have dual RNA/DNA-binding activity, which may reflect that HIV-1 genome transitions from RNA to DNA. Using single particle imaging we confirmed that the identified RBPs are loaded into viral particles and that they accompany the viral genome in its journey across the cell. Moreover, functional assays revealed that ivRBPs are critical for HIV-1 infectivity. Our data thus opens new avenues to understand the roles of cellular RBPs in HIV-1 infection.

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60 Misregulation of splicing factors in breast cancer initiation and metastasis

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

We identified splicing factors that are amplified and/or overexpressed in a collection of >1100 human breast tumors. Specific splicing factors were frequently altered in tumors and associated with the expression of distinct spliced isoforms, suggesting that splicing-factor alterations are likely to play a role in disease pathogenesis. We used an in vitro organotypic 3D culture model to dissect the functional consequences of splicing-factor overexpression on transformation of human mammary epithelial. By screening ten splicing factors we identified a subset of splicing factors that are oncogenic, differentially affecting cell proliferation, apoptosis, and/or mammary acinar architecture, and thus suggesting non-redundant functions. We defined the repertoire of splicing-factor regulated alternatively spliced isoforms using RNA-sequencing, and defined the target specificities of SR proteins in human breast cancer cell lines, patient-derived xenograft models and breast tumors. Strikingly, SR proteins promoting a similar cell phenotype regulate a shared subset of spliced isoforms in breast cancer. Furthermore, we identified specific splicing factors controlling cell invasion and metastasis in breast cancer models in vitro and in vivo, and correlated their expression with increased metastatic incidence in breast cancer patients. Finally, we defined the transcriptional and post-transcriptional regulatory mechanisms that control splicing factor expression in normal mammary cells and investigated their misregulation in cancer cells.

In summary, we gained new insights into the regulatory mechanisms of SR proteins and identified novel oncogenic spliced isoforms that represent potential biomarkers and targets for therapeutics development.

61 Human-specific circular Tau RNAs as new causes for Neurodegeneration leading to Alzheimer's Disease and Frontotemporal Dementia

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The Tau protein, encoded by the Microtubule-Associated Protein Tau (*MAPT*) gene, is the major component of the intracellular filamentous deposits that define many neurodegenerative diseases (Tauopathies) and is a critical component in the etiology of Alzheimer's disease. Tau exon 10 is alternatively spliced and its usage is deregulated in Alzheimer's disease. Further, mutations changing alternative splicing of *MAPT* exon 10 cause Frontotemporal Dementia linked to chromosome 17 (FTDP-17), underscoring the importance of tau pre-mRNA processing.

We have identified human-specific circular RNAs (**circRNAs**) formed by the tau-encoding pre-mRNA from the *MAPT* locus generated by backsplicing of exon 12 to either exon 10 or 7. 46 of the 53 mutations leading to FTDP-17 will affect exons 10 to 12. The circular RNAs formed by these exons contain an open reading frame without a stop codon. Thus, if translation initiates, both circRNAs could form high molecular weight multimers containing the microtubule binding domains of the tau protein.

We found that human tau circRNAs are concentrated in the synaptosomes of human brain tissue and might be upregulated in late stages of Alzheimer's disease. We generated transgenic zebrafish expressing only the tau circRNAs, which resulted in aberrant neuronal sprouting and neurite shape suggesting a physiological relevance of tau circRNAs. Importantly, the introduction of an in-frame start codon (K317M) that causes frontotemporal dementia in humans causes early neurodegeneration in zebrafish, suggesting translation of the tau circRNAs may be occurring in neurons.

The significant difference between the human tau gene generating circular RNAs and rodent species lacking these circRNAs are human-specific Alu-elements. To identify RNA elements controlling exon 10 usage and circRNA formation, we generated reporter genes containing all human exons and up to 20 Alu-elements. Exon 10 usage can form both linear and circRNAs, which is influenced by downstream exons (exon 13) and the presence of Alu-elements.

In summary, our data suggest that circular tau RNAs might be involved in tauopathies, possibly by being aberrantly translated at the synapse. Aberrant usage of exon 10, long known to be associated with various neurodegenerative disorders could act through circular RNAs, which is a new and human-specific disease mechanism.

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62 Metabolic and chemical regulation of tRNA modification under physiological and pathological conditions

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It has been generally thought that tRNA modifications are stable and static, and their frequencies are rarely regulated. We previously reported that lack of tRNA modification causes major classes of mitochondrial diseases including MELAS and MERRF. Deficient tRNA modification results in defective protein synthesis, leading to mitochondrial dysfunction. These findings provided the first evidence of human disease caused by an RNA modification disorder. We call "RNA modopathy" as a new category of human diseases. I am going to show our recent studies on tRNA modifications associated with human diseases and their dynamic regulation by sensing intracellular metabolites, such as aminoacids, AdoMet, and CO_2, under physiological condition.

63 Effect of tRNA composition in ORF selection and proteostasis in *Drosophila*

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Genome expression strongly relies in the accurate flux of information from DNA to proteins. In this process tRNA composition plays an instructive role decoding the transcriptome of the cell. Consequently, how isoacceptor abundance and tRNA modifications differentially influence protein translation is a relevant question that only recently has begun to be addressed. Furthermore, the importance of this layer of regulation in the development of multicellular organisms is almost completely unknown. We have explored the expression of tRNA loci in different cell types of *Drosophila*, and also investigated the impact of t6A and m6t6A modifications in ANN decoding tRNAs in open reading frame (ORF) selection and proteostasis. Our previous results show that levels of t6A, particularly in the initiator tRNA, determine cellular and animal growth by regulating mTOR activity. Also, t6A deficiencies impair translation inducing the unfolded protein response (UPR). Here we show that similar results are obtained decreasing the levels of TrmO, enzyme responsible of m6t6 modification. Expression and functional analysis of the enzymes that synthetize t6A and m6t6 reveal that they are not transcribed and required equally in different cell types. Additionally, *in vivo*experiments show that levels of t6A affect ORF selection in a Kozak site dependent manner and this selectivity could be modulated by functional interactions with eIF1 and eIF5. Together our results highlights the variations in tRNA composition in different cell types and suggest how the integration of this layer of translational control in multicellular organisms could be part of the expression program during development as it is in response to environmental conditions.

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64 Expanded tRNA Diversity in Mammals Indicates New Conserved Functions

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A "grand challenge" in tRNA biology is to identify and study the full range of functions of all human tRNA genes and their hyper-processed RNA transcripts. With the advent of comparative genomics, chromatin state analysis, and powerful new tRNA-seq methods, we now have a first glimpse at the complexity of human tRNA evolution, function, and gene regulation. Of the ~600 human tRNA loci, we estimate just 19% are constitutively transcribed, 38% are tightly regulated, and the remaining apear mostly silent according to broad chromatin immunopreciptation data. These different genes are influenced by chromosomal neighborhood (transcription factor binding sites, variation in transcription termination signals, and nearby protein coding genes), as well as subtle differences in internal sequences that affect transcription, folding, and modification. For example, out of 15 Gly-GCC genes, only one is constitutively active, four are regulated, and ten appear to be silent. However, four "silent" Gly-GCC genes are deeply conserved, suggesting other biologically important functions including transcriptional interference, establishment of chromosomal insulators, and possible production or interaction with regulatory tRNA-derived RNAs (tDRs). New high-throughput sequencing of full length tRNAs and tDRs across human and mouse tissues bears out previously suggested diversity of function among different isodecoders (tRNAs with the same anticodon but different sequences), based on different relative abundances and RNA modification profiles across cell types and genetic backgrounds. Intriguingly, it appears that mammalian evolution has produced conserved new isodecoder mixes of multiple but consistently present variants, expanding functional profiles of Gly, Leu, Arg, and other isotypes that appear to enable newly-evolved but tightly-regulated, specialized functions. Taken together, we are building a comprehensive new comparative atlas of tRNA function that integrates these many different types of computational and experimental data to help piece together the increasingly complex picture of tRNA function and evolution.

65 Discovery of a unique RNA 2′,3′-cyclic phosphatase in human cells

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RNA terminal 2’,3’-cyclic phosphates arise as a result of endonuclease cleavage, exonuclease trimming, or *de novo* synthesis. These RNA species are essential intermediates in mammalian cells as they are substrates for the tRNA ligase complex during pre-tRNA processing and *XBP1*mRNA splicing in the Unfolded Protein Response. Terminal 2’,3’-cyclic phosphates can be hydrolyzed into 2’-phosphate, 3’-hydroxyl or 2’-hydroxyl, 3’-phosphate. The only enzyme known to fully convert 2’,3’-cyclic phosphates into 2’,3’-hydroxyl is T4 polynucleotide kinase-phosphatase, but such activity has not yet been detected in eukaryotic cells. Here, we report the identification of the first RNA 2’,3’-cyclic phosphatase in eukaryotic cells through activity-guided purification from HeLa cell extracts, that converts 2’,3’-cyclic phosphates as well as 2’-phosphates present in single- and double-stranded RNA termini into 2’,3’-hydroxyl. Given that 2’,3’-cyclic phosphates are the preferred substrates of the human tRNA ligase complex, we show that mis-expression has a measurable effect in the efficiency of pre-tRNA processing and *XBP1* mRNA splicing during the UPR. Our results foresee a general impact of this unique mammalian enzyme on RNA stability and turnover, as well as in other RNA pathways that rely on the ligation or hydrolysis of 2’,3’-cyclic phosphates.

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66 Modulation of mammalian translation by the tRNA^{Pro} half

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In recent years our group analyzed the small ncRNA interactome of translating ribosomes in various model organisms. A major fraction of these ribosome-associated ncRNAs are processed from longer functional precursors, such as mRNAs, rRNAs, snoRNAs or tRNAs. Functional analyses of selected tRNA-derived RNAs (tdRs) revealed an unexpected functional heterogeneity [1]. One of the investigated archaeal tdR associates with the small ribosomal subunit and inhibits translation initiation by competing with mRNA binding [2]. In the parasite *T. brucei* another tdR associates with polysomes under nutritional stress to stimulate global translation during stress recovery [3]. In several mammalian cell lines we uncovered tRNA^{Pro} 5’ half which has a completely different function. Northern blot analysis revealed the presence of the tRNA^{Pro} 5’ half in the polysomal fractions. The ribosome association was also confirmed via filter binding assays *in vitro* and showed preferential binding to the large ribosomal subunit. Addition of synthetic tRNA^{Pro} half in *in vitro* translation reaction showed unique effects on translation in several species/cell lines including yeast, CHO, HEK, rabbit reticulocytes, and HeLa cells. Addition of the tRNA^{Pro} half inhibits global translation and causes upregulation of a specific low molecular weight translational product consisting of both RNA and amino acids. Transfection of the synthetic tRNA^{Pro} half into HeLa cells lead to formation of the same product *in vivo*. The migration of the product in acidic gels, insensitivity to copper sulfate treatment, insensitivity to 3’ polyadenylation, and association with 80S monosomes suggest that the accumulated product is peptidyl-tRNA. Our data suggest that binding of the tRNA^{Pro} 5’ half to the ribosome leads to ribosome stalling, consequent translation inhibition and formation of peptidyl tRNA. Originally considered meaningless degradation products, tdRs have proven over the recent past to be crucial players in orchestrating gene regulation. Our findings reveal ribosome-bound tdRs as functionally multifaceted ribo-regulators capable of fine-tuning protein biosynthesis.

(1) Cristodero M. & Polacek N., *Non-coding RNA Invest*. (2017)

(2) Gebetsberger J. et al., *RNA Biol.* (2017)

(3) Fricker R. et al., *Nature Comm.* (2019)

67 Conserved uridines 54 and 55 of human tRNAs are modified by different enzymes in the nucleus and the cytoplasm

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The conserved uridine (U) 55 of tRNAs is nearly always modified to pseudouridine (Ψ). Orthologs TruB and Pus4 produce Ψ55 in Bacteria and yeast, respectively. Humans have two paralogs of TruB, TruB1 and TruB2 that produce Ψ55. TruB1 is reported to be present in both nucleus and cytoplasm. TruB2 is predicted to produce Ψ55 in mitochondrial tRNAs. Nearly conserved U54 of tRNAs is commonly modified to ribothymidine (T) in Bacteria and eukaryotes and mostly to Ψ in Archaea. The T54 is produced by the orthologs TrmA and Trm2 in Bacteria and yeast, respectively. Again, human cells contain two paralogs of Trm2, TrmT2A and TrmT2B that are active in the nucleus and mitochondria, respectively. Archaeal Pus10 produces both Ψ54 and Ψ55. Certain human tRNAs contain Ψ54 instead of T54. Previously, we showed that, although human Pus10 is present in both nucleus and cytoplasm, the cytoplasmic Pus10 produces Ψ54 in these tRNAs by recognizing specific structural features of their TΨC loops.

Here we show that recombinant TruB1, Pus10 and TruB2 as well as nuclear, cytoplasmic and mitochondrial extracts of human cells can produce Y55 in most tRNAs. Individual knockdown of TruB1, Pus10 and TruB2 causes a reduction in Ψ55 activity of nuclear, cytoplasmic and mitochondrial extracts, respectively. Requirements of the structure of TΨC loop vary for TruB1, Pus10 and TruB2 activities, and these structural requirements are reflected in the activities of nuclear, cytoplasmic and mitochondrial extracts, respectively. These data suggest that there is a compartmentalization of Ψ55 synthase activities amongst these three proteins. In vitro, Pus10 specifically binds the tRNAs in which U54 can be converted to Y54. Pus10 also inhibits TruB1-mediated U55 to Y55 conversion in these tRNAs. Therefore, we suggest that the human tRNAs that contain Ψ54 are blocked from the actions of nuclear TruB1 and TrmT2A, probably by the nuclear Pus10, and both U54 and U55 in these tRNAs are converted to Ψ's by the cytoplasmic Pus10. Overall our results suggest that U54 and U55 of certain tRNAs are modified in the cytoplasm and those of the others in the nucleus.

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68 Time-resolved NMR monitoring of tRNA maturation

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Although the biological importance of post-transcriptional RNA modifications in gene expression is widely appreciated, methods to directly detect the introduction of these modifications during RNA biosynthesis are rare and do not easily provide information on the temporal nature of events. Here we introduce the application of NMR spectroscopy to observe the maturation of tRNAs in cell extracts. By following the maturation of yeast $tRNA^{Phe}$ with time-resolved NMR measurements, we found that modifications are introduced in a defined sequential order, and that the chronology is controlled by cross-talk between modification events. In particular, we uncovered a strong hierarchy in the introduction of the T54, Ψ55 and m^1A58 modifications in the T-arm, and demonstrate that the modification circuits identified in yeast extract with NMR also impact the tRNA modification process in living cells. The NMR-based methodology presented here could be adapted to investigate different aspects of tRNA maturation and RNA modifications in general.

69 Crystal structure of an Adenovirus Virus-Associated RNA

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Adenovirus Virus-Associated (VA) RNAs are the first discovered viral noncoding RNAs. These multifunctional pro-viral RNAs interfere with essentially all host systems that interface with double-stranded RNAs (dsRNAs), from their sensing by protein kinase R (PKR), export by Exportin-5, processing by Dicer, editing by ADAR, to activation of oligoadenylate synthetases (OASs). Dicer-processed terminal strands of VA are further assembled into functional RISC complexes. Collectively, VA RNAs contribute ~60 fold to viral titers and confer adenoviruses general resistance to interferon-mediated antiviral defense.

However, how VA-I suppresses PKR activation despite its strong dsRNA character, and inhibits the crucial antiviral kinase to promote viral translation remains unclear. Here, we report a 2.7 Å crystal structure of VA-I RNA. The acutely bent VA-I features an unusually structured apical loop of exceptional stability, a wobble-enriched, coaxially stacked apical and tetra-stems necessary and sufficient for PKR inhibition, and a central domain pseudoknot that may facilitate VA-I folding and prevent PKR activation by VA-I. Interestingly, the VA-I central domain exhibits structural similarities to the tRNA anticodon stem loop and codon-anticodon interactions in the ribosome and T-box riboswitches. This finding lends support to the tRNA origin hypothesis of VA RNAs. Together, this study provides molecular insights into RNA-mediated viral evasion of host innate immunity.

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References:

1. Reich, P.R., et al. J Mol Biol, 1966; 2. Soderlund, H., et al. Cell, 1976; 3. Thimmappaya, B., et al. Cell, 1982; 4. O'Malley, R.P., et al. Cell, 1986; 5. Korostelev, A., et al. Cell, 2006; 6. McKenna, S.A., et al. J Mol Biol, 2006; 7. Selmer, M., et al. Science, 2006; 8. Nallagatla, S.R., et al. Curr Opin Struct Biol, 2011; 9. Grigg, J.C., et al. Structure, 2013; 10. Zhang, J., et al. Nature, 2013; 11. Launer-Felty, K., et al. Biophys J, 2015; 12. Youssef, O.A., et al. Proc Natl Acad Sci U S A, 2015; 13. Grosjean, H., et al. Nucleic Acids Res, 2016; 14. Dzananovic, E., et al. PLoS One, 2017; 15. Kim, Y., et al. Mol Cell, 2018; 16. Bou Nader, C., et al. RNA, 2019.

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70 The Elongator subunit Elp3 is a non-canonical tRNA acetyltransferase

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Transfer RNAs are heavily decorated with different chemical moieties to maintain their correct folding and proper functionality in protein translation. Among the over 100 kinds of modifications of tRNAs, the cm^5 modification catalyzed by Elongator complex (Elp) at U34 of tRNA is particularly important in many aspects. The cm^5U_{34} modification represents the initial step in a cascade that allows the subsequent conversion into mcm^5U, ncm^5U, mcm^5s^2U by other modification enzymes. The lack of this enzyme activity was found to promote cancer formation and neurodegenerative diseases, but the molecular details of the enzymatic reaction remained elusive. Here, we present apo and ligand bound crystal structures of Elp3, the catalytic subunit of Elongator, at atomic resolution. We not only provide evidence that structures of Elp3 proteins from evolutionary diverse species are almost identical but also their biochemical and biophysical activities, especially the acetyl-CoA (ACO) binding and hydrolysis reaction. Furthermore, we found that tRNAs (and not peptides) represent the genuine substrate for Elp3, showing the highest binding affinity and the potential to induce ACO hydrolysis. The pre-requisite modifications on other positions of tRNA seem to be dispensable for the enzyme activity in most tRNA species. More importantly, U34, residing in the anti-codon stem loop of the tRNA, is the crucial element for triggering the ACO hydrolysis. In summary, we provide strong biochemical, biophysical and structural insights into the molecular details of the Elp3-mediated U34 modification reaction. Our results pave the way for further clinically related studies to develop therapeutic and diagnostic tools for Elongator-related diseases.

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71 Chemical availability of tRNA fragments containing modified nucleosides from 34 and 37 positions of anticodon domain

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More than 100 modified nucleosides have been identified in cellular tRNAs, wherein most of them are located at positions 34 (wobble) and 37 (3'-adjacent to the anticodon).[1] Recently, modern methods for analysis of isolated tRNA molecules have significantly increased the progress in the discovery of new modified units, in particular from the anticodon loop domain (*e.g.* ct^6A, ms^2ct^6A, $msms^2i^6A$, mnm^5ges^2U, $cmnm^5ges^2U$).[2-5] Modifications within the anticodon loop are known to be essential for accuracy of protein biosynthesis, however their contribution to the translation process and other tRNA biological functions is still not fully understood. Important insights into the role of individual nucleotide modification come from the studies of chemically synthesized modified oligonucleotides.[6-9]

Herein, we present our contribution to improving chemical access to site-specifically modified, model oligoribonucleotides useful for further biological and structural studies. Single and multiple modified tRNA fragments containing hypermodified 5-substituted uridines and 2-thiouridines (R^5U_{34}-RNAs and $R^5s^2U_{34}$-RNAs, R=nm, mnm, cmnm, tm, inm, cn),[10] *S*-geranylated 2-thiouridines ($R^5ges^2U_{34}$-RNAs, R=H, mnm)[6] or cyclic form of N^6-threonylcarbamoyladenosines (ct^6A_{37}-RNA and ms^2ct^6A-RNA)[11] were obtained by post-synthetic modification of appropriate precursor oligoribonucleotides. Final oligomers were purified using chromatographic methods and their structures were confirmed by MS and analysis of enzymatic digestion using HPLC comparison with possessed standards of modified nucleosides: 5-substituted uridines, 2-thiouridines and N^6-substituted adenosines.

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References:

[1] Boccaletto et al., *Nucleic Acids Res.*, **2018**, *46*, D303; [2] Matuszewski *et al*., 2017 ; *Nucleic Acids Res.,* **2017**, *45*, 2137; [3] Kang *et al*., 2017, *Nucleic Acids Res.,* **2017**, *45*, 2124; [4] Dal Magro *et al., Angew. Chem. Int. Ed. Engl.,* **2018**; *57*, 7893; [5] Dumelin *et al*., *Nat. Chem. Biol.* **2012**, *8*, 913;[6] Sierant et al., *Nucleic Acids Res.*, **2016**, *44*, 10986; [7] Sierant et al., *FEBS Lett*, **2018**, *592*, 2248; [8] Wang et al., Chem. Commun., **2015**, *51*, 16369; [9] Cantara et al. *J. Mol. Biol.*, **2012**, *416*, 579; [10] Bartosik et al., *Org. Biomol. Chem.,* **2017**; *15*, 2097; [11] Matuszewski et al., *Chem Commun,* **2017**, *53*, 7945-7948.

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72 A microfluidic-based assay recapitulates eukaryotic ribosomal translation and identifies toxic tRNAs

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Ribosomes translate the genetic information into proteins. Translation fidelity relies essentially on the ability of ribosomes to accurately recognize triplet interactions between codon and anticodon of aminoacylated tRNAs. To determine the codon-anticodon pairing combinations efficiently accepted by the eukaryotic ribosome in the absence of modification in the anticodon loop and bias from competition between tRNAs or protein amino acid sequence, we took advantage of the IRES from the intergenic region (IGR) of the Cricket Paralysis Virus. It contains an essential pseudoknot PKI that structurally and functionally mimics a codon-anticodon helix. With a reporter containing the IGR upstream of the GFP coding sequence, we screened the entire set of 4,096 possible combinations using ultrahigh-throughput screenings combining *in vitro* coupled transcription/translation and droplet-based microfluidics. Only 97 combinations are efficiently accepted and accommodated for translocation and further elongation: 38 combinations involve cognate recognition and 59 involve near-cognate recognition. A/U-rich codon-anticodon combinations did not promote GFP translation and the majority of missing combinations have a U at the first anticodon position. These data comfort the contribution of nucleotide modifications to the stability of triplet formation. Most of the near-cognate combinations (51) contain a G at the first position of the anticodon (numbered 34 of tRNA). G34-containing tRNAs translating 4-codon boxes are virtually absent from eukaryotic genomes. We reconstructed these missing tRNAs *in vitro*, reintroduced them in HeLa cells, and could demonstrate that these tRNAs are toxic for cells probably due to their miscoding capacity in eukaryotic translation systems. These data suggest that the absence of G34-containing tRNAs in eukaryotic systems might be an evolutionary response to specific structural and dynamic properties of the eukaryotic translation machinery.

73 Ribosome Heterogeneity in Translating the Genetic Code

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In the transmission of biological information, the ribosome has been perceived to serve an integral but largely passive participant in the synthesis of all proteins across all kingdoms of life. Our research has changed this view, by demonstrating that not all of the millions of ribosomes within each cell are the same and that ribosome heterogeneity provides a novel means for diversity of the proteins that can be produced in specific cells, tissues, and organisms. I will present our work centered on developing a roadmap for the characterization of ribosome composition at a single cell level and during cellular differentiation. We employed a highly quantitative mass spectrometry-based approach to precisely quantify the abundance of each ribosomal protein (RP) as well as a large cohort of ~ 400 ribosome associating proteins (RAPs) belonging to actively translating ribosomes. This led to the identification of subsets of ribosomes that are heterogeneous for RP composition. To further address the functional role of ribosome heterogeneity in translational control of the mammalian genome, we employed CRISPR/Cas9 to endogenously tag and purify heterogeneous ribosome populations. We then developed an adapted ribosome profiling method to precisely quantify and characterize the nature of mRNAs translated by distinct heterogenous ribosomes genome-wide. This led to the identification of subpools of transcripts, critical for key cellular processes including cell signaling, metabolism, growth, proliferation and survival, which are selectively translated by specific types of ribosomes. Most interestingly, there are specific signaling pathways where almost every single component is selectively translated by specialized ribosomes demarcated by a single RP. I will further present recent findings on the mechanisms by which ribosome-mediated control of gene expression is encoded by structured RNA elements within 5'UTRs visualized through Cryo-EM. Together, these studies reveal a critical link between ribosome heterogeneity and specialized translational control of the mammalian genome, which adds an important layer of control to the post-transcriptional circuitry of gene regulation.

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74 Specialisation of ribosomes in gonads

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Our understanding of ribosomes and translational control has recently been transformed by the discovery that specialised ribosomes exist and regulate the translation of specific mRNA pools. One key means of ribosome specialisation is the variation of substitutions of ribosomal protein (RP) paralogs. Transcriptomic and proteomic data has revealed that many RP paralogs are expressed in a tissue-specific manner e.g. RpS4Y2 in human testis and prostate cells. mRNA translational control and ribosome biogenesis are key to gametogenesis and many RP mutations in *Drosophila* melanogaster result in infertility. Therefore, we have sought to profile ribosome specialisation in the gonads of *D. melanogaster*.

We are dissecting the function-structure relationship of *in vivo* ribosomes from *D. melanogaster* gonads, to understand how specialised ribosomes control of the translation of particular mRNA populations. We have purified ribosomal complexes from *D. melanogaster* testes, ovaries, heads, embryos and S2 tissue culture cells. Comparing the protein composition of these different ribosome populations has revealed distinct ribosome populations between these tissues. The testis shows the most extensive and widespread specialisation through RP incorporation. Specialisation is also evident in the ovary. We have identified variations in both paralog and canonical RP incorporation. To probe the structural consequences of the differences in composition we have used cryo-EM. We have solved the structures of ribosome populations from testis and ovary to 3.9 Å and 3.4 Å respectively. These provide a significant improvement to the previous 80S structure from *Drosophila melanogaster* embryos at 6 Å. These structures have revealed that paralog changes occur within intact 80S ribosomes and suggest functional consequences of ribosome specialisation *in vivo* to gametogenesis.

75 The mechanism of inhibition of cap-dependent translation by the Translational Inhibitory Elements (TIE) a3 and a11 in Hox mRNAs

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In eukaryotes, most cellular mRNAs undergo the canonical cap-dependent translation since the initiation is guided by the 5' m7G cap.This process requires several factors termed eukaryotic Initiation Factors (eIFs). Homeobox (Hox) genes are evolutionarily conserved genes that encode for transcriptional factors controlling embryonic body plan along the head-tail axis. Recent reports suggest that subsets of HoxA mRNAs are translated in a cap-independent manner due to the presence of 5'UTR RNA regulon termed Translational Inhibitory Element (TIE) that inhibits cap-dependent translation.(1)

The objective of our project is to decipher the inhibitory mechanism of two TIE elements: TIE a3 and TIE a11 in Hox a3 and Hox a11 mRNAs respectively. For that, we established an in vitro cell-free translation system with rabbit reticulocytes lysate that faithfully recapitulates TIE-mediated translation inhibition. We were able to map the minimal functional domain of each TIE element. We also established a model of the secondary structure for each TIE element using chemical probing methods with DMS and CMCT reagents. By sucrose gradient fractionation, we analysed the ribosomal assembly profile on each TIE element. Further experiments were conducted by 'Toe Printing' assay and site-directed mutagenesis to better understand TIE-mediated inhibition. Next, we purified translation initiation complexes to identify the trans-acting factors using an approach developed in our laboratory for mass spectrometry analysis (2). Interestingly, the two TIE elements function by radically distinct mechanisms. Our current model for TIE a3 suggests that it inhibits translation due to an upstream Open Reading Frame (uORF) which translates through the 5' UTR with the involvement of eIF2D, a non-canonical GTP-independent initiation factor (3). In contrast, TIE a11 sequesters 80S ribosome by an unknown mechanism.

Further experiments will be conducted to validate the trans-acting factors involved in TIE-mediated inhibition.

(1)(Xue et al., 2015) (2)(Chicher et al., 2015) (3)(Dmitriev et al., 2010)

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76 Dissecting the roles of eIF2 and eIF3 during translation using TCP-seq

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The Translation Complex Profile sequencing (TCP-seq) method is related to the popular ribosome profiling approach with the key difference that small ribosomal subunit (SSU) footprint libraries are generated in addition to the usual full ribosome (RS) footprints. TCP-seq was initially implemented in budding yeast and the transcriptome-wide SSU footprints were used to interrogate the mechanism and regulation of translation initiation and termination [1]. We have now developed TCP-seq further, first, by readjusting the approach to work with mammalian cells in culture, and second, by combining it with a prior immuno-purification step to selectively isolate translation intermediates containing affinity-tagged factors of choice (IP-TCP-seq).

Regarding the former, metagene footprint alignments indicate that mammalian and yeast SSU complexes distribute in a broadly similar manner across mRNA 5'UTRs, consistent with strong evolutionary conservation of the translation initiation machinery and mechanism.

Regarding the latter, we focussed on yeast eIF2 and eIF3, components of the proposed multi-factor complex (MFC). Distribution patterns along mRNA 5'UTRs and at start codons of eIF-tagged SSUs compared to the full complement of mRNA associated SSUs showed interesting quantitative differences in that eIF-tagged SSU complexes were over-represented in 5'UTRs relative to start codons. This is consistent with the prevailing model that eIF2 and eIF3 travel along the 5'UTR with the SSU and are dissociating from the complex as a consequence of start codon recognition. SSU complexes at start codons generate several classes of footprints of different size, due to complex rearrangements during start codon recognition. Here, eIF-tagged SSU complexes showed a distinct footprint size distribution profile suggestive of a staged dissociation of these factors during this process.

Finally, and surprisingly, when inspecting patterns of eIF-tagged RS footprints, we found ample evidence for co-translational assembly of eIF3 subunits and potentially even the MFC. Collectively, these results show that TCP-seq is a versatile method of broad utility in studying translation.

[1] S.K. Archer, N.E. Shirokikh, T.H. Beilharz, T. Preiss
Dynamics of ribosome scanning and recycling revealed by translation complex profiling, Nature, 2016

77 Distinct H/ACA small nucleolar RNA-guided ribosome modifications in control of lipid metabolism and ribosome dynamics

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H/ACA small nucleolar RNAs (snoRNAs) are responsible for converting hundreds of specific uridine residues to pseudouridine within the ribosome and are found altered in numerous cancers. However, it remains unknown whether H/ACA snoRNA expression and function is regulated to control the pattern of rRNA modifications on the ribosome in health and disease. To our surprise, we have uncovered that specific subsets of H/ACA snoRNAs, guiding modifications within distinct regions of ribosomal RNA (rRNA), are rapidly remodeled during the earliest cellular response to cellular transformation. To address whether individual snoRNAs play a role in tumor suppression, we assessed whether a reduction in specific H/ACA snoRNAs may alter cellular senescence, a critical barrier to oncogenic transformation. Interestingly, we observed that a reduction in distinct H/ACA snoRNAs including SNORA24, which guides pseudouridine modifications within 18S rRNA, leads to a bypass of senescence induced by oncogenic RAS and promotes the development of liver cancer *in vivo*. Histological analysis of tumor nodules revealed a dramatic increase in lipids that closely resembles human steatohepatitic hepatocellular carcinoma, a liver cancer variant characterized by lipid deposition. From a clinical perspective, hepatocellular carcinomas with low SNORA24 expression exhibit increased lipid content and are associated with poor patient survival. We next employed single-molecule fluorescence resonance energy transfer (smFRET) imaging to monitor the functional dynamics of ribosomes isolated from HCC cells lacking SNORA24. We find that ribosomes lacking two pseudouridine modifications guided by SNPRA24 display an increased propensity to adopt hybrid tRNA conformations during translation elongation and exhibit alterations in peptidyl-tRNA engagement within the A site. These findings provide the first evidence that ribosomes from cancer cells, lacking specific pseudouridine modifications, are biophysically distinct. Interestingly, HCC cells lacking SNORA24 display increased rates of stop codon readthrough from reporter constructs suggesting that changes in the biophysical properties of ribosomes induced by loss of two specific pseudouridine modifications may impact translation fidelity. Altogether, these studies show that the pattern of rRNA modifications is dynamically regulated to safeguarding the genome against oncogenic insult.

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78 Transcriptome-wide sites of collided ribosomes reveal sequence determinants of translational pausing

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During translation, ribosomes traverse along mRNA coding sequences at non-uniform speeds. Conceivably, when an elongating ribosome is locally slowed down, or even pausing, subsequent ribosomes would collide and stack behind it. While this effect was observed to occur in *in vitro* translation reactions already some decades ago (Wolin & Walter, 1988, EMBO J. 7:3559-69), it has still remained unclear (1) to what extent such pausing and stacking also occurs *in vivo*, (2) whether it is associated with regulation of gene expression, and (3) how it relates to a variety of processes potentially influencing translation elongation, such as structural features and codon usage of the mRNA, tRNA abundances, amino acid composition and interactions of the nascent peptide sequence.

By selectively purifying and sequencing the ≈60 nt-footprints from two stacked monosomes ("disomes"), we have now mapped the precise locations of collided ribosomes transcriptome-wide at nucleotide resolution. We will present a comprehensive study from mouse liver, in which we demonstrate that disome footprints identify *bona fide* translational pausing and collision events. Our data indicate that at steady state ca. 10% of translating ribosomes are trapped in the state of the disome, showing that ribosome collisions during translation are frequent. Moreover, disome sites are associated with specific amino acid and peptide motifs, and with structural and functional features of the nascent polypeptide (e.g. charge, presence of signal sequence, transmembrane and other domains). Interestingly, we find that the translation of several ribosomal proteins involves inter-domain pausing events, and using reporter assays, we provide experimental verification for functional relevance of translational pause sites. Taken together, our disome profiling strategy allows for novel and unexpected insights into gene expression regulation occurring at the level of translation elongation.

79 Mechanisms of mammalian mitochondrial translation

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Mitochondria are eukaryotic organelles that critically influence the process of aging and defects in mitochondrial protein homeostasis are involved in a number of severe human pathologies such as cancer and cardiomyopathies. Mitochondria originate from alpha-proteobacterial ancestors and have undergone a rapid evolution in their eukaryotic environment. This has also affected the mitochondrial translation apparatus, which has become very specialized producing only a few polypeptide chains. Despite their small number these proteins are essential since they are parts of the respiratory chain complexes rationalizing why defects in mitochondrial translation are detrimental to human health.

The mitochondrial ribosome and its surrounding regulatory elements have undergone a striking re-composition in comparison to bacteria, including alterations in mitochondrial messenger RNAs, adaptions of the genetic code and the lack of translation factors that are otherwise essential. This resulted in highly distinct and specialized mechanisms of translation. In order to gain mechanistic insights into the mitochondrial translation cycle, our lab has set out to reconstitute multiple key translation intermediates and determine their high-resolution structures by cryo-electron microscopy. Our structural observations are complemented with biochemical assays and define how peculiar adaptions in central translation factors promote faithful initiation as well as termination of translation in mammalian mitochondria.

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80 Capturing ribosomal translocation by EF-G using cryo-EM

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During protein synthesis, tRNAs and mRNA are translocated from the A to P to E sites of the ribosome. This essential step is catalyzed by a universally conserved ribosome GTPase, elongation factor G (EF-G, in bacteria). Understanding how the ribosome and EF-G maintain the correct open reading frame during this highly dynamic process requires visualization of structural intermediates. Most of the translocation trajectory remains uncharacterized, as structural studies have reported a limited number of EF-G-ribosome complexes with two translocating tRNAs. Furthermore, capturing such states often depends on antibiotics or mutated EF-G, which may report off-pathway states.

Here, we present our cryo-EM work aimed at characterizing translocation without using antibiotics. We report a previously unseen structural state, which shows the transition of two tRNAs from the A and P to P and E sites, respectively, in the presence of new intermediate translocation conformations of the 70S ribosome and EF-G. The structure suggests how EF-G unlocks intra-subunit ribosome rearrangements that reform the interactions with tRNAs and mRNA during translocation. This work uncovers a missing link in the understanding synchronous movement of tRNAs and mRNA during elongation.

81 Anatomy of a standby site: an essential role for ribosomal protein S1 and a secondary structure element for ribosome binding

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Translation initiation involves binding of 30S, fMet-tRNA^{fMet}, and initiator factors to an accessible mRNA ribosome-binding site (RBS). Stable structures at RBS's inhibit initiation, yet in some cases, "ribosome standby" can overcome this (1): transient, sequence-non-specific binding to a single-stranded region enables 30S subunits to compete with rapidly folding secondary structure masking an RBS. Standby can work over considerable distances. The best-characterized example is the *tisB*/*istR*-1 locus in *Escherichia coli*. Here, a standby site ≈100 nts upstream of the sequestered RBS in *tisB* mRNA is required for translation of TisB (2). Under normal growth conditions, the sRNA IstR-1 blocks this site, inhibiting translation. Though standby is strongly supported, *direct* evidence of ribosomes on standby has been elusive. Here, we report on the anatomy of the *tisB* mRNA standby site, its requirements and functional elements.

Fluorescence anisotropy experiments with a fluorescein-labeled *tisB* mRNA were conducted. 30S subunits bind the standby site independently of tRNA^{fMet} with high affinity, and addition of IstR-1, or competition with unlabeled mRNA (with standby site), impairs 30S binding. Ribosomal protein S1 is required for standby. We found that S1-alone binding is reminiscent of that of 30S, being affected by IstR-1 inhibition and mRNA competition. Furthermore, 30S subunits depleted of S1 neither bind the *tisB* mRNA, form 30S initiation complexes (30S-IC), nor support TisB translation *in vitro*. To directly map standby binding, *in vitro* CLIP experiments were conducted with 30S subunits, or S1-alone, on *tisB* mRNA. As expected, an abundant cluster of reads covered the single-stranded standby region, but unexpectedly also a structural element at the mRNA 5'end. 5'-truncations inactivated functional standby and TisB translation. Anisotropy changes revealed that this was due to decreased affinity of 30S and S1. In conclusion, our results define the anatomy of the natural standby site in *tisB* mRNA and its functional requirements: an RNA structure element, a single-stranded region, and ribosomal protein S1. The long-distance effect of this standby binding event is tentatively explained by S1-dependent directional unfolding towards the downstream RBS.

1. de Smit & van Duin (2003) *J. Mol. Biol.* 331:737-743.

2. Darfeuille et al. (2007) *Mol. Cell* 26:381-392.

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82 The timing of splicing

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Expression of genetic information in eukaryotes involves a series of interconnected reactions that ultimately control the production of proteins in cells. Many individual steps in gene expression are kinetically coupled, but how these temporal relationships affect the final gene output remains unclear. Some models posit that kinetic competition during gene expression contributes to quality control, since delays in completion of a pre-mRNA processing reaction such as splicing may promote degradation of the RNA. To study how long does it take to splice, we developed a strategy that permits direct measurements of intron dynamics in single pre-mRNA molecules in live cells. This approach revealed that splicing can occur within a few seconds after splice site transcription. To further determine the timing of pre-mRNA splicing relative to the position of RNA polymerase II (Pol II), we used Native Elongation Transcript sequencing (NET-seq). We found an accumulation of transcripts mapping precisely to the 3' end of exons, as expected for intermediates formed after the first transesterification splicing reaction, indicating that splicing must occur within a stable complex formed between the spliceosome and Pol II. However, in mammalian cells we did not detect spliced products associated with polymerases transcribing the exon downstream of a 3' splice site as seen for budding yeast. This may be because mammalian introns are much longer than yeast introns and therefore rely on an exon definition splicing mechanism. To study cells from an organism with a distinct exon-intron architecture, we optimized the NET-seq approach to analyze nascent transcripts in the developing *Drosophila* embryo. We found widespread evidence for recursive splicing taking place shortly after Pol II transcribes past a recursive splice site within long introns. We also detected spliced products associated with polymerases transcribing the first 145 nucleotides of the downstream exon. Thus, as observed in yeast, a subset of *Drosophila* introns are excised immediately after transcription of the 3' splice site. The physiological implications of immediate splicing will be discussed.

83 Bacterial and eukaryotic RNA gets an NAD-cap - But How to Remove it?

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The complexity of the transcriptome is triggered by the specific interplay of transcription initiation, termination and enzymatic RNA processing and decay. The selective degradation of RNA is a crucial component within the regulation of intracellular RNA levels, thus enabling the cell to respond quickly to changing environmental conditions. Especially the removal of a 5'-RNA cap structure is essential for the initiation of RNA decay. Besides the canonical 5'-N7-methyl guanosine cap in eukaryotes, the redox cofactor nicotinamide adenine dinucleotide (NAD) was identified as a new 5'-RNA cap structure in prokaryotic as well as in eukaryotic organisms.

Recently, two classes of NAD-decapping enzymes have been identified, that remove the NAD-cap using different mechanisms. We identified the Nudix hydrolase NudC as the first bacterial NAD-RNA decapping enzyme which converts NAD-RNA into 5'-monophosphorylated-RNA in vitro and in vivo. Crystal structures of E. coli NudC in complex with NAD reveal the catalytic residues lining the binding pocket and principles underlying the specific molecular recognition of NAD-RNA. Using biochemical mutation analysis tools, we identified an RNA-binding platform that specifically interacts with the RNA, triggering the hydrolysis of the NAD-cap. These studies demonstrate clearly that NAD-RNA is NudCs primary biological substrate.

In addition to the NudC-mediated RNA decapping pathways, we present here a new decapping mechanism that is specific for NAD-RNAs in eukaryotes. We discovered that the well described, eukaryotic enzyme CD38 converts NAD-capped-RNA into ADP-ribose-modified RNA. Afterwards ADP-ribose-RNA is processed by the human Nudix enzyme hNudt5 into 5'-monphosporylated-RNA triggering exonucleolytic decay. Neither CD38 nor hNudT5 show decapping activity on m7G-RNA, which demonstrates their specificity for 5´-NAD-capped RNA.

Our findings indicate that the decapping of cofactor-modified-RNAs like NAD-RNA is a highly regulated process in the cell. Given the central role of NAD in redox-biochemistry, protein-modification, and signalling, its specific attachment and removal to RNA points to unknown roles of RNA in these processes and to undiscovered pathways in RNA metabolism and regulation.

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84 Cap-specific terminal *N*^{6}-methylation of RNA by an RNA polymerase Ⅱ-associated methyltransferase

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RNA molecules are enzymatically modified, and more than 160 chemical modifications have been found in various RNAs across all domain of life. *N*^{6}-methyladenosine (m^{6}A) is a major modification of mRNAs, and plays critical roles in various biological events. Biogenesis and dynamics of m6A have been studied extensively; the modification is introduced by the writers (METTL3 complex and METTL16), and demethylated by eraser proteins (ALKBH5 and FTO). Internal m^{6}As are decoded differently by several reader proteins including YTH family proteins, thereby leading to diverse fates of mRNAs. In addition to the internal m^{6}A, *N*^{6},2′-*O*-dimethyladenosine (m^{6}Am) is present at the transcription start nucleotide of capped mRNAs in vertebrates. Although a recent study investigated the demethylation pathway of m^{6}Am and the link to mRNA destabilization, biogenesis and physiological significance of this modification have not been fully understood. Using a reverse genetics approach, we here identified cap-specific adenosine methyltransferase (CAPAM/PCIF1) responsible for *N*^{6}-methylation of m^{6}Am. CAPAM specifically interacts with the Ser5-phosphorylated C-terminal domain of RNA polymerase II, resulting in the formation of m^{6}Am at the early stage of transcription cycle. The crystal structure of CAPAM in complex with substrates revealed the cap-specific m^{6}A formation mediated by a novel helical domain of CAPAM. Furthermore, a transcriptome-wide analysis revealed that *N*^{6}-methylation of m^{6}Am promotes the translation of capped mRNAs, instead of stabilizing the A-starting capped mRNAs.

85 Construction and screening of an RNA binding protein CRISPR/Cas9 knockout library identifies RNA degradation as a specific vulnerability in Myc-dependent cancer

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c-Myc (Myc) is a frequently amplified oncogene in triple-negative breast cancer, in which cancer cells become dependent on Myc for sustained proliferation and survival. However, Myc has proven to be challenging to inhibit pharmacologically. Since Myc activation increases transcription and global pre-RNA synthesis, we hypothesized that RNA-binding proteins (RBPs) that regulate mRNA levels may be limiting in Myc-dependent cancer cells. We therefore have exploited the concept of synthetic lethality to specifically identify RBPs that are essential for growth and survival of Myc-dependent breast cancer cells but not in Myc-independent cells. We constructed a lentiviral CRISPR/Cas9 knockout library targeting over one thousand human RBPs and transduced human mammary epithelial cells expressing an inducible MYC transgene (Myc-ER HMECs). Our screen revealed depleted CRISPR single guide RNAs in the Myc-induced cell populations and identified RBPs that cause apoptotic cell death upon knockout. Among these were several RBPs that mediate RNA degradation including the N6-Methyladenosine (m6A) reader protein YTH N6-Methyladenosine RNA Binding Protein 2 (YTHDF2), which to date has no identified role in Myc-dependent cancer. We show that depletion of YTHDF2 impairs survival and tumorigenicity in established Myc-dependent, triple negative breast cancer cell lines, but not in established Myc-independent, receptor-positive breast cancer cell lines. Enhanced CLIP-seq (eCLIP) of YTHDF2 in Myc-ER HMECs revealed binding sites enriched for the 5'-DRACH-3' sequence motif in 3'UTRs near stop codons, with significant overlap with m6A sites, as expected. YTHDF2 target transcripts are enriched for growth factor response pathways and are up-regulated in YTHDF2 depleted cells, suggesting that YTHDF2-mediated degradation of this subset of tumor suppressing mRNAs is a specific vulnerability in Myc-dependent breast cancer. A pooled shRNA knockdown assay in triple-negative subcutaneous xenograft models revealed a significant depletion of YTHDF2 shRNAs in solid tumors, thus validating this vulnerability *in vivo*. Together, our RBP-focused CRISPR/Cas9 knockout library provides an unbiased approach to investigate the function of RBPs in disease and points to mRNA degradation pathways, specifically through destabilization of m6A containing mRNAs, as new therapeutic targets for aggressive Myc-dependent cancers.

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86 New insights into mechanisms and functions of m^{6}A-YTH modules: lessons from plants

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Methylation of N6-adenosine (m^{6}A) in mRNA has emerged as an important mechanism of post-transcriptional gene regulation in eukaryotes. m^{6}A provides a binding site for effector proteins ("readers") that may influence pre-mRNA splicing, mRNA degradation or efficiency of translation. The biological importance of such m^{6}A-dependent regulation is considerable, because stem cell differentiation and embryonic development require m^{6}A. YTH domain proteins are important m^{6}A readers with clearly established functions in animals and yeast. They contain an effector part, typically an intrinsically disordered region, in addition to the m^{6}A-binding YTH module.

The flowering plant *Arabidopsis thaliana* has provided a valuable system to find components of the m^{6}A methyltransferase complex, as well as to highlight the importance of this pathway in embryonic development. However, the study of YTH domain proteins in plants has turned out difficult, as plants contain more YTH domain proteins than other eukaryotes. Due to their redundancy, the potential involvement of plant YTH domain proteins in gene regulation by binding to m^{6}A remained unknown until very recently. Here, I will present our freshly published data on the role of a small clade of *Arabidopsis* YTH domain proteins to control the timing of organ initiation and morphogenesis of leaves via binding to m^{6}A. In addition, I will provide new insights into 1) how this mechanism affects organogenesis of every differentiated tissue in the plant, namely leaves, roots, flowers and fruits, by very specific expression patterns in proliferating and differentiating cells, 2) the molecular pathways that m^{6}A-YTH controls to shape organogenesis in eukaryotes, thanks to data obtained using techniques such as iCLIP or HyperTRIBE, and 3) the molecular mechanisms underlying the functionality of YTH domain proteins, for which we are using strategies only accessible to model organisms suitable for screening such as *Arabidopsis*.

87 Mechanisms controlling the dynamics of A to I editing

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RNA editing by adenosine deaminases acting on RNA converts adenosine to inosine in double-stranded and structured RNAs. As inosines are primarily interpreted as guanosines, A to I editing can affect proteins by changing splicing patterns or by recoding of mRNAs. Today, about 20 abundant and conserved editing-mediated recoding events are known in mammalian mRNAs. These mRNA recoding events frequently change protein function and are therefore important for normal life and development.

Frequently, editing-induced changes in mRNAs are regulated in a spatio-temporal manner. However, to this point it is not known how RNA-specific editing events are controlled and to which physiological stimuli they respond. Here we investigate the editing of the mRNAs encoding the actin crosslinking proteins Filamin A and Filamin B. We show that filamin A editing is most abundant in smooth muscle cells while Filamin B is highest in cartilage. To this end we have identified splicing as a major regulator of Filamin editing. Most interestingly, we can show that changes in cell culture conditions can modulate editing levels in Filamins. This describes for the first time that changes in cellular physiology can directly impact on RNA editing. Currently, we are dissecting the pathway underlying dynamic control of editing following physiological changes.

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88 Purifying Selection of long dsRNA is the first line of defense against false activation of innate immunity

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Mobile elements comprise a large fraction of the genome, across metazoa. Accumulation of mobile elements is bound to produce multiple putative dsRNA structures within the transcriptome. Endogenous cellular dsRNA structures resemble viral RNA, and may trigger false activation of innate immune response, leading to a severe damage to the host cell. It was recently shown that a key function of A-to-I RNA editing by ADAR1 is to unwind the dsRNA and suppress this response. Here we show a strong selection against endogenous dsRNAs, resulting in their purification from the canonical transcriptome. This general evolutionary mechanism, found in dozens of species across metazoa, is the main protection against false triggering of an immune response. The critical targets of ADAR1 editing are, likely, to be found in non-canonical transcript.

89 RNA editing - an alternative to DNA editing

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Programmable RNA-guided tools for genome editing attracted a lot of interest during the last years. However, there are safety concerns related to off-target editing, immunogenicity, and gene editing fails when the introduced mutation is either lethal or genetically compensated. During the last years, our lab has been pioneering several alternative strategies that are based on site-directed A-to-I editing of the transcriptome.

Specifically, we developed the SNAP-ADAR approach that applies a unique and novel assembly strategy to generate artificial RNA-guided riboproteins. Recently, we demonstrated the simultaneous A-to-I editing of several endogenous transcripts, with high efficiency (up to 90%), high potency, sufficient duration, and high precision.[1] Yet unpublished, we will report on an updated version of the approach with largely improved properties and the application on the manipulation of signaling proteins. We aim to point out that the SNAP-tag approach is a highly convenient general RNA-targeting approach, orthogonal to current Cas approaches.

Furthermore, we develop genetically encodable [2] and chemically stabilized [3] oligonucleotides that enable the recruitment of endogenous ADARs for site-directed RNA editing. Compared to all other apporaches, this approach enables to reprogram the transcriptome with a short oligo only, circumventing the ectopic expression of any additional editase. Consequently, the editing reaction is very precise. We demonstrated editing of disease-related transcripts like STAT1 and Serpina1 [3].

[1] P. Vogel, et al. *Efficient and Precise Editing of Endogenous Transcripts with SNAP-tagged ADARs*. *Nature Methods* 15, 535 (**2018**)

[2] J. Wettengel, et al. *Harnessing human ADAR2 for RNA repair - Recoding a PINK1 mutation rescues mitophagy. Nucl. Acids Res.* 45, 2797-2808 (**2017**).

[3] T. Merkle, et al. *Precise RNA editing by recruiting endogenous ADARs with antisense oligonucleotides. Nature Biotech.* **2019**, in press.

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90 PPR-based Mechanisms of Mitochondrial Editing Surveillance in Trypanosomes

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Most mitochondrial mRNAs in Trypanosoma brucei undergo massive U-insertion/deletion editing to create open reading frames. These sequence changes disrupt collinearity between the genome and transcriptome and introduce considerable heterogeneity into mRNA pool. Here, we report recent advances in understanding the roles of pentatricopeptide (PPR) RNA binding proteins in orchestrating polyadenylation-based surveillance of mRNA editing. These mechanisms ensure translation of correctly edited transcripts, which represent a minor fraction of the entire mRNA pool. Addition of short 3′ A-tail by mitochondrial KPAP1 poly(A) polymerase prior to editing protects mRNA from 3′-5′ degradation during the editing process. Conversely, completion of editing is manifested by A-tail extension into long A/U-heteropolymer. This unconventional mRNA modification stimulates translation by increasing mRNA affinity to the small ribosomal subunit. The distinct roles and editing-dependent temporal separation of A-tailing and A/U-tailing events imply existence of sequence-specific factors that sense the mRNA's editing status and regulate 3′ additions. We identified pentatricopeptide-repeat containing (PPR) RNA binding proteins responsible for monitoring mRNA editing status, 3′ modifications, and direct binding to the ribosome. We show that Kinetoplast Polyadenylation Factor 3 (KPAF3) specifically recognizes 3′ end of pre-edited transcripts thereby stabilizing mRNAs, and stimulates polyadenylation. Initiation of editing displaces KPAF3 leaving mRNA reliant on short A-tail as stability determinant. We further show that Kinetoplast Polyadenylation Factor 4 (KPAF4) recognizes a stretch of five adenosines acting as poly (A) binding protein. In this capacity, KPAF4 blocks 3′-5′ mRNA degradation of adenylated mRNAs by the mitochondrial processome and limits their premature uridylation by RET1 TUTase. The latter prevents translational activation of partially-edited mRNAs. Furthermore, we demonstrate that upon completion of editing KPAF1/2 heterodimer recruits KPAP1 and RET1 thereby inducing long A/U-tail addition specifically to fully-edited mRNA. Finally, we identify PPR factors that represent integral ribosomal proteins involved in direct recognition of specific mRNAs. Collectively, our findings reveal previously unappreciated roles of PPR proteins as edited sequence readers, polyadenylation factors, and poly(A) binding proteins.

91 Expanded functional repertoire of tRNA modifying enzymes

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tRNAs are the most highly modified RNAs, and pseudouridines are the most abundant RNA modifications. Many tRNA pseudouridine synthases are conserved from bacteria to humans such as the E. coli TruB / yeast Pus4 enzyme targeting U55 in the T arm of all tRNAs and E. coli TruA / yeast Pus3 modifying uridines in the anticodon loop. tRNA methylations are similarly abundant and conserved; for example, E. coli TrmA / yeast Trm2 methylates U54 in all tRNAs. However, none of these tRNA modifying enzymes is essential raising the question to their cellular function.

My group is investigating the hypothesis that many tRNA modifying enzymes function as RNA chaperones and fold tRNAs, but also modify and fold other coding and non-coding RNAs in the cell. Combining biochemical, genetic and transcriptomic studies, we demonstrate here the tRNA chaperone function and wide target range of representative tRNA modifying enzymes.

First, we have proven that the pseudouridine synthase TruB is a tRNA chaperone; this function is critical for E. coli fitness. Similarly, we show here that the methyltransferase TrmA also folds tRNA. Therefore, both enzymes, that modify all tRNAs in all domains of life, act as tRNA chaperones. Importantly, we further demonstrate that both TruB and TrmA act early during tRNA maturation preferring unmodified RNA as substrate and folding tRNA in preparation for modification by other enzymes.

Second, we tested the hypothesis that tRNA modification enzymes can target a wide range of cellular RNAs. Therefore, we have conducted a PAR-CLIP study with the yeast pseudouridine synthases Pus3 and Pus4. Our data confirm the handful of Pus3 and Pus4 target RNAs revealed by pseudouridine sequencing and identify more than thousand new mRNA and non-coding RNA targets. Biochemical characterization confirms that many of the identified RNAs are not only bound, but also modified by Pus3 and Pus4.

In conclusion, we prove that tRNA modifying enzymes act as RNA chaperones and reveal that they may modify and fold many more RNAs in the cell. This wide repertoire of target RNAs, that benefit from the interaction with tRNA modifying enzymes, can explain the conservation of these proteins

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92 Structure and Function of the eukaryotic Elongator Complex

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All types of cellular RNAs are post-transcriptionally modified, constituting the so called "epitranscriptome". In particular, tRNAs and their anticodon stem loops represent major modification hotspots. The attachment of small chemical groups at the heart of the ribosomal decoding machinery can directly affect translational rates, reading frame maintenance, co-translational folding dynamics and overall proteome stability. The variety of tRNA modification patterns is driven by the activity of specialized tRNA modifiers and large modification complexes. Notably, the absence or dysfunction of these cellular machines is correlated with several human pathophysiologies, like cancer and neurodegenerative diseases. I will present our latest structural and biochemical analyses comparing the enzymatic core of the highly conserved Elongator complex in eukaryotes, bacteria and archaea. In addition, I will present data on Elongator's unfortunate role in human diseases and our data on the regulatory network surrounding this large macromolecular machine in eukaryotic cells. I aim to focus on our most recent work that has allowed us to understand this large RNA modification complex and its regulatory factors at atomic resolution.

· Krutyhołowa R, Zakrzewski K and Glatt S, COSB 2019

· Krutyhołowa R et al., NAR 2019

· Lin TY et al., Nat Comm 2019

· Kojic M et al., Nat Comm. 2018

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93 Identification of large noncoding RNA-protein interactions and their effects on stress response in bacteria

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Noncoding RNAs (ncRNAs) are responsible for a remarkable variety of biological functions. Large ncRNA classes such as rRNAs, RNase P, and tmRNAs accomplish biochemical functions that rival the activities of proteins. However, large ncRNA classes are rare in bacteria. In the last decade, ~20 additional large, structured ncRNAs classes have been uncovered in bacteria that are unique, highly conserved, and likely to have interesting functions. Of these, the OLE (Ornate, Large, Extremophilic) RNA class is among the most complex and well-conserved ncRNAs discovered to date. This class includes 795 distinct representatives, each ~600 nucleotides long, from a wide range of species, including several human pathogens. These RNAs are abundantly expressed and lack homology to known RNA classes, suggesting they have an important and novel function. One possibility is that OLE RNAs have a role related to bacterial cell membrane stress. OLE RNAs localize to membranes through association with the transmembrane OLE-associated protein OapA. In *Bacillus halodurans,* Δ*ole*, Δ*oapA*, and Δ*ole-oapA* strains are less tolerant of cold temperatures, ethanol, and, surprisingly, Mg^{2+}. We have identified a dominant OapA mutant that reduces growth beyond the knockouts under cold, ethanol, and Mg^{2+} stresses. This defect can be rescued by mutations that render OLE RNA nonfunctional, suggesting that the dominant negative effect requires an intact RNA and that the ribonucleoprotein complex (RNP) interacts with other molecules. A genetic screen revealed several suppressor mutations in a gene for a protein of unknown function, named OapB. We found that OapB specifically binds OLE RNA *in vitro* with high affinity and specificity, whereas a mutation identified in the screen negates binding. These results indicate that the OapB-OLE RNA interaction is important for the RNP function. Furthermore, we investigated one of the first observations of Mg^{2+} toxicity in bacterial cells. By isolating suppressor mutants from cells exposed to Mg^{2+}, a potential strategy cells use to overcome Mg^{2+} stress was identified.

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94 Xist deletional analysis reveals an inter-dependency between Xist RNA and Polycomb complexes for spreading along the inactive X

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Mammalian X-chromosome inactivation (XCI) compensates sex-chromosome dosage imbalance by silencing one of the two X chromosomes in female cells. This gene silencing on a chromosome-wide scale makes XCI one of the best model systems to study epigenetic gene regulation. Xist RNA, the master regulator of XCI, spreads along an entire chromosome to establish silencing. However, the mechanism and functional RNA elements involved in spreading remain undefined. Here, we perform a comprehensive endogenous Xist deletion screen using CRISPR genome engineering and identify Repeat B motif as crucial for spreading Xist and maintaining Polycomb repressive complexes 1 and 2 (PRC1/PRC2) along the Xi. Unexpectedly, spreading of these three factors is inextricably linked. Deleting Repeat B compromises recruitment of PRC1 and PRC2. In turn, ablating PRC1 or PRC2 impairs Xist spreading. Therefore, Xist and Polycomb complexes require each other to propagate along the Xi, suggesting a feed-forward mechanism between RNA initiator and protein effectors. Perturbing Xist/Polycomb spreading causes failure of de novo Xi silencing, with compensatory down-regulation of genes on the active X, and also disrupts topological Xi reconfiguration. Thus, Repeat B is a multifunctional element that integrates inter-dependent Xist/Polycomb spreading, silencing, and chromosome architecture.

95 Riboregulation of mammalian autophagy by the non-coding vaultRNA 1-1

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Vault RNAs have been identified in 1986 through their association with a barrel-shaped 13MDa ribonucleoprotein complex, referred to as the vault particle. Still, the function and importance of this conserved and abundant small non-coding RNA species remained elusive for more than three decades. We have recently reported that *vault RNA1-1* directly binds to the autophagic receptor p62/sequestosome-1 and thereby affects autophagic flux in the human hepatocellular carcinoma cell line HuH-7[1] , and show that this regulatory interaction is relevant in other human cell lines and also forms between the mouse vault RNA and murine p62.

Functionally, *vault RNA1-1* binds the zinc finger domain of p62 which lies in close proximity to its PB1 oligomerisation domain. We show that *vault RNA1-1* prevents oligomerisation of p62, which is a prerequisite for its interaction with ATG8-like proteins and key to its function in autophagy. As a result, acute (LNA mediated knockdown) as well as stable (CRISPR/Cas9 mediated knockout) depletion of *vault RNA1-1* in HuH-7 cells leads to increased autophagic flux. In line with this, a small molecule (XIE62- 1004-A) that induces p62-ZZ-domain dependent autophagy has a distinctively higher effect on autophagy in *vault RNA1-1* knockout cells, further confirming the role of this non-coding RNA in p62-dependend autophagy. Interestingly, the total as well as the p62-bound levels of vault RNA1-1 decrease upon starvation following a time course that mirrors the increase in autophagic flux. How starvation mediates vault RNA1-1 level depletion is currently under investigation.

To delineate structure-function relationships in detail, we further determine the binding interface and employ structural characterisation of the interaction. Interestingly, the central region of *vault RNA 1-1* that is the least conserved between the four human vault RNA paralogs contributes strongly to p62 binding, possibly contributing to the specificity of the interaction. Riboregulation as exemplified by p62 and *vault RNA1-1* may well extend to other key processes in biology.

1. Horos, R. & Büscher, M. *et al.*The Small Non-coding Vault RNA1-1 Acts as a Riboregulator of Autophagy. **Cell** 176, 1054–1067.e12 (2019).

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96 You can't teach an old dog new tricks? Story about newly discovered lincRNA involved in seed dormancy.

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Choosing the best moment of germination is one of the most important decision in plants life. In nature the major regulator of the transition from a dormant seed to germination is *Delay of Germination 1* gene (*DOG1*).

DOG1 is regulated by a range of mechanisms including - alternative splice site selection, speed of Pol II elongation, alternative polyadenylation and antisense transcript (*1GOD* encoded within *DOG1* transcript unit).

Nevertheless, exciting things are taking place in *DOG1* genomic neighborhood. Firstly, expression of neighboring gene (*PKS18*) is regulated opposite way to *DOG1* during development. Secondly, we discovered a novel long intergenic non-protein coding RNA (lincRNA) - *3'1GOD* located between *DOG1* and *PKS18*.

3'1GOD region is needed for proper expression of both *DOG1* as well as *PKS18*. Detailed analysis show that *3'1GOD* inactivation leads to altered ratio between *DOG1* sense and antisense and affect choice of *DOG1* alternative polyadenylation site. Further, *3'1GOD* level is strongly induced in *cpl1* mutant which link levels of this lincRNAs with disturbance in Pol II elongation and recycling.

To explore the molecular mechanisms involved we mapped Pol II distribution at *3'1GOD* locus at a single-base resolution showing that Pol II is extensively pausing along this transcript. Our molecular dissection reveals that *3'1GOD* transcripts can be subjected to urydynalation and its expression is extensively upregulated in nuclear exosome mutant.

I will present how the discovery of this novel lincRNA originating from region next to *DOG1* locus opens the doors to exploring mechanistic of *DOG1* expression regulation and investigation of how upstream environmental signals control *DOG1* through *3'1GOD*.

97 Characterization of *Arabidopsis thaliana UGT73C6* natural *cis*-antisense long non-coding RNAs and analysis of their role in leaf size modulation.

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Long non-coding RNAs (lncRNAs) have been shown to be important modulators of gene expression in eukaryotes. In plants, lncRNAs are involved in a wide range of biological processes including flowering time and germination regulation, root development, and hormone and stress responses. Our research focuses on natural antisense long non-coding RNAs (lncNATs), a particular sub-type of lncRNAs transcribed from the opposite DNA strand of protein-coding genes. In the present work, we characterized two lncNATs of the model plant *Arabidopsis thaliana*, referred as *lncNAT1-* and *lncNAT2-UGT73C6*, which overlap the UDP-glycosyltransferase gene *UGT73C6*. It has been previously described that UGT73C6 and its closest homologue UGT73C5 play a role in plant development by inactivating steroid plant hormones (brassinosteroids, BRs). Reporter gene lines fusing the promoter region of *lncNAT1*-and *lncNAT2-UGT73C6* indicate independent promoter activity in roots and shoots, respectively. Analysis of *lncNAT1*-and *lncNAT2-UGT73C6* transcripts showed that they are stable and cytosol-localized. Overexpression or down-regulation of each lncNAT significantly affects the overall leaf area, whereas other developmental processes, including flowering time and seed yield are not affected. However, the observed phenotypes do not seem to correlate with changes in transcript levels of the overlapping protein-coding gene *UGT73C6*. Additionally, *lncNAT1-* and *lncNAT2-UGT73C6*, like *UGT73C6* and *UGT73C5*, remain un-responsive to BR treatment but expression and *in silico* data suggest that they can exert their function *via* microRNAs target mimicry. Peptides encoded by small open reading frames present in *lncNAT2-UGT73C6* can be detected after transient expression assays in *Nicotiana benthamiana* although its overexpression has no phenotypic effect in *A. thaliana*. However, the overexpression of a

non-peptide coding *lncNAT2-UGT73C6* variant, in which all the start codons were mutated, results in increased leaf area. These data indicate that *lncNAT1*-and *lncNAT2-UGT73C6* act as bona fide long non-coding RNAs modulating leaf size.

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98 Nopp140-mediated concentration of scaRNPs in Cajal bodies is required for telomere length regulation and spliceosomal snRNA modification

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Unlike the cytoplasm, the nucleoplasm of a cell does not contain membrane-bound organelles, yet subnuclear partitions exist, most conspicuously nucleoli and Cajal bodies (CBs). These and other nuclear bodies apparently form through liquid-liquid phase separation. Whereas nucleoli are well characterized as the ribosome factories of the cell, the function of CBs is less well defined, although two major classes of nuclear ribonucleoproteins (RNPs) are concentrated in them: small CB-specific (sca) RNPs and spliceosomal small nuclear (sn) RNPs. Stable knockdown cells indicate that the highly disordered nucleolar and CB phosphoprotein Nopp140 forms the underlying principle for concentrating scaRNPs, but not snRNPs, in CBs. Sequence complementarity of most scaRNAs points to their function as guides for snRNA modification. Indeed, we show that the presence of scaRNPs in CBs is required for snRNA modification. Mutations of the scaRNP protein WDR79 (aka TCAB1) in dyskeratosis congenita (DC), a bone marrow failure syndrome, dislocate all scaRNPs from CBs, including the telomerase scaRNP, causing severe telomere shortening. In contrast, removal of the intact telomerase scaRNP (with WDR79) from CBs causes gradual telomere lengthening, offering Nopp140 knockdown as an avenue for therapy in DC.

Our studies further reveal that the localization of Nopp140 to CBs requires the extreme phosphorylation of its central domain (~80 phosphates/molecule). This casein kinase 2-mediated phosphorylation of Nopp140 appeared to be constitutive. However, we now show that Nopp140 is specifically dephosphorylated in mitosis. This finding suggests that dephosphorylation of Nopp140 may be required for the well-documented dispersal of nucleoli and CBs in mitosis.

99 Nuclear export of circular RNA

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Circular RNAs (circRNAs), which function as regulators of eukaryotic gene expression, are formed by non-canonical back-splicing of precursor mRNAs (pre-mRNA) in the nucleus of mammalian cells. circRNAs are predominantly localized in the cytoplasm and can be translated, indicating that they must be exported from the nucleus to the cytoplasm. Here, we show that circular RNAs are not exported from the nucleus by canonical mRNA export pathways. Instead, circRNAs are exported by Exportin-2 (XPO2) and IGF2BP1 in a Ran-GTP dependent manner. Modulating the nuclear Ran-GTP gradient by depletion or chemical inhibition of the major protein exporter in human cells, CRM1, increases nuclear export of circRNAs but has no effect on linear mRNAs. Conversely, disrupting the nuclear Ran-GTP gradient reduces nuclear export of circRNAs, with no effect on linear mRNAs. Analysis of nuclear circular RNA binding proteins reveals that interaction of IGF2BP1 with circular RNA is enhanced by Ran-GTP. Depletion of Exportin-2 and IGF2BP1 inhibits nuclear export of circRNA while formation of an Exportin-2 circRNA export complex requires Ran-GTP and IGF2BP1. Our findings suggest that Exportin-2 is recruited to circular RNAs by adaptors such as IGF2BP1 that bind directly to circular RNAs in a mechanism analogous to protein export.

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100 Insights into the biogenesis and potential functions of exonic circular RNA

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Circular RNAs (circRNAs) exhibit unique properties due to their covalently closed nature. Models of circRNAs synthesis and function are emerging but much remains undefined about this surprisingly prevalent class of RNA. Here, we identified exonic circRNAs from human and mouse RNA-sequencing datasets, documenting multiple new examples. Addressing function, we found that many circRNAs co-sediment with ribosomes, indicative of their translation potential. By contrast, circRNAs with potential to act as microRNA sponges were scarce, with some support for a collective sponge function by groups of circRNAs. Addressing circRNA biogenesis, we delineated several features commonly associated with circRNA occurrence. CircRNA-producing genes tend to be longer and to contain more exons than average. Back-splice acceptor exons are strongly enriched at ordinal position 2 within genes, and circRNAs typically have a short exon span with two exons being the most prevalent. The flanking introns either side of circRNA loci are exceptionally long. Of note also, single-exon circRNAs derive from unusually long exons while multi-exon circRNAs are mostly generated from exons of regular length. These findings independently validate and extend similar observations made in a number of prior studies. Furthermore, we analysed high-resolution RNA polymerase II occupancy data from two separate human cell lines to reveal distinctive transcription dynamics at circRNA-producing genes. Specifically, RNA polymerase II traverses the introns of these genes at above average speed concomitant with an accentuated slow-down at exons. Collectively, these features indicate how a perturbed balance between transcription and linear splicing creates important preconditions for circRNA production. We speculate that these preconditions need to be in place so that looping interactions between flanking introns can promote back-splicing to raise circRNA production to appreciable levels.

101 Expression, alternative splicing and function of circRNAs in neuronal development and disease

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Circular RNA (circRNA) is a poorly understood class of non-coding RNAs with is particularly highly expressed expression in the brain. We have studied the expression of circRNA in various neuronal systems including human pluripotent stem cells at different developmental stages, fetal pig brain, which closely resembles the human brain in terms of developmental stages and brain morphology, as well as in human and mouse brains. To examine the global exon composition of circRNAs, we performed global long-read sequencing of single human and mouse brain-derived RNA using the Oxford Nanopore Technology. By applying an optimized circRNA enrichment protocol this method was established as a fast and reliable method to map the specific exon composition of full-length circRNA. In total, we were able to detect7,834 and 10,975 circRNAs in human and mouse brain, respectively, of which 2,945 and 7,052 were not reported before. Interestingly, alternative splicing was more prevalent in circRNAs than in linear spliced transcripts, and notably >200 not previously annotated exons were used in circRNAs. This suggests that properties associated with circRNA-specific features, e.g. the unusual back-splicing step during biogenesis, increased stability and /or their lack of translation, alter the general exon usage at steady state.

From the profiling of iPSCs during neuronal differentiation we identified unique sets of circRNAs that were sharply regulated in the course of neuronal development. Targeting circRNA expression by CRISPR/Cas or RNA interference we were able to confirm that a subset of the differentially regulated circRNAs play an important functional role in neuronal differentiation and that their action occurs at distinct stages of development.

We have studied the expression of circRNA in connection with various neuronal diseases and find that circRNA expression profiles may constitute reliable biomarkers.

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102 Large Scale Screenings of lncRNA Functions and Structures

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Our recent atlas of human long non-coding RNA (C.C. Hon, JA Ramilowski, et al., Nature, 543, 2017) shows computational evidence of function or implication in disease for nearly 20,000 lncRNAs. Experimental functional and structural annotations of lncRNA are further required to understanding multilayer regulation and function of the human genome. In the pilot phase of the FANTOM 6 project, we performed antisense oligo knockdowns of over 600 nuclear and cytoplasmic lncRNAs expressed in human dermal fibroblasts and in iPS cells. Next, we comprehensively compared resulting changes in cell growth and morphology to the transcriptional response quantified by CAGE. Molecular phenotyping predicted by CAGE recapitulated cellular phenotypes and suggested additional functions of lncRNAs, while revealing changes in underlying genes and pathways. In addition, we have been exploring structural elements of thousands of lncRNAs in cells cytoplasm and nucleus. We aim to integrate the findings with additional data sets and predictions (coexpression, HiC, RBPs, motifs, etc.) to infer potential functions of lncRNAs.

103 Ribosomes in unfamiliar territory: consequences of translation in noncoding sequences

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Ribosome occasionally finds itself in unfamiliar territory: translating a noncoding sequence that has not evolved to encode a protein, such as a lncRNA or an mRNA UTR or introns. In addition to the waste of resources, such noncoding translation events might produce peptides/proteins that are harmful to the cell. Because defective mRNAs accumulate in both normal ageing and pathological conditions, such as neurodegenerative diseases and cancer, it is important to understand the consequences of noncoding translation. We have generated a large pool of reporters that forced ribosomes to translate 12,000 noncoding regions of human transcriptome, and found that translation of those noncoding regions led to proteasome-mediated degradation in the absence of significant mRNA decay. Sequence analysis revealed a C-end rule of protein degradation similar to those recently reported by two other groups. Together with existing literatures, our results suggest a potential feedback loop formed by widespread mRNA processing defect, noncoding translation, and proteasome saturation in driving the progress of ageing and cancer.

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104 Non-canonical translation initiation in yeast generates a cryptic pool of mitochondrial proteins.

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Alternative translation initiation is an important source of additional protein isoforms and contributes to expanding the capacity of eukaryotic proteomes by allowing expression of multiple protein isoforms from a single gene. A more specific case concerns utilization of alternative translation initiation sites (aTIS), particularly at non-AUG codons. Non-AUG initiation has been reported for a number of proteins in higher eukaryotes, whereas in *Saccharomyces cerevisiae* only a cases have been described that are translated from upstream near-cognate start codons as N-terminally extended (NTE) variants that localize to mitochondria. We have performed a genomewide *in silico* screen in yeast for proteins that are generated as NTE isoforms by non-AUG initiation. Our bioinformatics analyses, backed up by *in vivo* investigations and ribosome profiling data, revealed that this phenomenon may apply to more than a thousand genes. We have focused on NTE proteins that gain a mitochondrial targeting signal (MTS) through their extensions. We have confirmed mitochondrial localization for a large number of candidates, which previously have not been predicted to reside in this compartment, and we have demonstrated that it depends on translation from the upstream start codon within the NTE. These analyses revealed that utilization of near-cognate start codons in yeast is much more widespread than previously anticipated and creates a large pool of proteins with different localization and possibly also other features. This strategy to increase the proteome is probably conserved in higher eukaryotes, since human homologs of several yeast candidates from our screen also have mitochondrial isoforms.

105 mRNA Translation Landscape Shapes the Biology of MYC in Cancer and Immune Response

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Aberrant activation of mRNA translation is emerging as a common theme in many malignancies however genome-wide regulation of translation at the transcript level is not well defined in cancer. We focused on deciphering the changes in translation landscape upon MYC activation using lymphoma models. The oncogenic c-MYC (MYC) transcription factor has broad effects on gene expression and cell behavior. We show that MYC alters the efficiency and quality of mRNA translation into functional proteins. Specifically, MYC drives the translation of most protein components of the electron transport chain in lymphoma cells and many of these effects are independent of changes in proliferation. Specific interactions of MYC-sensitive RNA binding proteins (e.g. SRSF1/RBM42) with 5'UTR sequence motifs mediate many of these changes. Our study establishes a previously unknown mechanism of translation regulation through splicing factor SRSF1 through binding to specific 5'UTR elements in a MYC dependent manner. Moreover, we observe a striking shift in translation initiation site (TIS) usage. MYC favors translation from upstream regions of mRNA. MYC also modulates the usage of downstream TIS resulting in truncated protein products. For example, in low MYC conditions lymphoma cells initiate translation of the CD19 mRNA from a site in exon 5. This results in the truncation of all extracellular CD19 domains and facilitates escape from CD19-directed CAR-T cell therapy. Together, our findings reveal MYC effects on the production of key metabolic enzymes and immune receptors in lymphoma cells.

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106 mTORC1 coordinates mRNA translation and mitochondrial dynamics and functions through 4E-BP

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Protein synthesis is the most energy consuming process in the cell. mRNA translation and energy metabolism are dysregulated in a variety of diseases, including cancer. However, the mechanisms that coordinate translation and energy metabolism are largely unknown. mTORC1 stimulates mRNA translation and other metabolic processes in response to a variety of extracellular signals, ultimately promoting cell proliferation. Our previous genome-wide polysome profiling analysis revealed that mRNAs whose translation is regulated by mTORC1 are enriched in those encoding mitochondrial proteins.

Here, by using a combination of pharmacological, genetic, biochemical and histological approaches, we identify a hitherto unrecognized link between nutrient-sensing mTORC1, mRNA translation, and mitochondrial functions and dynamics. The alterations in mTORC1 activity are paralleled by dramatic changes in mitochondrial organization as illustrated by hyper-fusion and branching. mTORC1 through the phosphorylation of the translation initiation factor 4E (eIF4E)-binding proteins (4E-BPs) promotes mitochondrial fission and activity by selectively enhancing translation of the mRNA encoding mitochondrial fission protein 1 (MTFP1). We demonstrate that mTORC1 coordinates energy consumption by translation machinery, and energy production by bolstering mitochondrial dynamics and functions. We further show that the suppression of MTFP1 translation upon inhibition of mTOR serves as a cytoprotective mechanism that prevents mitochondrial fragmentation and cell death. These findings provide a molecular basis for improving the anti-neoplastic effects of mTOR inhibitors in the clinic by targeting mitochondria. Indeed, we demonstrate that a combination of oncogenic kinase inhibitors and biguanides that inhibit the mitochondrial respiratory chain complex I exhibit synergistic anti-neoplastic effects across a number of cancer cells. In this presentation, we will introduce our recent data and discuss the role of mTORC1/4E-BP signaling pathway in mitochondrial dynamics, cellular metabolism and cancer.

107 Baseline translation ensures maintenance of a stable population of TOP mRNAs under starvation conditions

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The synthesis and maintenance of the translation apparatus is a major task of rapidly dividing cells and consumes a large proportion of their metabolic energy. In all domains of life, cells have therefore developed mechanisms that control this process based on the availability of nutrients and other growth stimuli. In contrast to a mostly transcription-based regulation in prokaryotes and unicellular eukaryotes, mammals coordinate the production of the proteins of the translation machinery at the post-transcriptional level via the master regulator of cell growth, the mTORC1 complex. Upon mTORC1 inactivation, a general decrease in cap-dependent translation and a particularly marked translational downregulation of TOP-mRNAs encoding components of the translation machinery is induced. The Larp1 protein was proposed to cause the sequestration of TOP mRNAs in translationally silenced sub-ribosomal mRNPs by directly interacting with a cis-acting regulatory sequence, termed the 5' Terminal OligoPyrimidine tract (5'TOP motif) and thereby preventing cap-dependent translation initiation via the eIF4F complex. However, little is known about the composition and functional status of these mRNPs and how degradation of the resident mRNAs is avoided. Here, we show that 5'TOP mRNAs are not sequestered in silenced mRNPs upon starvation. Rather, they perform baseline translation due to drastically reduced initiation rates. This mode of regulation ensures the maintenance of a stable population of mRNAs under starvation condition that guarantees a baseline production of their protein products. Furthermore, the generation of mRNPs with low translational activity explains the complete and fast reversibility of the translational repression of 5'TOP mRNAs and their increased stability compared to other mRNAs upon starvation.

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108 LARP1 is a novel mTORC1 substrate and a key repressor of TOP mRNA translation

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The mammalian target of rapamycin complex 1 (mTORC1) controls critical cellular functions such as protein synthesis, lipid metabolism, protein turnover and ribosome biogenesis through the phosphorylation of multiple substrates. In this study, we examined the phosphorylation of a recently identified target of mTORC1: La-related protein 1 (LARP1), a member of the LARP superfamily. Previously, we and others have shown that LARP1 plays an important role in repressing TOP mRNA translation downstream of mTORC1. LARP1 binds the 7-methylguanosine triphosphate (m^7Gppp) cap moiety and the adjacent 5'terminal oligopyrimidine (5'TOP) motif of TOP mRNAs, thus impeding the assembly of the eIF4F complex on these transcripts. mTORC1 plays a critical role in the control of TOP mRNA translation *via* LARP1 but the precise mechanism by which this occurs is incompletely understood. The data described herein help to elucidate this process. Specifically, it show that: (i) mTORC1 interacts with LARP1, but not other LARP superfamily members, *via* the C-terminal region that comprises the DM15 domain, (ii) mTORC1 pathway controls the phosphorylation of multiple (up to 26) serine and threonine residues on LARP1 *in vivo,* (iii) mTORC1 regulates the binding of LARP1 to TOP mRNAs and (iv) phosphorylation of S689 by mTORC1 is particularly important for the association of the DM15 domain of LARP1 with the 5'UTR of RPS6 TOP mRNA. These data reveal LARP1 as a major substrate of mTORC1.

109 Loss of tRNA modification i^6A_{37} leads to mitochondriopathy and increased +1 frame-shifting in mice

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tRNA modifications in the anticodon loop are crucial for translation efficiency and fidelity. The enzyme tRNA-isopentenyltransferase 1 (TRIT1) modifies adenosines at position 37 in tRNAs with an isopentenyl moiety at N6 (i^6A). We showed that Trit1 is the only enzyme capable to isopentenylate tRNAs in mice.

In mammals, cytoplasmic tRNAs carrying i^6A are the three $tRNA^{Ser(UCN)}$ and $tRNA^{Sec(UGA)}$. Mitochondrial isopentenylated tRNAs, Tyr, Trp, Phe, Cys and Ser(UCN), are further thiomethylated to ms^2i^6A (2-methylthio-N6-isopentenyl adenosine). While i^6A is among the tRNA modifications longest known, its significance in animals remained unclear. We have conditionally inactivated Trit1 (tRNA:dimethylallyl-isopentenyltransferase 1) in mouse hepatocytes and neurons accordingly abrogating tRNA isopentenylation in targeted cells.

Ribosomal profiling of nuclear transcripts in liver reveals increased +1 frame-shifting, starting at the initiation site. A-site occupancy of codons requiring wobble base pairs is changed in mitochondria. Expression and activity of mitochondrial electron transport chain subunits is impaired and the mitochondrial integrated stress response pathway is mounted. The cytokine Fgf21 is secreted into the plasma. The mitochondria specific protease Lonp1 as well as the chaperones Hspa8 and Hspa9 are induced. Amount of $(ms^2)i^6A$ modified tRNAs is increased in mitochondria. Neuron-specific *Trit1* knockout mice exhibit microcephaly and seizures and upregulate markers of the unfolded protein response pathway.

Inactivation of tRNA isopentenylation is more severe than inactivation of tRNA thiomethylation, underlining the fundamental role of i^6A in mammalian biology.

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110 Novel cross talk between tRNA and mRNA processes exerted by Aminoacyl tRNA synthetases

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Recent RNA interactome studies had identified many novel mRNA-binding proteins. Interestingly, a recurring family of proteins detected in these studies are the cytosolic aminoacyl tRNA synthetases (aaRSs). Association of this well-studied tRNA binding protein-family with mRNA raises intriguing questions regarding their possible targets, mode of interaction and the physiological significance of their dual interaction with tRNA and mRNA. We therefore investigated the repertoire of mRNAs bound by members of this family in yeast. Using RNA immunoprecipitation followed by deep sequencing (RIP-seq), we identified subsets of mRNAs associated with aaRSs. Interestingly, in all cases we observed strong association of each aaRS with its own mRNA ('self-association'). We aimed to determine the target mRNA motif bound by a representative aaRS and identified that association occurs through its cognate tRNA anticodon-mimic. Point mutations within the anticodon-mimic dramatically reduces self-association, concomitant with increased translation, suggesting that self-association autoregulates translation. Finally, we found that overexpression of the cognate tRNA alleviates mRNA association and increases protein production in an anticodon-mimic dependent manner. Overall, our study reveals a novel aaRS-dependent cross talk between tRNA and mRNA processes that affects aaRS translation in response to amino-acid charging demands. Furthermore, it emphasizes the importance of interactions through similar RNA elements to coordinate distinct cellular processes.

111 A large-scale functional tethering screen identifies UBAP2L as a translational enhancer protein to rescue neurodevelopmental defects in Fragile X Syndrome

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RNA binding proteins (RBPs) impact cellular protein levels by regulating messenger RNA (mRNA) levels. Assignment of function to hundreds of emerging, uncharacterized RBPs is a critical bottleneck to a complete understanding of gene expression control. Here, large-scale tethering of nearly a thousand RBPs discovers 74 RBPs that affect reporter RNA turnover and translation. Enhanced UV crosslinking and immunoprecipitation (eCLIP) identify hundreds of endogenous mRNA targets affected by manipulating levels of more than a dozen candidate RBPs. Among these candidates, we characterize the ubiquitin-associated protein 2-like (UBAP2L) gene. Polysome profiling assays indicate that UBAP2L enhances translation of target mRNAs, likely due to ribosome interactions as supported by eCLIP data. UBAP2L can also be found in complex with fragile X mental retardation protein FMRP, and 52% of UBAP2L mRNA targets are also FMRP targets. UBAP2L depletion in a cortical neuronal model of Fragile X Syndrome (FXS) corrects molecular, cellular and electrophysiological defects relevant to autism spectrum disorder. Reduction of the Drosophila ortholog of UBAP2L in a FXS fly model rescues the neurodevelopmental defects due to loss of FMRP. Our efficient and scalable method identifies proteins involved in RNA metabolism and detailed studies of UBAP2L provides a new therapeutic strategy into human disease.

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112 Structural insights into Ssd1p, a spatial and temporal regulator of mRNA translation in budding yeast

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Mechanisms of post-transcriptional control of gene expression by RNA binding proteins (RBPs) include inhibition of translation, mRNA localisation, mRNA degradation and sub-cellular redistribution of transcripts and RBPs in response to stress. The RNA binding protein Ssd1p, found in fungi, is an excellent paradigm for understanding the interplay of post-transcriptional control pathways. Ssd1 has been observed to localise to the bud and bud neck in dividing *S. cerevisiae* cells. Transcripts encoding cell wall remodelling factors have been shown to co-purify with Ssd1p and it is thought that Ssd1 suppresses their translation by binding to specific sequences in 5'UTRs. However, which RNA motifs are directly recognised by Ssd1 and how they are recognised is not well understood. Ssd1p shows sequence similarity to the DIS3 family of exoribonuclease but residues essential to catalytic function are altered. We present a 1.9 Å X-ray crystal structure of Ssd1p. In addition to loss of catalytic residues, loop sequences specific to Ssd1p block the route normally taken by RNA substrates of DIS3-related enzyme to access the active site. Using in vitro binding assays, we characterise specific Ssd1-associated RNA motifs identified by UV crosslinking and cDNA analysis (CRAC).These studies reveal how the exonuclease scaffold has adapted and evolved into an RNA binding protein that controls functionally related mRNA targets.

113 A learning model for RNA secondary structure prediction using SHAPE mapping and sequence alignment data

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Folding algorithms based on thermodynamics have proven to be useful in the prediction of RNA secondary structure. These algorithms are based on dynamic programming and allow the full ensemble of available secondary structures to be obtained, given the sequence. However, in benchmarks where three-dimensional structures are available and predictions are done based on thermodynamic models alone, the correct experimental secondary structures might be assigned a low population in the model ensembles. In order to improve the reliability of predictions, the model free energy defining these ensembles can be extended to integrate data from structure probing experiments [1]. In this work we modify the ViennaRNA [2] model free energy with two terms to simultaneously include SHAPE reactivities and co-evolutionary information from direct-coupling analysis of multiple sequence alignments [3]. The model is trained to reproduce secondary structures known from high-resolution X-RAY diffraction and allows for more precise and accurate predictions. The transferability of the parameters is assessed with a rigourous cross-validation procedure. The resulting model can be used to gain insight on the information content of SHAPE reactivity profiles and co-evolutionary data.

[1] R.Lorenz, D.Lunzter, I.L Hofacker, P.F Stadler and M. Wolfinger, Bioinformatics, 2016, **32**(1), 145-147.

[2] R.Lorenz, S.H. Bernhart, C. Höner zu Siederdissen, H. Tafer, C. Flamm, P.F. Stadler and I. L. Hofacker, Algorithms for Molecular Biology, **26,** 2011.

[3] F. Cuturello, G. Tiana, G. Bussi, arXiv:1812.07630, 2018.

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114 RNA structural ensembles at the exon-intron boundary *in vivo*

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The process of splice site selection by the spliceosome is dependent on the recognition of *cis*-sequence elements by trans-factors. These *cis*-elements include accessory sequences, like splicing enhancers or silencers, which are modulated by RNA structure around functional elements. RNA structures can promote or block *trans*-factor recognition and modify the effectiveness of *cis*-elements. The majority of disease-associated single nucleotide variants (SNVs) that occur close to splice junctions in intronic sequences cannot be explained by direct disruption of a *cis*-element. SNVs that change the structure of the RNA and impair splicing elements, but do not directly alter canonical splicing elements, are the focus of our study. Of 127,445 disease-associated SNVs within the Human Gene Mutation Database we predicted that 13.1% are predicted to disrupt splicing, and 1.6% (>2,000 SNVs) of those are likely to function by changing RNA secondary structure. One particularly salient example is in *MAPT*, a gene that codes for the Alzheimer's associated Tau protein. *MAPT* mRNA transcripts exist as 3R and 4R isoforms, based on exon skipping, which is thought to be in part regulated by an RNA hairpin forming at the exon-intron junction. Using *MAPT* as a model system for understanding how SNVs affect RNA structure and splicing, we determined pre-RNA secondary structure using chemical probing techniques, *in vivo,* with a novel pre-mRNA read sorting strategy that leverages the unique Mutational-Profiling (MaP) read-through reverse transcription step. Using mutational and ensemble-guided structural data, we have developed a quantitative model documenting how RNA structure, intronic and exonic splicing elements interplay to control the ratio of 3R to 4R *MAPT* isoforms. This model is unique in that it considers the ensemble of suboptimal structures existing *in vivo* as opposed to the minimum free energy of folding, and as such provides a structurally rigorous framework to reconcile splicing *cis* and *trans* elements with RNA structural ensemble characterization *in vivo.*

115 Improved boundary definition of RNA structures using multiple sequence alignments and its application on RNA motif detection.

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Self-contained structured domains of RNA sequences have often distinct molecular functions, such as regulating complex cellular processes or catalyzing biochemical reactions. The precise definition of the boundaries of RNA structures is essential for their characterization by modelling, e.g., through covariance models, or clustering for the search of common motifs. Previous efforts have focused on single sequences. In the first part of this presentation we compare the accuracy of boundary definition from single sequences and multiple sequence alignments. The performance of different boundary definition methods had been tested on a limited number of Rfam families using the annotated structured RNA regions in the human genome and their multiple sequence alignments created from 14 species. We introduce the program RNAbound (PMID: 30518121) for finding the boundaries that are based on probabilities of evolutionarily conserved base pairings. The results show that multiple sequence alignments improve the boundary prediction for branched structures compared to single sequences independent of the chosen method. The actual performance of the methods, however, differs on single hairpin structures and branched structures.

In the second part we discuss the application of boundary definition on RNA motif detection from computational predictions and experimental signals. First, we search the location of RNA structures inside non-coding RNAs predicted by the non-coding RNA gene finder program RNAz (PMID: 19908359) in pre-defined genomic windows of aligned vertebrate genomes. Second, we search structure boundaries around signals of RNA-protein binding sites from ENCODE immunoprecipitation data, which are further used for identifying and clustering of common RNA binding motifs through the clustering program DotAligner (PMID: 29284541). In both studies the improved boundaries of the detected RNA motifs help with their characterization and with assigning discrete biological functions to RNA.

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116 The importance of being RNASt

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Structural characterization of RNAs is a dynamic field, exposing many modelling possibilities. Every model is characterized by an encoding in which to include structural information of a molecule ranging from string representations to graphs. These mappings usually do not consider structure as an evolving concept, as it is instead well established with nucleotides and their substitution matrices.

In recent works, our lab developed a structural encoding, the BEAR alphabet, that allowed the improvement of pairwise alignments (Beagle) and motif discovery (BEAM) in the analysis of RNA secondary structure. Both have been tested on simulated and real data and show results consistent with previous findings.

BEAM is particularly efficient for high-throughput data, such as CLIP-Seq, in uncovering structural recurrences among sets of functionally related RNAs.

In particular, BEAM employs a simulated annealing approach to maximize the local alignment of primary and/or secondary structures, and the method has so far been successfully applied on more than 200 high-throughput CLIP-Seq (and similar) datasets, unveiling both known and putative motifs which act or co-exist as drivers in an interaction scenario. The method has been thoroughly tested on simulated data and Rfam as well, showing how its robustness let one investigate even with highly noisy datasets, which is to be expected in the experiments taken into consideration.

These results were made possible by the construction of a substitution matrix of Secondary Structure Elements, which measures the amount of structural variability in curated alignments of RNA molecules.

We are developing a framework for the custom creation of Secondary Structure Elements similarity matrices, inspired by classical formulations of Blocks. These substitution matrices are more than just a means to improve alignments, as the information they carry can emerge by exploiting other measures such as entropy and distance trees. In this regard, we show a way to group RNA families by their structure, and how those structures are locally conserved.

The importance of a well-balanced notation is hence called for, since an efficient encoding of the RNA secondary structure facilitates the extraction of meaningful information, being aware that different notations may perform best on different sets of tasks.

117 Automated and customizable identification of 3D modules and prediction of RNA 3D structures

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RNA structures are articulated around sophisticated 3D modules connecting together secondary structure elements (i.e. Watson-Crick helices). The classification, identification and analysis of these structural subunits is key to understand the folding principles, decipher evolutionary mechanisms, and design 3D structure prediction algorithms. Here, I will describe our progresses toward the implementation of a customizable computational platform to analyze, predict and build 3D structures of large RNA molecules. First, I will present BayesPairing [1], an automated, efficient and customizable tool for building Bayesian networks representing RNA 3D modules and rapidly identifying occurrences of these 3D modules in sequences. BayesPairing uses a flexible definition of RNA 3D modules that allows us to model complex architectures such as multi-branched loops, and features multiple algorithmic improvements enabling us to accelerate searches and scan full genomes. Then, I will show how to leverage this information to rapidly and accurately build 3D structures of RNAs with up to 150 nucleotides using RNA-MoIP [2,3]. More specifically, RNA-MoIP identifies and refines secondary structure predictions that are compatible with 3D modules predictions. Then, it assembles this information into 2.5D templates that are injected into the MC-Sym program to generate full 3D models. Our results show that this strategy significantly improves the speed and accuracy of 3D structure prediction software. We illustrate the potential of this technology on a set of selected examples and showcase our free online web services.

References:

[1] Sarrazin-Gendron, Reinharz, Oliver, Moitessier, Waldispühl (2019). Automated, customizable and efficient identification of 3D base pair modules with BayesPairing. Nucleic Acids Research.

[2] Reinharz, Major, Waldispühl (2012). Towards 3D structure prediction of large RNA molecules: an integer programming framework to insert local 3D motifs in RNA secondary structure. Bioinformatics.

[3] Yao, Reinharz, Major, Waldispühl (2017). RNA-MoIP: prediction of RNA secondary structure and local 3D motifs from sequence data. *Nucleic Acids Research (Web Server Issue)*.

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118 New classification of tetrads and quadruplexes in DNA and RNA structures

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Quadruplexes are unique tertiary structure motifs occurring in nucleic acid molecules. So far, they have been confirmed as promising therapeutic targets in many drug development strategies and contributors to various biological processes. Recent years, brought increasing interest in their structure and roles, especially in relation to biomedicine. Thus, new computational methods dedicated to these motifs started to appear. Most of them support quadruplex analysis on the sequence level. Some touch the 3D structure and its classification defined in [Webba da Silva, 2007]. Hereby presented approach is based on the secondary structure and was initiated by the development of RNApdbee 2.0 [Zok et al., 2018], a bioinformatics system for RNA secondary structure annotation. A new option in this tool to display and annotate non-canonical interactions in the secondary structure diagrams made us observe specific patterns in the visualization of RNA structures containing quadruplexes. Their observable recurrence in both RNAs and DNAs allowed for describing novel classes of tetrads and quadruplexes. We developed an algorithm to identify quadruplexes in the 3D structures and classify them according to our new nomenclature. We completed a statistical analysis of new classes' coverage by tetrads and quadruplexes included in the PDB-deposited structures. We introduced a multiline dot-bracket encoding optimized to represent the secondary structure of these motifs. Finally, we studied the relationship between our classification and the formalism defined in [Webba da Silva, 2007]. These two approaches address different properties of the structure, and - according to our discoveries - they are not totally complementary. We believe, our methodology creates a new perspective in the research focusing on quadruplex motifs and opens the new paths in their analysis.

Acknowledgements

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References

1.Webba da Silva M. (2007) Geometric formalism for DNA quadruplex folding. Chemistry, 13(35), 9738-9745.

2.Zok T., Antczak M., Zurkowski M., Popenda M., Blazewicz J., Adamiak R.W. and Szachniuk M. (2018) RNApdbee 2.0: multifunctional tool for RNA structure annotation. Nucleic Acids Research, 46(W1), W30-W35.

119 Rosetta modeling and M2-seq for coordinate inference in rapidly determined RNA-only cryo-EM maps

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Recent advances in RNA computational modeling and high-throughput biochemistry offer the prospect of rapid determination of RNA structures when coupled to modest resolution experimental data. Here we describe integration of the Rosetta DRRAFTER algorithm and mutate-and-map-seq (M2-seq) secondary structure determination with advances in cryoEM. The resulting hybrid method has enabled the all-atom 3D coordinate determination of 14 RNAs of previously unknown structure on the timescale of a few months. The targets range from ribozymes to riboswitch aptamers to synthetic RNA nanostructures designed *de novo*; and the sub-nanometer resolution of the models give a wealth of functional insights. The use of computer methods allows for unbiased estimates of coordinate errors, which we have validated through blind challenges, recovery of 'internal control' structures, and mutate-map-rescue experiments.

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120 Integrating SAXS, chemical probing, motif-modeling and cryo-EM fitting: two-dimensional and three-dimensional structure-function relationships for individual long non-coding RNAs

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Fundamental questions regarding the structure of lncRNAs are just beginning to be explored, including: (1) are lncRNAs organized into modular sub-domains or linear chains of stem loops?, (2) what do detailed structure-function relations look like in the case of lncRNA systems?, and (3) do lncRNAs have tertiary structure? We use a combination of 2-D chemical probing, 3-D small X-ray scattering (SAXS) and CRISPR-Cas9 to elucidate structure-function relations in individual lncRNAs. We have produced 2-D models by applying chemical probing in a hierarchical fashion (Shotgun Secondary Structure, or 3S) to derive secondary structures of intact, individual lncRNAs and identify modular sub-domains. We applied this method to the mouse Braveheart, plant COOLAIR, human SRA1, HULC, and Gas5 lncRNAs and the 3'-end of MALAT1. Braveheart, COOLAIR and SRA1 each show highly structured sub-domains and share a unique expansive internal loop (r-turn motif). The structures were combined with other data (CRISPR-Cas9 and SNP data) to inform on structure-function relationships. For Braveheart, CRISPR-Cas9 analysis demonstrates that the expansive loop motif determined from 3S is necessary for cardiomyocyte lineage commitment. This loop was found to interact with a zinc finger transcription factor (CNBP). For 3-D models, we use a motif-based system to construct 3-D models from 2-D secondary structures for large systems. We then perform SAXS studies and use the resulting 3-D volume envelopes in our cryo_fit package to morph 3-D models into the SAXS envelop. 3D studies using SAXS demonstrate organized 3-D structure that is modified due to CNBP.

121 Modeling of ribonucleic acid-ligand interactions

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Currently, most of the registered drugs are small molecule compounds [1]. Computational methods play a pivotal role in the early stages of drug discovery and are widely applied in virtual screening, structure optimization, and compound activity profiling. Over the last decades, almost all the attention in medicinal chemistry has been directed to protein-ligand binding, and computational tools have been created with such targets in mind. However, with growing discoveries of functional RNAs and their possible applications, RNA have gained considerable attention as possible drug targets. This flow of discovery was followed by adapting existing protein-based computational tools for RNA applications, as well as active development of new RNA-tailored methods. However, due to the difference in nature of RNA from that of proteins, especially its tendency to use morphological plasticity (conformational change in ligand binding), the modeling of RNA remains a challenging task [2]. The evolution of 'protein-based' drug discovery and related computational methods offers some clues on possible future directions and developments in modeling RNA interactions with small molecule ligands.

We will present two new computational tools for predicting RNA-ligand interactions. One is a semi-automatic modeling procedure, which involves input data pre-processing (RNA and ligand files), molecular docking, re-scoring of models of complexes, clustering of poses and visualization of results. The second tool that we will present is a new scoring function, AnnapuRNA, for scoring RNA-ligand complexes obtained, e.g., from molecular docking. It is based on statistical data derived from the experimentally solved RNA-ligand complex structures and predictive models obtained using machine learning techniques. According to our benchmarking tests, AnnapuRNA outperforms other tested scoring functions, including rDock and our previous method LigandRNA. Taken together, our methods present an advancement over the existing tools in both accuracies of predictions and ease of use.

We will also present the plans for the future development of predicting methods that take into account the full flexibility of the RNA and ligand.

References:

[1] Newman D.J. et al., J. Nat. Prod., 2016, 79 (3), pp 629-661.

[2] Stefaniak F. et al., WIREs Comput Mol Sci 2015, 5:425-439.

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122 Computational approaches to targeting RNA with small molecules

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The challenge of drugging RNA with small molecules is exemplified in part by the emerging view that there are ligandable *structured* segments within RNA that can be targeted to modulate desired biology. Recent small-molecule modulators of RNA pathology such as branaplam from Novartis and ribocil from Merck shine an opportunistic light on a path forward for drugging RNA. New biology around RNA is also emerging that gives us a better understanding of the problem that we are facing. Drugging RNA remains a complex problem; to understand the role of computation better, we present some approaches to tackling the problem through a model aptamer system and mRNA. We will discuss questions such as: Where and how does the small molecule bind? How does RNA dynamics play a role in ligand binding? And how well do computational techniques provide an atomistic or nucleotide-level view of complex chemical probing data.

123 An Integrator 'Phosphatase Module' Remodels the RNAPII CTD to Attenuate Transcription

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The Integrator complex (INT) consists of at least 14 highly conserved subunits, and is associated with phosphorylated RNA polymerase II (RNAPII). Key functions ascribed to INT are the co-transcriptional cleavage of UsnRNA and eRNA as well as regulating the activity of paused RNAPII at many genes critical for development. Our recent work revealsthat INT drives promoter-proximal termination of transcription and gene attenuation at a broad set of stimulus-responsive mRNA genes in *Drosophila*. Using nascent RNA sequencing, we find that INT-attenuated genes successfully recruit RNAPII to their promoters, but the paused polymerase is unstable and fails to enter productive elongation. Importantly, the endonuclease activity of Integrator subunit 11 (IntS11) is essential for INT-mediated transcription attenuation.

Here, we report that protein phosphatase 2A (PP2A) is stable component of INT that does not associate using any of the known canonical PP2A B regulatory subunits. Rather, we find a small, highly conserved motif within Integrator subunit 8 (IntS8) is required for the PP2A catalytic core (PR65 and PP2Ac) to interact with Integrator. Using both in vitro and in vivo approaches, we find that a key phospho-substrate for INT-PP2A is ser7P within the CTD as purified INT-PP2A is highly specific to this modification and cells depleted of specific INT subunits have increased levels of ser7P within the CTD. We observe that human or fly cells expressing RNAi-resistant IntS8 harboring a mutation that specific disrupts PP2A association have defects in UsnRNA biogenesis and, importantly, are deficient to attenuate RNAPII at INT-regulated gene promoters. Our results suggest that IntS8 utilizes PP2A to dephosphorylate ser7P preventing robust addition of ser2P by pTEF-b thereby preventing RNAPII pause-release. The unstable, dephosphorylated RNAPII is then induced to terminate through nascent RNA cleavage by IntS11. Overall, our work indicates that Integrator contains two distinct catalytic activities that are both involved in promoting the promoter-proximal termination of RNAPII and likely explain the previously reported affinity of Integrator toward ser7P.

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124 Gene-Specific Variation in Co-Transcriptional Pre-mRNA Processing

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Gene expression requires cooperation between RNA polymerase II (Pol II) and the RNA processing machinery. While 5' end capping and cleavage at the polyA site are absolutely coupled to transcription initiation and termination, co-transcriptional splicing takes place during elongation and is likely more variable. For example, we have assumed that the longer the gene, the greater the chance that introns will be spliced out before termination. To identify regulatory transitions in co-transcriptional RNA processing, we previously developed single molecule nascent RNA sequencing methods that detect and quantify splicing precursors, intermediates and products. In *Schizosaccharomyces pombe*, such data revealed a positive correlation between intron splicing and polyA cleavage; notably, unspliced transcripts failed to undergo polyA cleavage, displayed transcriptional readthrough, and were degraded by the nuclear exosome1. Here, we postulate functional coupling between splicing and polyA cleavage and investigate this further in *Saccharomyces cerevisiae*. Using Single Molecule Intron Tracking (SMIT) and long-read sequencing, we show that unspliced transcripts also undergo transcriptional readthrough in budding yeast, indicating conservation of regulatory mechanisms. Interestingly, in depth SMIT analysis of 87 budding yeast genes revealed diverse co-transcriptional splicing kinetic profiles that proceed in two phases: first, a sharp rise in spliced nascent RNA when Pol II is ~50nt downstream of all introns and, second, a downstream plateau referred to as the saturation value that is independent of gene length. Saturation values vary considerably from gene to gene. These observations therefore invoke a new model, in which a discrete level of splicing is set early in the transcription of each gene. To identify the regulator(s) controlling splicing and readthrough within this "window of opportunity", we employed machine learning algorithms capable of predicting co-transcriptional splicing kinetic parameters from gene-specific features, including DNA and RNA sequence and structure, chromatin profiles, regulatory binding proteins, and transcriptional dynamics. The model selected 29 feature groups which contributed significantly to prediction performance, including several prominent polyA cleavage and transcription termination factors. Follow-up SMIT experiments comparing WT to strains mutant in identified factors suggest that Pol II-associated polyA cleavage factors may regulate the efficiency of splicing prior to 3' end formation.

125 Isoform-specific translational control is conserved across higher primates

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Alternative splicing is an important regulatory process that controls gene expression. Recent work from our lab and others demonstrates that splicing influences translational control of alternative mRNA isoforms by remodeling cis-regulatory elements 1–3. However, the mechanisms responsible for the coordination of alternative splicing coupled with translational control (AS-TC) is still unknown. To understand the evolutionary origins of AS-TC, we employed a comparative transcriptomics approach to identify sequence elements associated with isoform-specific mRNA translation. In order to test this hypothesis, we employed a comparative transcriptomics approach to identify sequence elements associated with isoform-specific mRNA translation. We sequenced polyribosome-associated mRNA from different subcellular fractions (Frac-seq) of human, chimpanzee, and orangutan induced pluripotent stem cells (iPSCs). After identifying orthologous alternative splicing events and calculating the interspecies Manhattan distance across all fractions, we discovered groups of alternative mRNA isoforms, mainly defined by skipped exon events, with ribosome association patterns that were conserved between the human, chimpanzee, and orangutan iPSCs. Remarkably, we identified hundreds of isoforms, generated predominantly through the use of alternative first exon or exon skipping events, with lineage-specific ribosome engagement. We show that isoforms with conserved patterns of ribosome association show higher sequence conservation near the edges of the cassette exon than those with differential ribosome association. These data demonstrate that AS-TC is a conserved mechanism for post-transcriptional gene regulation and suggests the intriguing hypothesis that genetic variation underlies lineage-specific translational control of alternatively spliced mRNA isoforms.

1. Sterne-Weiler, T. et al. Frac-seq reveals isoform-specific recruitment to polyribosomes. Genome Res. 23, 1615–1623 (2013).

2. Floor, S. N. & Doudna, J. A. Tunable protein synthesis by transcript isoforms in human cells. Elife 5, (2016).

3. Wong, Q. W.-L. et al. Embryonic Stem Cells Exhibit mRNA Isoform-Specific Translational Regulation. PLoS One 11, e0143235 (2016).

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126 MCL1 alternative 3'UTRs play a role in Mcl-1 protein localization and function

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Alternative polyadenylation in the 3' untranslated region (3'UTR-APA) generates mRNA isoforms that display alternative 3'UTRs. Myeloid cell leukemia 1 (Mcl-1) is an anti-apoptotic member of the Bcl-2 family that plays an essential role in cell survival. We have found two MCL1 mRNA isoforms generated by 3'UTR-APA in human T lymphocytes, named pA1 and pA2, which only differ in the length of their 3'UTR. Here we investigated the role of the alternative 3'UTRs in Mcl-1 protein function and subcellular localization. Our confocal microscopy results clearly show that Mcl-1 encoded by the pA1 mRNA isoform localizes in the mitochondria, the conventional localization of Mcl-1. Surprisingly, Mcl-1 derived from pA2 mRNA localizes throughout the cell. Constructs harboring deletions of the 3'UTR showed that a region around 800 nt upstream pA2 is responsible for Mcl-1 mitochondria delocalization. We quantified EGFP-Mcl-1 fluorescence intensity and showed that the Mcl-1 pA2 encoded protein is less expressed that pA1, in accordance with luciferase assays. To investigate the individual function of pA1 and pA2 isoforms, we used CRISPR/Cas9 genome editing technology to delete pA1 or pA2 poly(A) signals in Jurkat cells. To study the physiological impact of each MCL1 APA isoform on cell viability, we quantified the apoptosis levels and the results demonstrated that both pA1 and pA2 contribute to the anti-apoptotic function of Mcl-1. Taken together, our results provide new insight on the function of the two MCL1 alternative polyadenylation isoforms by demonstrating that both contribute for the Mcl-1 anti-apoptotic function but have a distinct role on Mcl-1 protein subcellular localization.

127 Post-translational modifications of Rpb4 are required for the linkage between mRNA synthesis, translation and decay

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The yeast Rpb4/7 heterodimer plays roles in all the major stages of the mRNA life cycle: mRNA synthesis, export, translation and mRNA decay. Previously we reported that Rpb4/7 binds RNA Polymerase II (Pol II) transcripts co-transcriptionally and accompanies the resulting mRNAs throughout their life. By virtue of its capacity to interact with key regulators (e.g., eIF3, Pat1) - temporally and spatially, Rpb4/7 regulates each of these stages. We proposed that Rpb4/7 integrates all stages into a system, thus functioning as an "mRNA coordinator" (Harel-Sharvit et al., *Cell* **143**, 552 [2010]). Using 2-dimensional gel electrophoresis and mass spectrometry, here we show that Rpb4/7 carries ~100 combinations of post-translational modifications (PTMs). Most PTMs occur on Rpb4, while Rpb4/7 is engaged in post-transcriptional stages. These modifications are biologically significant as they are responsive to the environment and are required for proliferation under stress. Remarkably, the PTMs repertoire of Rpb4 changes as the mRNP progresses from one stage to the next. Thus, each stage is characterized by unique combination of PTMs. We mutated a number of residues that underwent PTMs and found that specific PTMs are required for efficient transcription, efficient mRNA decay or translation. Interestingly, some of our mutants uncouple mRNA synthesis from degradation, indicating that Rpb4 PTMs play a key role in the linkage between mRNA synthesis and decay. This function argues against a prevailing model whereby mRNA synthesis and decay are linked by default. Analyses of interacting partners of either Rpb4 or its mutant derivatives highlighted a novel type of interactions that could be discovered only with mutants that mimic constitutive modifications. For example, some specific, but transient, PTMs are required for recruiting the capping enzyme or TFIIS to Pol II. Taken together, we propose that numerous transient Rpb4/7 PTMs are involved in the cross talks among the various stages of the mRNA life, in agreement with our previously proposed role of Rpb4/7 as an mRNA coordinator.

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128 Defining requirements for PKR activation by snoRNAs

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Protein Kinase RNA-activated (PKR) is an interferon-inducible kinase that is potently activated by long double-stranded RNA (dsRNA). In a previous study we found that snoRNAs exhibit increased association with PKR in response to metabolic stress. Follow up studies demonstrated that snoRNAs can directly bind and activate PKR in vitro. This was unexpected as snoRNAs do not adopt the canonical structure known to potently activate PKR, perfectly base-paired dsRNA. To further interrogate the mechanism of PKR activation by snoRNAs we selected a representative snoRNA, SNORD113, for use in downstream studies. In vitro transcribed SNORD113, containing a 5'-triphosphate, is a potent activator of PKR, while chemically synthesized SNORD113, containing a 5'-hydroxyl, does not activate PKR. However, in vitro transcribed SNORD113 treated with Calf Intestinal Phosphatase (CIP), Antarctic Phosphatase, or RNA 5´Pyrophosphohydrolase, to remove the 5'triphosphate, activated PKR to a similar extent as mock treated SNORD113, which retains the 5'-triphosphate. Indeed, we have tested additional snoRNAs and ss-dsRNA(9,11), and see no effect of the 5'phosphorylation status on PKR activation.

In hopes of understanding why the T7 transcript, but not the synthetic substrate, activated PKR, we analyzed the T7 transcript by native PAGE. This analysis revealed that SNORD113 adopts three main conformers, "top", "middle", and "bottom", which re-equilibrate after native PAGE purification. Correlating analytical ultracentrifugation data with native gel analyses indicates the "top" band represents a dimer of SNORD113, and the "bottom" band represents a monomer of SNORD113. While SNORD113 conformers re-equilibrate after native PAGE purification, they exhibit different properties regarding PKR activation. The two predominant conformers ("top" and "bottom") do not activate PKR, while the "middle" conformer is a potent PKR activator. As above, we see no significant difference between mock or CIP treated SNORD113 after PAGE purification in regards to PKR activation.

In summary, we observe distinct differences between chemically synthesized SNORD113 and in vitro transcribed SNORD113 that are independent of the 5'-triphosphate. In ongoing experiments, we hope to determine if the activating "middle" conformer is an alternate conformer of SNORD113, or a species related to an aberrant T7 side product, as well as provide ways to minimize such activating species.

129 The role of RNA-binding proteins in regulating the RIG-I/interferon type I signalling pathway

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The retinoic-acid-inducible gene-I (RIG-I) signalling pathway is one of the body's first lines of defence against RNA virus infection. Upon detecting viral RNAs bearing 5'-triphosphate (5'ppp), RIG-I undergoes tri-partite motif 25 (TRIM25)-mediated ubiquitination. This initiates a signalling cascade that culminates in the phosphorylation of IRF-3, IRF-7 and NF-κB, which translocate to the nucleus and induce type I interferon expression, triggering an innate immune response.

We and others have recently revealed that the E3 ubiquitin ligase TRIM25 is a novel RNA-binding protein (RBP). However, little is known about TRIM25's RNA-related roles in innate immunity. Additionally, it is unknown if other cellular RBPs play a role in RNA sequence and structure-specific regulation of the RIG-I/interferon type I signalling pathway.

To address these questions, we engineered human TRIM25 knock out cells to express wild type or mutant TRIM25 proteins and challenged them by infection with an attenuated influenza A virus (IAV), or by transfecting an IAV-derived 5'-pppRNA. As expected, cells lacking TRIM25 were more susceptible to IAV infection, but surprisingly, host RNA-binding deficient mutant TRIM25ΔRBD (lacking 38 amino acids of the C-terminal domain) and TRIM25ΔRING, which does not have E3 ubiquitin ligase activity, fully rescued IAV inhibition. This inhibition did not result from direct repression of viral transcription, as judged by viral "minireplicon" assays. Furthermore, activation of the RIG-I/interferon type I signalling pathway, triggered by the IAV-derived 5'-pppRNA, did not require TRIM25 activity. Unexpectedly, using CLIP-seq on IAV-infected cells showed that both wild type TRIM25 and TRIM25ΔRBD directly bound viral RNAs. In addition, quantitative RNA-pull down SILAC mass spectrometry (RP-SMS), comparing proteins bound to viral and host RNAs, identified sequence-specific RBPs that could regulate the RIG-I/interferon type I signalling pathway.

In conclusion, our data put into question the previously reported primary role of TRIM25 in activating RIG-I and suggest that TRIM25 has yet uncharacterised mechanisms by which it can inhibit an RNA virus and bind to viral RNAs. Finally, our results provide the first evidence that other cellular RBPs that bind to virus-derived RNAs could act as auxiliary factors for the RIG-I/interferon type I signalling pathway.

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130 Ubiquitination efficiency of E3 ligase TRIM25 is regulated by RNAs

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The tripartite motif (TRIM) protein family is one of the largest family of E3 ubiquitin ligases responsible for mediating modification of substrates with ubiquitin or ubiquitin-like modifiers. They consist of their trademark RBCC motif at the N-terminus (RING, 1 or 2 B-boxes, followed by a coiled-coil domain) but feature a variety of domains at the C-terminus, responsible for substrate recognition. In recent years, some of the TRIM members have been shown to be involved in RNA related pathways, foremost the TRIM-NHL subfamily. RNA binding has also been demonstrated for the TRIM-SPRY subfamily member TRIM25, which is involved in many disease-related pathways, but possibly best known for its ubiquitination of RIG-I, thereby triggering antiviral defense. We have accumulated direct evidence from X-ray crystallography, NMR, biochemical and biophysical experiments, that clearly confirms binding of RNA to TRIM25 at the SPRY-Coiled-coil interface. The bound RNA keeps both domains in close proximity and thus brings the bound substrate close to the E2-bound RING domain, which in turn increases ubiquitination efficiency. We have further data, which suggests that viral RNA undercuts this process, thereby preventing the initiation of antiviral defense. This mechanism presents a prime example of regulatory RNAs directly influencing protein function.

131 Cytoplasmic polyadenylation regulates the innate immune response in animals

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Innate immunity is an evolutionally ancient system that provides the first line of non-specific host protection against pathogens. It employs effector cells and a battery of antimicrobial peptides, cytokines, complement factors, proteases, and many other molecules to combat the infection. Since an effective innate immune response is critical for health, it is highly complex and tightly coordinated at multiple levels. Although the importance of post-transcriptional regulation of innate immunity is well appreciated, the particular role of cytoplasmic polyadenylation in this process has never been addressed. Here we show that members of the poorly described family of cytoplasmic poly(A) polymerases TENT5 modulate translation and stability of numerous mRNAs encoding secreted proteins involved in innate immune response. Using *Caenorhabditis elegans*, we performed a whole transcriptome analysis of worms lacking non-canonical poly(A) polymerase TENT5 (PQN-44). Among the transcripts downregulated in a mutant strain, majority encode short secreted proteins with a role in the antibacterial defense. Importantly, these transcripts exhibit shorter poly(A) tails in the *pqn-44*-defective worms, suggesting that they are direct targets for polyadenylation by PQN-44. Moreover, PQN-44 localizes mainly to the intestine which in worms serves as one of the major surfaces of host-pathogen interaction. At the cellular level, PQN-44 is enriched in the endoplasmic reticulum. In agreement with the observed molecular phenotype, *pqn-44*-defective worms are more susceptible to infection with pathogenic bacteria. Furthermore, the function of PQN-44 in innate immunity is evolutionally conserved, as its orthologs, TENT5A/TENT5C, polyadenylate and enhance expression of secreted anti-bacterial proteins in macrophages, as revealed by nanopore direct RNA sequencing combined with standard transcriptomic analysis. Taken together, this study demonstrates for the first time that cytoplasmic polyadenylation is essential for innate immunity and plays a much broader role than previously anticipated.

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132 Structural basis of G-quadruplex unfolding by the DEAH/RHA helicase DHX36

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Guanine-rich nucleic acid sequences challenge the replication, transcription, and translation machinery by spontaneously folding into G-quadruplexes, the unfolding of which requires forces greater than most polymerases can exert. Eukaryotic cells contain numerous helicases that can unfold G-quadruplexes. The molecular basis of the recognition and unfolding of G-quadruplexes by helicases remains poorly understood. DHX36 (also known as RHAU and G4R1), a member of the DEAH/RHA family of helicases, binds both DNA and RNA G-quadruplexes with extremely high affinity, is consistently found bound to G-quadruplexes in cells, and is a major source of G-quadruplex unfolding activity in HeLa cell lysates. DHX36 is a multi-functional helicase that has been implicated in G-quadruplex-mediated transcriptional and post-transcriptional regulation, and is essential for heart development, haematopoiesis, and embryogenesis in mice. Here we report the co-crystal structure of bovine DHX36 bound to a DNA with a G-quadruplex and a 3′ single-stranded DNA segment. We show that the N-terminal DHX36-specific motif folds into a DNA-binding-induced α-helix that, together with the OB-fold-like subdomain, selectively binds parallel G-quadruplexes. Comparison with unliganded and ATP-analogue-bound DHX36 structures, together with single-molecule fluorescence resonance energy transfer (FRET) analysis, suggests that G-quadruplex binding alone induces rearrangements of the helicase core; by pulling on the single-stranded DNA tail, these rearrangements drive G-quadruplex unfolding one residue at a time.

133 Alternative catalytic strategies employed by the nucleolytic ribozymes

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The nine nucleolytic ribozymes provide an opportunity to dissect alternative catalytic strategies, and we find that all available resources are used in various combinations by different ribozymes. For example, the hairpin, VS and twister ribozymes use guanine and adenine nucleobases as general base and acid respectively, with no direct role for a metal ion. By contrast the HDV and TS ribozymes use a hydrated metal ion as general base.

We have determined a new crystal structure of the pistol ribozyme. The core structure has strong resemblance to that of the hammerhead ribozyme, and both use a guanine nucleobase as general base. However, 2'-aminoribose substitution coupled with pH dependence measurements show that they differ in the nature of the general acid. While the hammerhead ribozyme uses a 2'-hydroxyl group (activated by a metal ion), the pistol ribozyme uses a hydrated metal (positioned by a 2'-hydroxyl group) as general acid. Both ribozymes use two further catalytic strategies, facilitating in-line nucleophilic attack and stabilizing the transition state. The pistol ribozyme is the first known example of a ribozyme using a hydrated metal ion as the general acid in the cleavage reaction.

Comparison of the structure and mechanisms of the pistol and hammerhead ribozymes by T. J. Wilson, Y. Liu N. S. Li, Q. Dai, J. Piccirilli and D. M. J. Lilley (submitted for publication).

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134 An atomic structure of RNase MRP

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Ribonuclease mitochondrial RNA processing (RNase MRP) is a multicomponent ribonucleoprotein complex that is involved in the metabolism of a wide range of RNA molecules including rRNA and mRNA. It is composed of a catalytic RNA subunit and nine to ten protein components which are all required for cell viability. RNase MRP closely resembles RNase P and shares most of their protein components as well as multiple features of their catalytic RNAs. Here we report the cryo-EM structures of RNase MRP from *Saccharomyces cerevisiae* alone and in complex with a truncated rRNA substrate containing the MRP cleavage site A3 at a resolution of 3.7 Å and 2.9 Å, respectively. The RNA subunit adopts an extended conformation that maintains a central helical core, in which three coaxially stacked helices P2-P3-P19, P1-P4-P15 and P8-P9 packed against each other. Stems ymP6 and ymP7 in the S domain fold into another coaxially stacked helix. The overall RNA adopts a slightly curved conformation with an open pocket in the catalytic center of the RNA. All the proteins are intimately connected together to cradle the RNA. Notably, the pseudoknot structure of the catalytic center in RNase MRP highly resembles that of RNase P. Strikingly, the N-terminal domain of Pop1 refolds into a different conformation to stabilize the C domain of the RNA. The MRP specific protein Rmp1 lies in the major groove of P15 and sits right above the pseudoknot structure of the C domain. The single stranded RNA substrate sits into the open pocket and three nucleotides pack against with CRIV of the RNA subunit through continuous base-stacking interactions, suggesting a conserved substrate recognition mechanism as that of RNase P. Our structures reveal the catalytic mechanism of RNase MRP and provide unprecedent insights into the differences and evolutionary relationships between RNase P and MRP.

135 Riboswitch-ligand structure determination using an integrative structural biology approach

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Riboswitches are regulatory 5' untranslated regions of mRNA molecules that specifically bind small molecules, resulting in conformational changes that regulate the production of proteins encoded by the mRNA. In recent years, riboswitches came under the spotlight as they are promising new drug targets.

The yjdF riboswitch, present in many bacteria, regulates the gene expression of the yjdF protein-coding gene. The function of the yjdF protein is unknown, although it is possible that its function will be related to detoxification for the ligand that regulates the gene expression. Although the yjdF riboswitch is known to bind a variety of compounds, its natural ligand remains unknown (Li et al, 2016).

To understand how the yjdF riboswitch regulates gene expression in bacteria, we decided to determine and validate its structure through an integrative approach involving X-ray crystallography, SAXS, chemical probing, gene reporter assays, and associated experimental and computational analyses. I will present the structure of the yjdF riboswitch in complex with one of its ligands. The ligand-bound structure is stabilized through long-range tertiary interactions, differing only mildly from the apo structure. The computational modeling was performed with SimRNA (Boniecki et al, 2015), developed in our laboratory. This computational approach was independently validated in the RNA puzzles experiment (Miao Z et al, 2017), where yjdF was also one of the targets.

This structure unveils the ligand-binding site of the yjdF riboswitch, providing the first insight into the ligand binding mode of its ligands.

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136 *In vivo* identification of critical structural motifs in Influenza A virus mRNAs

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The influenza A virus (IAV) is a continuous health threat to humans as well as animals due to its recurring epidemics and pandemics. The IAV genome is segmented and the eight negative-sense viral RNAs (vRNAs) are transcribed into positive sense complementary RNAs (cRNAs) and viral messenger RNAs (vmRNAs) inside infected host cells. A role for the secondary structure of vmRNAs has been hypothesized and debated for many years, but knowledge on the structure vmRNAs adopt *in vivo* is currently missing. Here we solve, for the first time, the *in vivo* secondary structure of IAV vmRNAs in living infected cells. We demonstrate that, compared to the *in vitro* refolded structure, *in vivo* vmRNAs are less structured but exhibit specific locally stable elements. Moreover, we show that the targeted disruption of these high-confidence structured domains results in an extraordinary attenuation of IAV replicative capacity. Collectively, our data provide the first comprehensive map of the *in vivo* structural landscape of IAV vmRNAs, hence providing the means for the development of new RNA-targeted antivirals.

137 Structural and functional studies of a novel interaction between poly(A) and ENE elements

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Long noncoding RNAs (lncRNAs) have been suggested to play important roles in tumorigenesis. The element for nuclear expression (ENE) contains a U-rich internal loop flanked by short helices and is found in viral noncoding RNA such as polyadenylated nuclear (PAN) RNA expressed in oncogenic Kaposi's sarcoma virus (KSHV)-infected cells. The ENE serves as a cis element for stabilization of lncRNA by sequestering the poly(A) tail via formation of a triplex structure that protects it from a fast deadenylation-dependent decay pathway. Structure-based bioinformatic studies have identified the presence of ENE-like elements that sequester the poly(A) tail in evolutionarily diverse genomes including a class containing two ENE motifs (double ENE elements) separated by an 11-bp double-stranded region (middle stem). Interestingly, a significant pyrimidine/purine strand bias is observed in the composition of the stems. A double ENE element identified in a rice transposable element was further investigated to evaluate the role of the pyrimidine/purine strand bias on the function of the motif. Importantly, double ENE variants containing horizontally flipped stems completely lost their stabilization function as well as their ability to bind poly(A). Our EMSA and β-globin reporter assay results strongly suggest that a novel interaction occurs between poly(A) and the pyrimidine/purine biased stems of double ENEs. Currently, we are using X-ray crystallography to gain further insights into the exact mode of interaction between poly(A) and the double ENE motif.

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138 Bespoke mechanisms of RNA remodelling by viral RNA chaperones

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To maintain genome integrity, segmented double-stranded RNA viruses of the *Reoviridae* family must accurately select and package a complete set of up to a dozen distinct genomic RNAs. Such high fidelity genome assembly involves multiple sequence-specific RNA-RNA interactions mediated by viral RNA-binding proteins with RNA chaperone-like activities, including reovirus sigmaNS and rotavirus NSP2. Despite the growing realisation of the importance of these proteins in the process of virus assembly, the nature of how these non-specific ssRNA-binding proteins facilitate selective genome packaging remains a mystery.

To understand the mechanisms underlying such selectivity in promoting inter-molecular duplex formation, we compared RNA-binding and helix-unwinding activities of both proteins. We demonstrate that octameric NSP2 binds structured RNAs with high affinity, resulting in efficient intramolecular RNA helix disruption. Hexameric sigmaNS oligomerizes into an octamer that binds two RNAs, yet it exhibits only limited RNA-unwinding activity compared to NSP2. Thus, the formation of inter-segment RNA–RNA interactions is governed by both helix-unwinding capacity of the chaperones and stability of RNA structure.

Furthermore, using cryo-EM, we have visualised the both NSP2 and sigmaNS oligomers and their ribonucleoprotein (RNP) complexes. The structures of these complexes exhibit dramatic differences in oligomeric organisation between these two proteins, and reveal both the molecular basis for chaperone-mediated RNA remodelling and the unique mechanism underlying sigmaNS oligomerisation.

We propose that this protein-mediated RNA selection mechanism may underpin the high fidelity assembly of multi-segmented RNA genomes in *Reoviridae.*

139 Requirement for multi-protein recruitment during co-transcriptional assembly of nascent ribosomal RNA

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We have developed a novel single-molecule assay for directly observing the early steps of co-transcriptional 30S assembly. In this assay, single molecule fluorescence colocalization and protein induced fluorescence enhancement (smPIFE) generate a real-time picture of protein binding to the nascent RNA during and immediately following transcription on a microscope slide. Using this system, we studied the co-transcriptional binding dynamics of E. coli ribosomal protein uS4 to a 2 kb transcript comprising the complete pre-16S rRNA. Classic assembly mapping experiment showed that protein uS4 stably binds to the 16S rRNA and nucleates assembly of the 5' and central domains of the 30S ribosomal subunit. Surprisingly, protein uS4 binds poorly to nascent pre-16S RNA, dynamically sampling the RNA nonspecifically during transcription, and only forming specific interactions several minutes after transcription has ended. In the presence of proteins uS17, bS20, and bS16, however, uS4 forms specific interactions much earlier during transcription and often shortly after its binding site is transcribed. Long-lived S4 binding is only observed in the presence of all 5' domain proteins (uS17, bS20, bS16, uS8, uS12, and uS5), suggesting that other events in assembly are required for stable association of S4 with newly transcribed RNA. To our knowledge, this is the first example of single-molecule observation of protein binding to a RNA during transcription providing unprecedented insight into the mechanism of co-transcriptional protein association. We propose a general model in which primary ribosomal proteins bind dynamically to nascent RNA, sampling the RNA structure until a sufficient number of specific RNA-protein interactions are achieved to commit the transcript for assembly. This single-molecule system is uniquely suited for observing the assembly large, complex RNA-protein complexes and can be adapted for studying other essential co-transcriptional processes.

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140 Real-time Imaging of Cotranscriptional Folding and Translational Regulation

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Cotranscriptional RNA folding is crucial for the timely and selective control of biological processes. Recently, single-molecule Förster Resonance Energy Transfer (smFRET) has been unique in monitoring RNA structural dynamics using strategically positioned fluorescent dyes. However, since bacterial and eukaryotic RNA polymerases (RNAP) do not incorporate fluorescent nucleotides during transcription elongation, smFRET analysis of RNA structures within such transcriptional complexes has been a challenge.

In order to follow transcriptional folding of nascent transcripts we used the tbpA riboswitch. Upon thiamine pyrophosphate (TPP) binding, this riboswitch controls translation initiation by sequestering the Shine-Dalgarno sequence in a stem loop. Here, we describe an approach allowing us to study the folding of nascent transcripts and visualizing the binding of the 30S subunit to the nascent mRNA. We found that riboswitch sensing is efficiently performed by elongating complexes that are located within a narrow transcriptional window upstream of the coding region. Our results show that the RNAP directly assists TPP binding by controlling nascent transcript folding at a specific transcriptional pause site, clearly showing the central role of RNAP in riboswitch regulatory mechanisms. Furthermore, our preliminary results show that the recognition of the Shine-Dalgarno sequence is performed transiently in the context of elongating complexes, suggesting that the kinetics of 30S binding and transcription elongation are crucial for the outcome of genetic regulation. Our results also suggest that the RNAP can specifically bind to the 30S subunit before translation initiation, which might play a role in facilitating the recognition of the SD sequence.

Overall, our approach allows to better understanding of how cotranscriptional folding and transcriptional pausing are used to regulate translation initiation.

141 Hidden codes of NEAT1 lncRNA for biophysical properties of phase-separated paraspeckles

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Many recent studies focus on formation and function of membraneless organelles by liquid-liquid phase separation (LLPS), and their relationship to diseases such as neurodegenerative diseases and cancer. A class of lncRNAs termed architectural RNAs (arcRNAs) have been identified as essential structural scaffolds for distinct nuclear membraneless organelles, also known as nuclear bodies. RNA is a suitable scaffolding molecule for multiple RNA-binding proteins (RBPs) carrying intrinsically disordered region (IDR), which can form weak, multivalent interactions, to induce LLPS by increasing their local concentration. We have been elucidating the molecular functions of arcRNAs by identifying functional RNA domains interacting partner RBPs, especially through our work on NEAT1 arcRNA that is an architectural core of nuclear body, paraspeckle. We have established more than 200 NEAT1 mutant clones in human haploid HAP1 cells by CRISPR/Cas9 system. These analyses combining with various methods including artificial tethering experiments have revealed multiple NEAT1 lncRNA domains required for several key aspects of paraspeckles. First, we identified that the interactions between multiple NEAT1 middle domains and NONO/SFPQ proteins are essential for LLPS. In addition, we found that spatial organization of NEAT1 within phase-separated nuclear bodies and shapes of the paraspeckles are determined by 5' and 3' ends of NEAT1. Interestingly, segregation of paraspeckles from other nuclear bodies is determined by several NEAT1 domains and their partner RBPs, which, as far as we know, is first example of the underlying mechanism how many nuclear bodies co-exist in the nucleus. Together, we uncovered the hidden codes in the NEAT1 for formation of distinct, highly ordered, phase-separated paraspeckles. Thus, our findings would be an important step to understand how RNAs and their partner RBPs determine the structural organization/shapes, biophysical properties, and functions of the complex cellular RNA-scaffolding nuclear bodies.

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142 Structural studies of the role phase-separating gene regulatory proteins and the formation of membraneless organelles

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Paraspeckles are subnuclear bodies that form when a specific group of nuclear RNA binding proteins are brought into close proximity by binding the long noncoding RNA, NEAT1, through a process of liquid-liquid phase condensation. This process of paraspeckle formation is coordinated and dynamic, with a high nuclear concentration of soluble paraspeckle proteins 'poised' to condense onto NEAT1 as soon as it is transcribed.

Proteins that build paraspeckles are enriched in a type of intrinsically disordered domain termed the 'prion like domain' as observed in proteins including FUS and HNRNPA1. We have been carrying out studies in vitro to characterise the biophysical properties of proteins required for paraspeckle formation in order to learn more about mechanisms involved. In addition to gel- and liquid-formation assays and crystallographic studies, we have used small-angle neutron scattering to seek structural detail on the essential paraspeckle protein HNRNPK which forms a variety of aggregates, droplets and fibrils when made recombinantly. Our experiments suggest that formation of small regular nanoaggregates of HNRNP occurs as a precursor to droplet formation, and that the structure of individual HNRNPK molecules change on forming these aggregates.

143 New repeat-enriched RNAs regulating subcellular localization and activity of RNA-binding proteins

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Many RNA-binding proteins (RBPs) can accumulate in non-membrane-bound compartments but the mechanisms underlying this process as well as its biological consequences are only beginning to be elucidated. We developed a customized workflow to systematically identify mammalian RNAs containing repeated RBP-interaction motifs. Most of the transcripts discovered by this approach were enriched in so-called short tandem repeats that consist of 2-12 nt-long sequence units concatenated in a head-to-tail manner. A top-scoring example of this category was a long noncoding RNA predicted to bind multiple copies of polypyrimidine tract-binding protein (PTBP1), an important regulator of pre-mRNA splicing in the nucleus and pro-apoptotic mRNA translation in the cytoplasm. The new transcript, which we named PNCTR, is expressed at elevated levels in various types of cancer cells where it localizes to the perinucleolar compartment (PNC), a nuclear body previously shown to sequester PTBP1 by an unknown mechanism. Loss of PNCTR abrogates PTBP1 localization to the PNC thus suggesting that this RNA plays a critical role in the PNC assembly. Importantly, PNCTR knockdown reduces cancer cell proliferation at least in part by altering PTBP1 splicing regulation activity and promoting programmed cell death. We will present results of our published and currently ongoing work in this area.

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144 DEAD-box ATPases are global regulators of phase-separated organelles and RNA flux

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The ability of proteins and nucleic acids to undergo liquid-liquid phase separation (LLPS) has recently emerged as an important molecular principle of how cells rapidly and reversibly compartmentalise their components into membraneless organelles such as the nucleolus, processing bodies or stress granules. How the assembly and turnover of these organelles is controlled, and how these biological condensates selectively recruit or release components is poorly understood. We present results demonstrating that members of the large and highly abundant family of RNA-dependent DEAD-box ATPases (DDXs) are global regulators of RNA-containing phase-separated organelles in pro- and eukaryotes. Using *in vitro* reconstitution and *in vivo* experiments we demonstrate that DDXs promote phase separation in their ATP-bound form, and ATP hydrolysis induces compartment turnover and RNA release. This mechanism of membraneless organelle regulation reveals a novel principle of cellular organisation that is conserved from bacteria to man. We further show that DDXs control RNA flux between phase-separated organelles, and thus propose that a cellular network of dynamic, DDX-controlled compartments establishes biochemical reaction centres that affords cells spatial and temporal control of various RNA processing steps regulating the composition and fate of ribonucleoprotein particles.

145 Bacterial RNP-bodies organize substeps of mRNA decay

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Bacteria have distinct challenges to organize their cellular pathways as they generally lack membrane-bound organelles. In eukaryotes, membraneless organelles called biomolecular condensates provide distinct liquid-liquid phase separated structures that organize cellular components. We identified bacterial ribonucleoprotein bodies (BR-bodies) as the first biomolecular condensate characterized in bacterial cells composed of the RNA degradosome multi-protein complex and cellular RNAs (1). The unstructured C-terminal domain of Ribonuclease E is both necessary and sufficient to form the core of the BR-body which is required to recruit RNA binding proteins and exoribonucleases into the bodies. Ribonuclease E requires mRNA to form a BR-body and disassembly requires mRNA cleavage, suggesting BR-bodies can form localized sites of RNA degradation. BR-body formation accelerates both the initial cleavage of mRNA by Ribonuclease E and subsequent exonucleolytic steps of mRNA decay intermediates. Cellular isolation and functional mRNA decay experiments demonstrate that mRNAs are the main substrate of BR-bodies and that ribosomes and tRNAs are excluded. The condensation of the RNA degradosome and mRNA substrates into BR-bodies, and the exclusion of ribosomes and tRNAs which catalyze mRNA translation from BR-bodies, provides an effective strategy for promoting mRNA decay on poorly translated mRNAs. Therefore, these selectively permeable biomolecular condensates yield a highly-adapted solution to organize the bacterial cytoplasm without the use of a membrane and likely represent a more broadly utilized mechanism of subcellular organization in prokaryotes.

References:

1. Al-Husini N, Tomares DT, Bitar O, Childers WS, Schrader JM. 2018. alpha-Proteobacterial RNA Degradosomes Assemble Liquid-Liquid Phase-Separated RNP Bodies. Mol Cell 71:1027-1039 e14.

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146 Regulation of RNA Granules by caliciviruses, from hijacking to novel paracrine induction

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Infection by viruses imposes major stress on the host cell. In response to this stress, infected cells can induce several defense mechanisms, which include the activation of stress response pathways and the innate immune response that can both be linked to translational stalling. This inhibition of translation culminates in the sequestration of transcripts into specialised cytoplasmic ribonucleoprotein complexes called stress granules (SGs). SGs are non-membranous organelles which nucleate from the accumulation of stalled mRNA interacting with aggregation-prone proteins such as G3BP1 and TIA-1. Because this threatens viral gene expression, viruses need to evade these stress response pathways to propagate. Human norovirus is responsible for gastroenteritis outbreaks worldwide. Here we dissect how related caliciviruses, model viruses for norovirus, interact with stress granule responses. Having previously shown that Feline Calicivirus (FCV) impairs SGs assembly by cleaving the scaffold protein G3BP1, we now present evidence that Murine norovirus infection results in repurposing of G3BP1. Using affinity purification of G3BP1 foci and proteomics, we demonstrate a redistribution of G3BP1 cellular partners within virus-specific granules, markedly different from arsenite-induced stress granules, thereby preventing the assembly of SGs. Therefore two related viruses rely on different strategies to counteract SGs responses. Furthermore a thorough analysis of SGs assembly during FCV infection reveals that infected cells signal to uninfected bystander cells triggering SGs assembly to promote SGs assembly. Our data suggest that infection results in paracrine signalling and release of small molecular weight signal that promote SGs assembly in uninfected cells. Biochemical isolation and characterisation using -omics approaches supports that these SGs differ from SGs induced in response to oxidative stress, results in storage of different mRNAs and are antiviral. We propose that this paracrine induction reflects a novel cellular defense mechanism to limit viral propagation and promote stress responses in bystander cells. Dissecting how caliciviruses interact with SGs, therefore, reveals how related viruses manipulate the scaffolding protein G3BP1 through different mechanisms to impair SGs assembly but also shed light on novel paracrine signalling that promotes SGs assembly.

147 GC content shapes mRNA storage in human P-bodies

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Regulating gene expression is a combination of fine tuning RNA synthesis, decay and translation. These processes can be linked to the localization of mRNAs in cytoplasmic RNP granules such as P-bodies. While major progress has been made in understanding the biochemical and biophysical mechanisms that lead to their formation, their contribution to the control of mRNA storage and decay remains uncertain.

We have recently purified P-bodies from mammalian cells and identified hundreds of proteins and thousands of mRNAs that structure a dense network of interactions separating P-body from non-P-body RNPs. Altogether, our data indicated that P-bodies are not primarily involved in RNA decay, but rather in the coordinated storage of poorly translated mRNAs, which encode regulatory functions.

Here, we will show that P-body mRNAs correspond only to the AU-rich fraction of the human transcriptome. As a consequence, their CDS contain the less frequently used codons, which probably explains why their translation is inefficient while their accumulation in P-bodies is quantitatively modest. Combined with other transcriptomic analysis and available CLIP and miRNA target data, this study allows us to propose an integrative model of post-transcriptional regulation in human cells, where GC content plays a central role.

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148 mRNA localization to translation factories for regulation, complex formation or inheritance

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The localization of mRNA within a cell can serve various key functions from the generation of localized protein to mRNA storage and/or degradation. Recently we have shown that highly expressed and translated mRNAs where the protein product is ubiquitously expressed are localised to specific translation factories. More specifically, we find that mRNAs encoding glycolytic enzymes or translation components are housed in different flavors of RNA granule. Our recent work defines at least three classes of granule: CoFe (Core Fermentation) granules that contain the majority of the glycolytic mRNAs, AFe granules (Accessory Fermentation) that house a smaller number of remaining glycolytic mRNAs and Translation Factor (TF) granules that contain many translation factor mRNAs. We postulate that these granules play roles in highly efficient yet co-ordinated translation, allowing cells to manage and harmonize the production of components from the same protein complex or metabolic pathway. In addition, for the TF granules, we identify a mechanism by which the protein synthetic machinery is inherited by the daughter cell and targeted to regions of active growth. Such a feedforward mechanism would ensure adequate provision of the translation machinery where it is to be needed most over a coming cellular growth cycle.

149 Direct RNA sequencing offers a comprehensive view of polyadenylation status in mammals thanks to an unprecedented range of poly(A) tail lengths measurement.

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Poly(A) tail, an essential part of almost all mRNAs, is a dynamic entity whose length is the factor playing a pivotal role in the control of the transcript's stability and translatability. However, due to its homogenous character, transcriptome-wide analysis of polyadenylation is challenging. Methods based on modified Illumina sequencing are available, but they are difficult to implement and have limitations coming from PCR bias on homopolymeric tracts. Such an obstacle does not apply to recently developed direct RNA sequencing using Oxford Nanopore Technologies (ONT), which allows reading full-length RNA molecules, with intact poly(A) tails.

We were interested if ONT sequencing can be reliably used for the transcriptome-wide analysis of poly(A) tails. Thus, we reanalyzed the existing dataset for NA12878 human cell line and sequenced activated primary B cells isolated from WT and non-canonical poly(A) polymerase TENT5C KO mice.

Our analysis led to several general conclusions regarding the poly(A) tails landscape. A broad range of tails lengths was observed, with inter- and intra-transcript differences. Although median poly(A) lengths were in agreement with existing data, numerous transcripts with poly(A) tails exceeding 200 nt could be detected, which were unavailable with existing methods. Interestingly the longest tails were observed for non-coding RNA species. Mean poly(A) length in B cells positively correlated with transcript levels except for ribosomal proteins transcripts which had high expression despite short tails. This is in contrast to previous, TAIL-seq-based reports, which suggested a general lack of correlation.

In the case of primary B cells, we were able to identify immunoglobulins transcripts as the main substrates of TENT5C. Their median poly(A) length decreased from 83 in wild-type to 67 in TENT5C KO samples, whereas other transcripts remained mainly unchanged. Concordantly, the amount of mature immunoglobulins was decreased. Such relatively subtle changes in the lengths of poly(A) could not be observed by other existing methods. Moreover, sequencing of the entire mRNAs allowed for precise identification of immunoglobulin repertoire.

Concluding, we showed that nanopore direct RNA sequencing allows for transcriptome-wide analysis of polyadenylation. To help with further development, we created the generally available software allowing for visualization and exploration of poly(A)-related experiments.

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150 Poly(A)-ClickSeq as a tool enabling simultaneous genome-wide poly(A)-site identification and differential expression analysis.

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The use of RNA-seq as a generalized tool to measure the differential expression of genes has essentially replaced the use of the microarray. Despite the acknowledged technical advantages to this approach, RNA-seq library preparation remains mostly conducted by core facilities rather than in the laboratory due to the infrastructure, expertise and time required per sample. We have recently described two 'click-chemistry' based library construction methods termed ClickSeq and Poly(A)-ClickSeq (PAC-seq) as alternatives to conventional RNA-seq that are both cost effective and rely on straightforward reagents readily available to most labs. ClickSeq is random-primed and can sequence any (unfragmented) RNA template, while PAC-seq is targeted to poly(A) tails of mRNAs. Here, we further develop PAC-seq as a platform that allows for simultaneous mapping of poly(A) sites and the measurement of differential expression of genes. We provide a detailed protocol, descriptions of appropriate computational pipelines (DPAC), and a proof-of-principle dataset to illustrate the technique. PAC-seq offers a unique advantage over other 3' end mapping protocols in that it does not require additional purification, selection, or fragmentation steps allowing sample preparation directly from crude total cellular RNA. We have shown that PAC-seq is able to accurately and sensitively count transcripts for differential gene expression analysis, as well as identify alternative poly(A) sites and determine the precise nucleotides of the poly(A) tail boundaries.

151 GRADitude: a computational tool for Grad-seq data analysis

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RNA-RNA and RNA-protein interactions play an important role in the post-transcriptional gene regulation. A global map of such interactions is a relevant contribution for a holistic understanding of the regulatory networks of an organism.

Grad-seq is a high-throughput profiling approach for the organism-wide detection of RNA-RNA and RNA-protein interactions in which molecular complexes are separated in a gradient by shape and size (Smirnov *et al*., 2016 PNAS). It offers new means to study the role of different RNA and protein components in various macromolecular assemblies by analyzing fractions of a glycerol gradient by a high-throughput sequencing approach combined with mass spectrometry. So far, Grad-seq has been used to globally study RNA-RNA and RNA-protein interactions in *Salmonella* Typhimurium and by that helped to identify ProQ as a new RNA-binding protein that affects numerous genes.

For a better analysis and visualization of Grad-seq based in-gradient profiling data, we now present the computational tool GRADitude. This open source tool (ISC license), developed in the programming language Python, performs all required steps to translate sequencing data of a Grad-seq experiment into a list of potential molecular complexes. It offers different methods to normalize read counts of fractions and generates numerous statistics.

Furthermore, GRADitude integrates several machine learning approaches to predict interactions and creates interactive visualizations to explore a given data set. Methods to process mass-spectrometry data generated from the different Grad-seq fractions and to find potential interactions between proteins and RNAs are also implemented. GRADitude's source code and documentation can be found at https://github.com/foerstner-lab/GRADitude.

REFERENCES:

Smirnov A, Förstner KU, Holmqvist E, Günster R, Becher D, Reinhardt R, Vogel J. 2016. Grad-seq guides the discovery of ProQ as a major small RNA binding protein. Proc Natl Acad Sci USA, 2016 Oct 11;113(41):11591-11596

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152 Assessing the specificity of protein-RNA binding sites identified with CLIP technologies

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UV crosslinking and immunoprecipitation (CLIP) identifies endogenous protein-RNA interactions by sequencing RNA fragments that copurify with a selected RNA-binding protein under stringent conditions. Optimisation of both experimental and computational methods is required to identify protein–RNA interactions with high specificity (Chakrabarti et al., 2018). Here we appraise multiple available methods for peak calling and background subtraction in order to understand how the choice of method and the tuning of its parameters affect the coverage and specificity of identified protein–RNA binding sites. For this purpose, we evaluate sequence motif enrichment at peaks of crosslinking, and visualise RNA splicing maps, which examine the positional distribution of peaks around regulated landmarks in transcripts. Moreover, we present comparative analyses that assess how the optimal computational approach depends on the type of protein studied, the particular variant of CLIP protocol, and the complexity of data. We conclude by presenting how these comparative visualisations can be performed via the web platform iMaps, available at https://imaps.genialis.com.

Reference:

Chakrabarti, A.M., Haberman, N., Praznik, A., Luscombe, N.M., and Ule, J. (2018). Data Science Issues in Studying Protein–RNA Interactions with CLIP Technologies. Annu. Rev. Biomed. Data Sci. 1, 235–261.

153 Methods for RNA splicing analysis using large heterogeneous datasets

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The ever growing availability of RNA-Seq datasets, including large scale projects such as ENCODE and TCGA, create an opportunity for mapping transcriptome variations and RNA function across diverse conditions. However, these datasets also pose formidable computational challenges when researchers try to combine them. Specifically, known confounders such as batch effects, as well as unknown ones can easily obscure the underlying biological signal of interest. In addition, the sheer size of the datasets and the inherent heterogeneity associated with human, not replicates, samples, need to be addressed. Notably, while much work has been done to address such challenges for gene expression analysis, little work has been dedicated to alternative splicing.

We present methods and pipelines developed in our lab to address the above challenges for splicing analysis. Using data from over 600 publicly available primary hematopoietic and acute myeloid leukemia RNA-Seq samples, we demonstrate how such data can be unified, corrected for batch effects, and subsequently clustered to identify meaningful biological subgroups associated with differential splicing. We also offer specific measures for the community to use for assessing how well a given method is able to rid datasets of confounding factors so that researchers can test alternative approaches on their datasets of interest.

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154 The NEXT complex controls the levels of miRNA precursors in Arabidopsis

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The SERRATE protein (SE) is an important factor of the miRNA biogenesis machinery in plants. We found that SE is associated with the Nuclear Exosome Targeting complex (NEXT), which directs RNA substrates for degradation by the nucleoplasmic RNA exosome. We identified the subunits of NEXT that directly mediate the SE/NEXT interaction. We show that NEXT is involved in the degradation of the pri-miRNA 5' remnants that accumulate after excision of miRNAs from their primary precursors. Moreover, the disruption of NEXT complex activity leads also to accumulation of full-length miRNA precursors (pri-miRNAs), suggesting that NEXT participates to the removal of superfluous miRNA precursors. Interestingly, the characteristic phenotype of the miRNA biogenesis mutants *se-2* and *hyl1-2* is partially restored by mutations of NEXT components. This complementation is due to higher levels of mature miRNAs in such double mutants, suggesting that the slower elimination of precursors or processing intermediates caused by loss of NEXT compensates for the slow processing caused by loss of SERRATE or HYL1. Our study uncovers a novel role for the NEXT complex and SE in controlling the levels of miRNA precursors in Arabidopsis.

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155 Nuclear poly(A) tail size is regulated by Cnot1 during the serum response

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The poly(A) tail removal from mRNAs introduces a delay between mRNA synthesis and decay. We measured levels and poly(A) tail sizes of serum-induced mRNAs and used mathematical modelling to correlate their deadenylation time with the delay in decay. Discrepancies between our data and the polyadenylation models after the peak of induction led us to investigate the size of the poly(A) tails on newly made mRNA. Surprisingly, new serum-induced mRNAs synthesised late in induction had short poly(A) tails (around A_{25}) in the nucleus. In addition, newly made constitutive mRNAs had medium sized poly(A) tails (around A_{50}). To see if deadenylation was responsible for the new short poly(A) tails, we depleted Cnot1, a subunit of the CCR4/NOT deadenylase. Cnot1 depletion led to slower deadenylation of cytoplasmic mRNAs, as expected, but also decreased transcription and stabilised longer nuclear poly(A) tails. These observations implicate CCR4/NOT in regulating both the transcription and the nuclear poly(A) tail size of serum-induced mRNAs. Detection of chromatin-associated mRNAs with long poly(A) tails suggests that nuclear deadenylation is an early event. Our data show that initial poly(A) tail size of mRNAs can be regulated and is not always 200-250 nucleotides, adding a novel layer to the control of gene expression.

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156 The role of CPEB4 during acetylation-induced mRNA turnover

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Acetylation is a prevalent post-translational modification critical for epigenetic and transcriptional control of gene expression through modification of histones and transcription-related factors. We have recently shown that acetylation also regulates gene expression at the post-transcriptional level by controlling global poly(A) RNA stability (Sharma et al., Mol. Cell, 2016). Inhibition of histone deacetylase (HDAC) 1 and 2 was found to induce widespread degradation of poly(A)-containing RNAs in mammalian cells. Acetylation-induced mRNA turnover critically depends on the integrity of the evolutionarily conserved CCR4-CAF1-NOT complex and involves p300/CBP-mediated acetylation of the exoribonuclease CAF1a. We propose that reversible acetylation acts as a major switch that promotes a dynamic mode of gene expression with high rates of transcription and mRNA turnover.

In order to identify additional factors that mediate acetylation-induced mRNA turnover, we performed poly(A) RNA interactome capture in conjunction with quantitative mass spectrometry. We found increased binding of cytoplasmic polyadenylation element-binding protein 4 (CPEB4) to poly(A) RNA upon treatment with the class I-specific HDAC inhibitor Romidepsin (RMD). CPEB4 is a sequence-specific RNA-binding protein known to activate translation of mRNAs through poly(A) tail lengthening during meiotic and mitotic cell division, and contribute to cancer progression. Expression of CPEB4 is strongly upregulated following RMD treatment as well as after HDAC1 knock-down. We identified p300/CBP as the acetyltransferases responsible for acetylation of CPEB4, and found that CPEB4 recruits the CCR4-CAF1-NOT complex via TOB1. Moreover, we can show that CPEB4 promotes mRNA degradation in a tethering assay. Using knock-down and CLIP/PAR-CLIP approaches, we are currently analyzing the spectrum of mRNAs targeted by CPEB4, and the impact of CPEB4 acetylation on its functions. The discovery of acetylation-induced mRNA turnover adds an unexpected post-transcriptional component to our fundamental concept of how acetyltransferases and deadenylases regulate gene expression.

Sharma S, Poetz F, Bruer M, Ly-Hartig TBN, Schott J, Séraphin B, Stoecklin G. Acetylation-Dependent Control of Global Poly-A RNA Degradation by CBP/p300 and HDAC1/2. Mol Cell 2016;63:927-38.

157 Dynamic regulation of m^{6}A deposition, poly(A) tails and CCR4-Not complex links transcription to mRNA stability

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Stability of mRNA molecules is generally considered to be an intrinsic feature of every distinct transcript, mainly regulated by its tendency to bind various regulatory factors and the efficiency of its translation. In this study, we investigated the effect of transcription on the stabilities of multiple human and mouse mRNAs. We found that transcription positively regulates mRNA stability, rendering efficiently transcribed messengers less prone to degradation. This regulation is independent of either translation or expression levels. We found this phenomenon to be based exclusively on the co-transcriptionally deposited m6A modification, length of poly(A) tails, and the preferential activity of the CCR4-Not complex toward m6A-marked transcripts.

Moreover, we demonstrate that upon large-scale transcriptional changes, such as during stress response or differentiation, the cell dynamically regulates its degradation machinery to buffer the global levels of mRNAs. This mechanism is based mainly on the tightly regulated expression of the CCR4-Not complex, which responds to global transcriptional fluctuations. We found this phenomenon to affect the stabilities of virtually all tested mRNAs, thus providing transcription an additional regulatory pathway to globally impact mRNA stability. Overall, we conclude that transcription is a primary regulator of mRNA degradation in eukaryotic cells. We postulate that mRNA stability is a flexible epigenetic feature that is continuously and dynamically adjusted to transcriptional fluctuations in order to fine-tune gene expression in the ever-changing conditions.

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158 Cryo-EM structure of a poly(A)-Pab1 ribonucleoprotein bound to the Pan2-Pan3 deadenylase

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The stability of eukaryotic mRNAs is dependent on a ribonucleoprotein (RNP) complex of poly(A)-binding proteins (PABPC1/Pab1) organized on the poly(A) tail. This poly(A) RNP protects mRNAs from premature degradation but also stimulates the Pan2-Pan3 deadenylase complex to catalyze the first step of poly(A) tail shortening. We reconstituted this process *in vitro* using recombinant proteins and show that Pan2-Pan3 associates with and degrades poly(A) RNPs containing two or more Pab1 molecules. The cryo-EM structure of Pan2-Pan3 in complex with a poly(A) RNP composed of 90 adenosines and three Pab1 molecules shows how the oligomerization interfaces of Pab1 are recognized by conserved features of the deadenylase and thread the poly(A) RNA substrate into the nuclease active site. The structure reveals the basis for the periodic repeating architecture at the 3' end of cytoplasmic mRNAs. This illustrates mechanistically how RNA-bound Pab1 oligomers act as rulers for poly(A) tail length over the mRNAs' lifetime.

159 LARP4 is a general factor involved in mRNA poly(A) tail length homeostasis and enhances induced levels of innate immune mRNAs

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LARP4 is a metazoan protein that independently binds to poly(A) RNA, the 40S ribosome-associated signaling protein RACK1, poly(A)-binding protein (PABPC1) and is found associated with polyribosomes. Messenger RNA function is controlled by the 3' poly(A) tail (PAT) and PABPC1. Prior work showed that LARP4 promotes net lengthening of the PAT and associated stabilization of mRNAs, including ribosomal protein mRNAs (RPmRNAs) and other mRNAs that were examined using northern blot analysis. Mouse embryonic fibroblasts (MEFs) from which LARP4 is deleted exhibit shorter PAT length and decreased RPmRNA stability[1]. Here we report PAT lengthening and shortening in LARP4 over-expressing and deletion cells, respectively, for an extensive set of mRNAs using a novel global PAT length-seq technique. By contrast PAT lengths of the mitochondrial-encoded mRNAs are unchanged. Observations that mRNAs from co-transfected reporter genes consistently showed the greatest increases in PAT length upon LARP4 overexpression, led to a hypothesis that the PATs of newly transcribed mRNAs benefit most from LARP4 protection. To begin to test this, we performed RNA-seq after stimulating the Type-I Interferon pathway and found that interferon-stimulated genes (ISGs) are indeed increased in mRNA levels ~3 fold with LARP4 over-expression. Importantly, LARP4 mutants that are defective for PABPC1 association and mRNA binding, are deficient in enhancing the increase. Interestingly, LARP4 KO MEFs that display a global shortening of endogenous mRNA PATs unexpectedly show longer PATs on mRNAs from transfected reporter genes as compared to the WT MEFs. We propose a model to account for these paradoxical results wherein global shortening of endogenous mRNA PATs limits availability of the mRNA deadenylation machinery in the LARP4 KO MEFs and this spares the mRNAs from newly transcribed genes. Aspects of this model and their implications for gene expression are being tested. In addition, we are also testing LARP4 KO and WT MEFs and splenocytes for differences in innate immune function.

1. Mattijssen et al. 2017. *Elife*, PMCID:PMC5626478

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160 Analysis of *upf1*-null mutants in Arabidopsis revealed central role of UPF1 in transcriptional and translational homeostasis

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Nonsense mediated RNA decay (NMD) is an evolutionary conserved mechanism attributed to degradation of aberrant transcripts and fine tuning cellular transcriptome. While the mechanism and regulation of NMD has been extensively studied, deeper understanding of its biological relevance is still limited, partially because inactivation of key NMD factors confer lethality in a majority of model organisms. We discovered that impaired growth or lethality of Arabidopsis NMD mutants can be subjugated by genetic abrogation of pathogen defense signaling. This allowed us to perform comparative analysis of plants lacking UPF3, UPF1, and SMG7, key proteins involved in different steps of NMD. UPF3 inactivation had the smallest effect on transcriptome, which is consistent with the previous observation in Drosophila suggesting peripheral role of UPF3 in NMD. Although SMG7 and UPF1 act non-redundantly on the consecutive steps of NMD and hence, should give a similar phenotype, we observed different impact of *smg7* and *upf1* null mutations on plant growth and transcriptome. UPF1 inactivation had much greater effect on accumulation of aberrantly spliced mRNA and transcriptome homeostasis than SMG7 indicating existence of yet unknown branch of NMD in plants that is parallel to SMG7/5/6 pathway. Transcriptome assembly in *upf1* plants revealed novel alternatively spliced isoforms never detected before underlying impact of NMD on transcriptome maintenance. Furthermore, the transcriptome analysis showed downregulation of processes related to translation. To examine the impact of UPF1 on translation, we analyzed RNA in monosomal and polysomal fractions of ribosomes in *upf1* mutant. We observed that UPF1 inactivation results in a global shift of mRNAs from polysomes to monosomes. Interestingly, NMD targets as well as low expressing mRNA with short half-life were shifted from monsomes to polysomes in *upf1* indicating key role of UPF1 in suppressing translation of aberrant RNAs. Particularly striking was UPF1-dependant increase in translation of plant immune receptors (TNLs). Regulation of TNLs via UPF1/NMD-mediated mRNA stability and translational de-repression offers a dynamic mechanism for rapid activation of TNLs in response to pathogen attack.

161 Insights into the molecular mechanisms of Staufen-mediated mRNA decay

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Eukaryotic gene expression is modulated at several steps such as transcription, mRNA processing and export, translation and mRNA turnover. The degradation of mRNA is an important step in post-transcriptional gene regulation as it serves to control the level as well as the quality of the mRNA being translated. Degradation is often triggered by distinct sequence or structural elements present in the 3'-untranslated region (UTR) of the target mRNA. These cis-elements are typically recognized by cognate protein factors, which in turn mediate mRNA decay through a specific pathway. One such well-investigated pathway is Staufen-mediated mRNA decay (SMD). The double-stranded (ds) RNA binding protein Staufen plays a critical role in mRNA transport and localization. The mammalian paralogs of Staufen, Stau1 and Stau2, additionally function in cellular mRNA turnover, selectively degrading mRNA containing complex secondary structures in the 3'-UTR of mRNA. In addition to Staufen, the RNA helicase UPF1 was shown to play a role in mediating mRNA decay. Similar to its function in nonsense-mediated mRNA decay, the helicase activity of UPF1 is also thought to be essential for SMD. We present here the biochemical reconstitution of a Staufen-mRNP comprising UPF1. We have mapped the interacting domains of the proteins within the complex and have identified novel protein-protein interactions that play a critical role in stabilizing the mRNP. Using cross-linking mass-spectrometry, we have obtained additional insights into the assembly and topology of the complex. Our studies elucidate the mechanisms of recruitment and activation of UPF1 in the context of SMD and shed light on the extent of cross-talk mediated by this RNA helicase in different pathways of mRNA turnover in cells.

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162 PTBP1 promotes ATPase-dependent dissociation of UPF1 from mRNPs to prevent NMD

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The nonsense-mediated mRNA decay (NMD) pathway selects its targets by assessing the ribonucleoprotein context of translation termination events. Selection of NMD substrates involves both degradation-promoting factors and sequence-specific RNA-binding proteins that safeguard specific mRNAs with long 3'UTRs and other NMD-inducing features. We have previously identified PTBP1 and hnRNP L as protective proteins capable of antagonizing UPF1 binding to 3'UTRs. Together, the protective proteins shape the target specificity of the NMD pathway, shielding hundreds of long 3'UTRs from recognition. However, the mechanism by which these sequence-specific RNA binding proteins can prevent sequence-independent UPF1 association with long 3'UTRs is unknown.

Here we elucidate the biochemical mechanism underlying PTBP1-mediated protection from NMD. Using in vitro helicase assays, we show that PTBP1 blocks UPF1 translocation and promotes its dissociation from RNA. In support of a model in which the protective proteins actively induce UPF1 displacement from potential target mRNAs, we identify a direct protein-protein interaction between PTBP1 and UPF1. Using a novel assay to track UPF1 dissociation from endogenously assembled affinity purified mRNPs, we show that PTBP1 recruitment to an mRNA promotes UPF1 displacement, in a manner dependent on UPF1's ATPase activity.

Our data suggest that PTBP1 exploits the tendency of UPF1 to release RNA when it binds and hydrolyzes ATP. Inhibition of RNA binding by ATP is coordinated by a regulatory loop in domain 1B of UPF1. Mammalian UPF1 undergoes alternative splicing to produce a protein isoform with an extended regulatory loop, which we now show impairs the ability of PTBP1 to block UPF1 translocation. Transcriptome-wide studies of UPF1 loop variant binding and function extend these findings to show that the regulatory loop controls the ability of UPF1 to bind and decay mRNAs normally protected by PTBP1 and hnRNP L. We propose that modulation of the UPF1 regulatory loop allows cells to tune the sensitivity of the NMD pathway to negative regulatory factors.

163 TUT7 is the uridyl transferase that uridylates histone mRNA and together with 3'hEXO cooperates to maintain histone mRNA structure in S-phase and degrade histone mRNA at the end of S-phase.

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Animal histone mRNAs are the only eucaryotic mRNAs that are not polyadenylated, ending instead in a conserved stemloop. Histone mRNAs are cell-cycle regulated, and are present at high levels only in S-phase. A protein, SLBP, binds the stem-loop and participates in all aspect of histone mRNA metabolism, including histone pre-mRNA processing and degradation of the histone mRNA. Altering the half-life of histone mRNAs is a major mechanism to rapidly adjust histone mRNAs levels when the rate of DNA synthesis changes. The stemloop at the 3' end of histone mRNA is the cis element that determines histone mRNA halflife. When DNA synthesis is inhibited in S-phase cells histone mRNAs are rapidly degraded.They are also rapidly degraded at the end of S-phase. In addition to SLBP, a 3' to 5'exonuclease, 3'hExo (Eri1) is bound to the stemloop.Histone mRNAs are uridylated at two different points in their life cycle. After processing 5 nts after the stemloop, 3'hExo trims 2 nts off the histone mRNA, leave an ACC tail. During its time in the cytoplasm, 3'hExo can remove one or two additional nts. These are then replaced by uridines, leaving a 3nt tail of ACU or AUU, maintaining the proper length of histone mRNA. Histone mRNA degradation initiates at the 3' end while the mRNA is being translated. Initial steps in degradation include recruitment of Upf1 and Smg1 to the 3' end of histone mRNA, likely as a result of defective translation termination. 3'hExo can then degrade into 3-4 nts into the stemloop, and degradation intermediates accumulate. These intermediates are uridylated with long U-tails (4-10 nts) many of which can bind Lsm1-Lsm7. Knockout of TUT7 using CRISPR prevents uridylation both on the tail of the histone mRNA in S-phase, and during histone mRNA degradation. The TUT7 knockout cells express 10x more TUT4 than control cells, but do not uridylate histone mRNA. Thus TUT7, but not TUT4, specifically interacts with the 3' end of histone mRNP. Knockout of 3'hExo by CRISPR also blocks rapid degradation of histone mRNA.

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164 Full-length mRNA sequencing reveals principles of poly(A) tail regulation

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We established FLAM-seq, a rapid and simple method for sequencing full-length mRNAs including their poly(A) tails. By combining a new cDNA library preparation method with single-molecule real-time sequencing, FLAM-seq delivers nearly complete sequence for thousands of transcripts per sample. We demonstrated that this method is capable of accurate profiling of poly(A) tails and applied it to human cell lines, brain organoids and *C. elegans*. We found that: (a) 3' UTR length is correlated with poly(A) tail length, (b) alternative polyadenylation sites and transcription start sites for the same gene are linked to different tail lengths and (c) tails can contain a significant number of cytosines. Moreover, we provide new insights into the temporal dynamics of poly(A) tail length regulation.

165 High-resolution quantitative profiling of tRNA pools by mim-tRNAseq

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Transfer RNAs are key determinants of accurate and efficient mRNA decoding, but remain refractory to quantitation by high-throughput sequencing: base modifications at the Watson-Crick face block tRNA reverse transcription, and high sequence similarity among tRNAs hinders accurate read alignment. Here, we present a novel workflow that overcomes these two chief hurdles through modification-induced misincorporation sequencing of tRNA (mim-tRNAseq). The workflow combines a sensitive method for cDNA library generation from low input with commercially available enzymes, and a novel data analysis pipeline in a user-friendly Python package. By identifying conditions that strongly favor misincorporation at modified bases during reverse transcription, we achieve uniform tRNA coverage in samples from yeast, mouse, and human cells. Accounting for misincorporations at modified sites dramatically increases read alignment rates, greatly improving estimates of intracellular tRNA abundance. The method's high resolution enables not only the sensitive detection of differentially expressed tRNAs, but also the quantitation of numerous base modifications, as well as the analysis of translationally available tRNA pools by assessing 3'-CCA completeness. We demonstrate the power of mim-tRNAseq by identifying a wide variation in tRNA isodecoder usage among different human cell lines. As our workflow is simple, robust, and applicable to any organism with a known genome, we anticipate that it will shed new light on many aspects of tRNA biology.

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166 A single molecule platform that allows comprehensive analysis of native RNA molecules

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Analysis of RNA molecules involves converting RNA into complementary DNA (cDNA). This, however, may not always reflect the state of native RNA molecules from which they are derived from. Moreover, epigenetic modifications such as m6A and m5C are lost during this conversion process. We developed sample preparations as well as reagents that allow us to both directly sequence and map epigenetic modifications on native RNA molecules with near single nucleotide resolution on the SIngle Molecule DEtection and Quantification platform (SIMDEQ™), which is based on magnetic tweezers. To demonstrate the exceptional capacity of this platform, we captured specific miRNAs molecules from HeLa cell and identified for the first time a m6A modification on native miRNAs. In addition, the long read-length capacity and single molecule nature of this platform offer the possibility to analyse splicing isoforms of individual transcripts. Taken together, this platform allows the analysis of hundreds of single molecules in parallel and acts as a powerful tool to analyse various aspects of RNA biology on native molecules with near single nucleotide resolution.

167 RNA In Situ Conformation Sequencing Reveals the Architecture and Regulation of the Human Transcriptome

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RNAs are highly structured and intertwined with each other to regulate critical biological processes, yet their in situ higher-order organization and regulation remain unknown. By coupling in situ proximity ligation with massively parallel sequencing, we report RNA in situ conformation sequencing (RIC-seq), a method that is used to probe the three-dimensional (3D) architecture and regulations of the whole transcriptome. Using RIC-seq, we construct 3D RNA maps in diverse cell types and identify millions of intra- and inter-molecular interactions. RIC-seq recapitulates known tertiary structures and reveals a fractal globule folding principal for both messenger RNA and long non-coding RNA (lncRNA). Surprisingly, RNAs are organized in different compartments and formed topological-like domains and even tissue-specific hubs. We further show that CCAT1-5L function as super-enhancer hub-RNA to synergize MYC promoter- and enhancer-RNAs to boost MYC expression. Our study demonstrates the power of RIC-seq technology and the wide applicability of the RNA-RNA interaction compendium.

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168 Imaging Single mRNAs and lncRNAs with Fluorogenic Mango Arrays

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RNA molecules play vital roles in many cellular processes. Visualising their dynamics in live cells at single-molecule resolution is essential to elucidate their role in RNA metabolism. RNA aptamers, such as Spinach and Mango, have recently emerged as a powerful background-free technology for live cell RNA imaging due to their fluorogenic properties upon ligand binding. Here, we report a novel array of Mango II aptamers for RNA imaging in live and fixed cells with high contrast and single-molecule sensitivity. Using both coding (β-actin mRNA) and long non-coding (NEAT1) RNAs, we show that the Mango array does not affect cellular localisation. Fluorophore exchange within the Mango array enables single mRNA transport to be tracked for extended time periods (minutes). Furthermore, the Mango II array is readily compatible with structured illumination super-resolution microscopy. Altogether, this new Mango array enables accurate determination of RNA transcription, nuclear export and subcellular localisation.

169 High-throughput biology of RNA using droplet-based microfluidics in tandem with next generation sequencing

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The extreme miniaturization allowed by microfluidics is currently revolutionizing many scientific areas by making possible the analysis of a large number of molecules in highly parallelized, quantitative and cost-effective ways. Among these technologies, droplet-based microfluidics is particularly attractive because of its ultrahigh-throughput capacity (several millions of analyses per day). Over the past years, our group has extensively used this technology to set-up *in vitro* evolution/selection pipelines aiming at improving the properties of ribozymes and light-up RNA aptamers. Yet, characterizing the selected genes was still a time-consuming and labor-intensive process. To overcome this major limitation, we recently integrated our ultrahigh-throughput droplet-based screening technology with next-generation sequencing and bioinformatics to set-up a semi-automated analysis pipeline.

In this presentation, I will exemplify the efficiency of this approach by three main application cases. First, I will show how, using a structure-guided strategy, we managed to identify a new mutant of the light-up aptamer Mango-III (an RNA we previously identified with our microfluidic screening) that was even brighter that the parental molecule. Then, I will show how the technology can be used to rapidly identify optimized RNA structural elements (in particular structure switching domains) by comprehensively screening large gene libraries. Indeed, by performing rounds of positive and negative selections we were able to deep screen a library of more than 65,000 different variants from which we identified those behaving as very efficient fluorogenic biosensors of metabolites. Finally, we also extended our procedure to the use of coupled *in vitro* transcription and translation extracts to set-up a strategy allowing for characterizing bacterial translation. In this last example, functionally important regions of an mRNA are randomized and each mutant of the resulting library (millions of mutants) is assayed for its capacity to support translation. Next, the most efficient variants are identified by next-generation sequencing and bioinformatics.

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170 DEEPEST-Fusion: Pan-cancer statistical gene fusion discovery and characterization

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Genomic instability is a hallmark of cancer that can lead to a variety of structural chromosome aberrations, including gene fusions. Chimeric RNA transcripts-- coding or non-coding RNAs-- formed by exons joined by distinct genes are known to be the main drivers in diverse cancers; as they are highly cancer-specific, fusions detected in the blood can be informative for liquid biopsies. Because of this, computational fusion detection has attracted significant attention in recent years. However, despite massive efforts and numerous methods, a scalable and highly-specific algorithm is still a challenging problem. Here, we present Data-Enriched Efficient PrEcise Statistical Fusion (DEEPEST-Fusion), a fusion detection pipeline tailored for large-scale screens. DEEPEST-Fusion consists of two components: (1) the junction nomination component, in which fusions are nominated by a modified version of MACHETE, a statistical fusion detection algorithm developed by our group in 2016; and (2) the statistical refinement step, which employs several statistical tests for prevalence of nominated fusions to address increased false positive rates due to multiple testing for fusions across thousands of datasets. DEEPEST-Fusion workflow, written in Common Workflow Language, can be run on any cloud platform without requiring further software modification. Extensive benchmarking analysis on simulated and real datasets demonstrates high specificity, achieving 100% positive predictive value on all simulated datasets generated by a third party (STAR-Fusion). We used DEEPEST-Fusion to analyze 9,946 tumors across all 33 tumor types in The Cancer Genome Atlas (TCGA) consortium and detected 31,007 fusions, higher sensitivity than other methods, including 12,196 novel fusions not previously reported in analysis of TCGA. DEEPEST-Fusion detects far fewer putative fusions than other methods when run on normal tissue samples, further evidence of its precision. In addition to the potential value of this method for diagnostics, the increased sensitivity of DEEPEST-Fusion reveals new cancer biology, including candidate driving long-non-coding RNA gene partners and protein-coding genes involved in RNA binding and metabolism. DEEPEST-Fusion is both a general statistically-driven method to precisely identify chimeric RNA in normal and cancer samples and in application, reveals new cancer biology including a role for RNA metabolism.

171 Artificial circular RNA sponges targeting oncogenic microRNAs inhibit tumor spheroid growth in 3D cell culture systems

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Circular RNAs (circRNAs) have recently become a focus of biomedical research. A cellular circRNA, CDR1as/ciRS-7, was identified as a neuronal microRNA (miRNA-) sponge that sequesters and functionally inhibits miRNA-7 and regulates its homeostasis. CDR1as knockout mice display dysfunctional synaptic transmission, misregulation of miRNAs and a behavioral phenotype that is associated with human neuropsychiatric disorders.

Due to their elevated stability compared to linear RNA, circRNAs represent a promising tool in biotechnological applications. Many human diseases are associated with miRNAs, which can be targeted by anti-miRNA drugs. The cellular miRNA-122 is hijacked by the Hepatitis C Virus (HCV) and appears essential for its propagation. Miravirsen, the first anti-miRNA drug has effectively inhibited HCV in patients. In an earlier proof-of-principle study, we demonstrated that in vitro transcribed and ligated circRNA sponges sequester miRNA-122 and impair HCV translation as effectively as Miravirsen in HCV cell culture model systems.

We are currently targeting miRNAs linked to other diseases, such as bacterial infection and cancer. Among promising targets are oncogenic miRNAs, which are upregulated in many tumor types and associated with poor prognosis in patients. We developed circRNA sponges targeting these miRNAs and assessed their effect on tumor growth in 3D cell culture systems. We observed a significant reduction in spheroid growth, spheroid invasion and anoikis resistance compared to control circRNAs lacking specific miRNA binding sites. In addition, we are applying strategies to bypass the innate immune response (foreign RNA recognition) of the cell. Current results suggest that RNA modifications within the circRNAs, alterations to secondary structure elements as well as different purification methods can be applied to circumvent cellular recognition. Overall, the described examples broaden the perspective on potential applications of artificial circRNA sponges in the field of molecular medicine and biology.

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172 Chemical Approaches to study the Epitranscriptome

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The properties of eukaryotic messenger RNA can be modulated by dynamic chemical modifications that occur post-transcriptionally (known as the "epitranscriptome"). These include N^6-methyladenosine (m^6A), which regulates mRNA turnover, translation, nuclear export, and splicing, as well as other modifications on the nucleobases. A major challenge is to identify the functional consequences of these modifications and elucidate the molecular mechanisms by which they affect gene expression programs in cells. To address this gap in our knowledge, we have developed and applied chemical biology strategies to characterize to study the effects of mRNA modifications on cellular processes. First, we describe a chemical proteomics approach (Arguello *et. al JACS* 2017) relying upon photocrosslinkable diazirine-containing synthetic RNA oligonucleotide probes and quantitative proteomics to profile readers of N^6-methyladenosine (m^6A), the most abundant internal mRNA modification in mammals. In addition to identifying known m^6A binders, including YTH-domain proteins and ALKBH5, we find that FMR1 and LRPPRC, two proteins associated with human disease, read this modification. Interestingly, we also find that m^6A disrupts RNA binding by the stress granule proteins G3BP1/2, UPS10, CAPRIN1, and RBM42, providing a link between mRNA modifications and the integrated stress response. Second, we describe a strategy for the metabolic incorporation of non-canonical nucleotides into cellular RNA (Zhang and Kleiner *JACS* 2019). We have applied protein engineering to uridine-cytidine kinase 2, an enzyme in the pyrimidine nucleotide salvage, to alter its substrate specificity. Remarkably, introduction of this mutant enzyme into mammalian cells enables the incorporation of bulky C5-modified pyrimidine nucleosides into RNA. We have used this approach for the metabolic incorporation of novel biorthogonal nucleotides for visualizing RNA synthesis and turnover, and we anticipate that it will serve as a general strategy for modifying RNA in living cells with diverse modified bases for probing RNA biology. Taken together, our work should improve our understanding of fundamental RNA regulatory mechanisms and provide powerful and general strategies for interrogating the function of mRNA modifications.

173 Global kinetic landscape of *Dazl* binding at individual RNA sites in cells

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The kinetics by which RNA binding proteins (RBPs) interact with their cellular RNA sites are thought to be critical for the biological function of RBPs. Yet, it has not been possible to measure these kinetic parameters in cells. We describe a new approach to determine transcriptome-wide kinetic parameters of protein binding to individual RNA sites in cells and we show how kinetic data quantitatively link RNA binding patterns to biological RBP function.

We combine time-resolved, UV-Laser induced, multi-photonic RNA-protein crosslinking with Immunoprecipitation, Next Generation Sequencing, and large scale kinetic modeling to determine rate constants for association, dissociation, crosslinking and fractional occupancy for thousands of individual binding sites of the RBP Dazl in mouse GC1 cells. Association and dissociation rate constants for Dazl vary by several orders of magnitude among different binding sites. Dazl resides at individual binding sites at most for few seconds or less, indicating exceptionally high dynamics of Dazl-RNA interactions. Kinetic parameters are highly similar between adjacent Dazl binding sites, revealing synergistic Dazl binding.

Using multivariate machine learning, we link ribosome profiling data at different Dazl concentrations to the obtained kinetic binding data. We find that the presence of only few Dazl proteins on a given RNA per time interval ultimately determines the impact of Dazl on the translation of the RNA. However, the Dazl presence on an RNA is controlled in a very complex fashion through the binding kinetics at individual binding sites, the collective, synergistic kinetics of Dazl clusters and the combination of these clusters on a given RNA. The data explain how similar Dazl effects on translation can be accomplished by very different Dazl-RNA binding patterns. Collectively, our results show how previously inaccessible, kinetic parameters for RNA-protein interactions *in vivo* allow the development of mechanistic models for cellular RNA-protein interactions.

End of Oral Abstracts

POSTER ABSTRACTS

174 Investigating the evolution of Dicer helicase function

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Dicer is a ribonuclease that produces miRNAs and siRNAs from longer double-stranded RNA (dsRNA) precursors. While Dicer is conserved across the eukaryote kingdom, different organisms possess different forms of the enzyme with different substrate specificities and functions. For example, Drosophila melanogaster has two Dicer enzymes: Dicer-1 is dedicated to miRNA processing, and Dicer-2 processes endogenous siRNAs and serves as a defense mechanism against viral infection. This defense involves cleavage of viral dsRNA replication intermediates and is mediated by the helicase domain of Dicer-2 in an ATP-dependent reaction. In contrast, Caenorhabditis elegans and mammals have a single Dicer, and while worm Dicer has an ATP-dependent helicase domain that is involved in antiviral defense, mammalian Dicer has never been observed to require ATP.

In most cases, the helicase domains are well conserved, and the reason for the differences in substrate preferences and ATP dependence is unclear. Using phylogenetic analyses, we are working to elucidate the evolutionary history of Dicer and understand how different selective pressures have contributed to gain or loss of helicase function from ancestral Dicers to extant proteins. Our goal is to reconstruct the most recent common ancestor Dicer protein, express this protein in the laboratory, and use biochemical assays to elucidate its ATP-dependence, substrate selectivity, and cleavage profile.

Concurrently, we are using biochemical methods to understand the source of the functional difference between mammalian Dicer and fly Dicer-2. We have constructed a chimeric protein where the helicase domain in human Dicer has been swapped out for the functional helicase domain of Drosophila Dicer-2. We are testing the chimera's function with electrophoretic mobility shift assays to measure binding. Subsequently, we will use cleavage and ATP hydrolysis assays to determine if human Dicer can work with an ATP-dependent helicase domain. We intend to generate different chimeras with smaller domain swaps until we find the accessory motif(s) responsible for the functional difference between these two different Dicers.

Our work will contribute to understanding the potential of the mammalian RNAi pathway as an antiviral defense system; a mammalian Dicer with an ATP-dependent helicase might unlock or enhance a dormant antiviral function *in vivo*.

175 tRNA-derived fragments target and regulate ribosome-associated aminoacyl-tRNA synthetases

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Ribosome-associated noncoding (ranc) RNAs are a novel class of short regulatory RNAs with functions and origins that have not been well studied. In this present study, we functionally characterized the molecular activity of Saccharomyces cerevisiae tRNA-derived fragments (tRFs) during protein biosynthesis. Our results indicate ribosome-associated tRFs derived from both 5' (ranc-5′-tRFs) and 3'-part of tRNAs (ranc-3′-tRFs) have regulatory roles during translation. We demonstrated five 3′-tRFs and one 5′-tRF associate with a small ribosomal subunit and aminoacyl-tRNA synthetases (aa-RSs) in yeast. Furthermore, we discovered that four yeast aa-RSs interact directly with yeast ribosomes. tRFs interactions with ribosome-associated aa-RSs correlate with impaired efficiency of tRNA aminoacylation.

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176 Plastid retrograde control of miRNAs expression in response to light stress

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Fluctuations in light intensity and spectra result in episodes of excess energy. Rapid adaptation to such dynamic changes requires coordinated communication at the cellular level but also between leaves and roots. It was shown that miRNAs regulate gene expression in a number of plant developmental processes and stress responses. Despite its significance, the role of miRNAs in the light stress signaling is not sufficiently studied. To determine if the expression of miRNAs is regulated by high light (HL) both in light-stressed shoots and dark-grown roots we used *Arabidopsis thaliana* plants growing in hydroponic conditions. The physiological response in low light acclimated plants exposed to HL stress was analyzed using chlorophyll fluorescence parameters. Additionally, we examined the expression level of several genes known as markers of redox changes. Micro-transcriptomic analysis of HL-triggered miRNAs expression changes confirmed that induced response varies between both organs. The validation of the obtained results was carried out using Two-tailed RT-qPCR. Chemical inhibition of the photosynthetic electron transport by either DCMU or DBMIB indicated that the plastoquinone pool contributes to this regulation connecting the redox signals with HL-induced miRNAs response. Interestingly, this response can be induced only in the rosette since the light had no effect on the expression of selected miRNAs when roots were separated from shoots before HL treatment. Linking the regulation of photosynthetic redox state to miRNAs abundance, we underpin the miRNA involvement in the nuclear-organellar crosstalk.

177 Molecular principles of Cajal body formation

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The cell nucleus is a highly heterogeneous environment crowded with numerous macro-molecules. Part of the nuclear complexity rises from the presence of a number of different bodies, non-membrane bound structures, which accumulate various proteins and, often RNAs. The molecular principles behind bodies assembly and maintenance are recently a matter of an intensive debate. One of the "classical" examples of a nuclear body is the Cajal body (CB). CBs are involved in biogenesis, quality control and recycling of spliceosomal snRNPs. Coilin, the essential scaffolding protein of CBs, self-oligomerize and interacts with numerous proteins including snRNPs, and these interactions are important for CB formation. However, the basic information regarding its structure and function are lacking. To uncover molecular principles of CB formation we determined coilin dynamics in the nucleoplasm and CBs and analyzed the snRNP influence on coilin self-oligomerization and CB formation. To eliminate the effect of endogenous coilin, we generated a coilin KO cell line. Then we prepared several different mutated versions of coilin that prevent interaction with snRNPs and express them in coilin KO cells. Our results show that coilin self-interaction does not depend on snRNP binding. However, the mutants that do not associate with snRNPs fail to reconstitute CBs in coilin KO cells. We also apply different fluorescence microscopy techniques (FRAP, single-point and imaging FCS and 3D-SIM SPT) to determine coilin dynamics in the nucleus and the CB. The data confirm our biochemical results and show that abolishing coilin interaction with snRNPs does not inhibit coilin self-association. Interestingly coilin dynamics inside the CB does not differ from the nucleoplasmic coilin, suggesting that the CB nucleation and maintenance are not based on different coilin mobility inside and outside CBs.

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178 The *Aspergillus fumigatus* RNAi machinery regulates secondary metabolite production

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The ubiquitous saprophytic fungus and opportunistic human pathogen *Aspergillus fumigatus* produces numerous well-characterized secondary metabolites from biosynthetic gene clusters scattered across its genome. Despite decades of experimentation, the regulation and even products of many of these clusters remain unknown. In this study, we hypothesized that the previously uncharacterized *A. fumigatus* RNA interference (RNAi) machinery regulates expression of secondary metabolite production. To test this hypothesis, we created single and double knockouts of key components of the RNAi machinery, including orthologs of dicer, argonaute, and RNA-dependent RNA polymerase proteins in *A. fumigatus*. Deletion of the argonaute proteins indeed resulted in a general upregulation of a number of previously described secondary metabolites during starvation. Surprisingly, the single knockout strains exhibited no major growth or stress defects, but a double knockout in the argonaute proteins did result in decreased sporulation. To gain insight into the active mechanisms of silencing in *A. fumigatus* and better characterize the observed phenotypes, we defined the contribution of each protein to silencing of exogenously applied, *in vitro* transcribed double-stranded RNA, which is readily internalized and processed by the fungus. We found that the dicer and argonaute proteins appear to contribute to exogenous dsRNA-dependent silencing, whereas deletion of the RNA-dependent RNA polymerases had no major effect. To better define the regulation of secondary metabolites and expand our knowledge of *A. fumigatus* small RNAs, we sequenced the small RNA population of fungal hyphae under starvation conditions. In parallel, we are performing LC-MS/MS-based proteomics analysis to correlate small RNA production with protein regulation. In short, this study has elucidated an additional layer in the complex regulation of *A. fumigatus* secondary metabolites, advances our understanding of fungal RNAi systems, and provides a platform for further investigations into the RNAi regulation of *A. fumigatus* virulence pathways.

179 Composition and processing activity of semi-recombinant holo U7 snRNP

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In animal cells, replication-dependent histone pre-mRNAs are cleaved at the 3' end by U7 snRNP, a multi-subunit endonuclease that contains two stably bound core components: a ~65-nucleotide U7 snRNA and a ring of seven Sm/Lsm proteins. Lsm11, one of the ring proteins, interacts with FLASH and they both recruit the catalytic component CPSF73 and other polyadenylation factors, forming active holo U7 snRNP. In this report, we assembled core U7 snRNP *in vitro* using recombinant U7 snRNA and the seven ring proteins and analyzed its ability to bind polyadenylation factors in mouse nuclear extract and to support histone pre-mRNA processing *in vitro*. We show that the reconstituted semi-recombinant holo U7 snRNP has the same composition and functional properties as endogenous U7 snRNP and faithfully cleaves histone pre-mRNAs and degrades the downstream cleavage product using the endonuclease and 5'-3' exonuclease activity of CPSF73, respectively. By varying the sequence of the 5' region of the U7 snRNA, we reprogrammed semi-recombinant holo U7 snRNPs to cleave and degrade RNA substrates that are not recognized by endogenous U7 snRNP. By adding or removing nucleotides upstream of the Sm site, we also changed the site of cleavage in histone pre-mRNAs, as predicted by the molecular ruler model. Finally, we demonstrate that the U7-specfic Sm ring assembles efficiently on a spliceosomal Sm site but the resultant semi-recombinant holo U7 snRNP fails to efficiently and faithfully cleave histone pre-mRNAs, likely due to incorrect geometry of the Sm ring.

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180 Disruption of the Caenorhabditis elegans Integrator complex triggers a non-conventional transcriptional mechanism beyond snRNA genes.

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Gene expression is generally regulated by recruitment of transcription factors and RNA polymerase II (RNAP II) to specific sequences in the gene promoter region. The Integrator complex mediates processing of small nuclear RNAs (snRNAs) as well as the initiation and release of paused RNAP II at specific genes in response to growth factors. Here we show that in C. elegans, disruption of the Integrator complex leads to transcription of genes located downstream of the snRNA loci via a non-conventional transcription mechanism based on the lack of processing of the snRNAs. RNAP II read-through generates long chimeric RNAs containing snRNA, the intergenic region and the mature mRNA of the downstream gene located in sense. These chimeric sn-mRNAs remain as untranslated long non-coding RNAs, in the case of U1- and U2-derived sn-mRNAs, but can be translated to proteins in the case of SL-derived sn-mRNAs. The transcriptional effect caused by disruption of the Integrator complex is not restricted to genes located downstream of the snRNA loci but also affects key regulators of signal transduction such as kinases and phosphatases. Our findings highlight that these transcriptional alterations may be behind the correlation between mutations in the Integrator complex and tumor transformation.

181 Deletion of a single miRNA binding site in the 3′ UTR of mRNA encoding synaptic protease MMP-9 results in enhanced learning in mice.

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Matrix metalloproteinase 9 (MMP-9) is an extracellular protease activated in synapses upon neuronal stimulation. By the cleavage of the extracellular matrix and adhesion molecules MMP-9 regulate the tructural plasticity of synapses located on dendritic spines. We have recently shown that the brain-enriched microRNA, miR-132 regulate local translation of MMP-9 in synapses.

In order to study what is an impact of this regulation on the organismal level, we have generated a knock-in mouse line with a mutated miR-132 binding site in the 3' UTR of MMP-9 mRNA (MutSeed). The mice did not display any visible morphological abnormalities in the brain, while the level of MMP-9 mRNA and protein in visual cortex and hippocampus during the development was unchanged for mRNA and only slightly incresed in the visual cortex (P21) on the protein level.

However, the behavioral tests in which we compared the formation of contextual memory (contextual fear conditioning) have shown significantly enhanced learning of the MutSeed knock-in mice. This indicates that regulation of local, synaptic synthesis of MMP-9 by miR-132, which does not have a substantial effect on total protein and mRNA levels, leads to changes of synaptic physiology manifested by altered behavior. This is, to the best of our knowledge, the first example of single target dysregulation by specific microRNA that leads to molecular changes on the synapse affecting brain physiology.

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182 A tissue-specific and transcription-dependent mechanism regulates primary microRNA processing efficiency of the human Chromosome 19 MicroRNA Cluster

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We investigated the maturation of microRNAs (miRNAs) from Chromosome 19 MicroRNA Cluster (C19MC), which is the biggest human miRNA cluster containing 46 miRNA genes. These miRNAs are currently believed to be expressed simultaneously and in terms of expression pattern, exclusively in human embryonic stem cells (hESC) and placenta. MiRNA maturation is a complex pathway, which is regulated both transcriptionally and post-transcriptionally. In case of a miRNA cluster, multiple miRNAs encoded by a single primary transcript (pri-miRNA) and regulated together on the transcriptional level. On the other hand, post-transcriptional processing alters each miRNA separately through sequential nucleolytic cleavages first by the Drosha/DGCR8 complex and then by Dicer to form mature miRNA molecules.

Comparing the expression of C19MC miRNAs in hESC and placenta samples revealed different level of expressional changes within the cluster. MiRNAs from the 5' region of the cluster have smaller disparity between the two tissues than downstream miRNAs. The correlation between expressional differences and miRNA positions is significant. This suggests a transcription-related mechanism, which is strengthened by the identification of two tissue-specific promoters, each with a diverse set of enhancers. However, the pri-miRNA level measurements do not correlate with the mature miRNA results, but we found a similar trend in pri-miRNA processing efficiency, which can explain the position related expressional differences. For further investigations, we used JAR, a choriocarcinoma cell line and characterized the connection between transcription and processing by manipulating these regulatory mechanisms by various transcription inhibitors and by knocking down Drosha.

Our data from examining multiple steps of the miRNA maturation pathway suggest that the expression of C19MC miRNAs is governed by a previously unknown tissue-specific post-transcriptional pri-miRNA processing mechanism, which is also connected to transcription regulation.

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183 Regulation of HIV latency by miR-34c-5p

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We have previously described miR-34c-5p as a novel regulator of naïve CD4 T cell activation and HIV-1 replication (Amaral et al 2017). In this work, stable overexpression of miR-34c-5p in the Jurkat T cell line coupled to HIV-1 infection resulted in increased viral replication defined by the increased production of viral mRNAs, proteins and viral particles, in spite of an apparent reduction of the levels of integrated proviral DNA. We have now started to investigate the effect of miR-34c-5p in the reactivation of a latent HIV infection in the Jurkat derived cell line J-Lat. In contrast to what is observed in infection, miR-34c-5p overexpression has a negative effect on the reactivation of the viral genome in response to TNF-alfa, which is consistent with reduced RNA Pol II recruitment to the viral LTR promotor. Our study of the underlying mechanisms suggests that the impact of miR-34c-5p in these processes results from a complex balance of positive and negative interactions that cannot be easily predicted from the simple identification of mRNA targets, thus requiring the incorporation of modeling approaches for a deeper understanding of the observed outcomes.

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184 PRP40 mediates the communication between RNA Polymerase II, spliceosome and microprocessor complex in plants

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MiRNA are short, non-coding RNA, which are engeged in the regulation of many genes. MiRNAs is transcribed by RNA Polymerase II (RNAPII) as precursors (pri-miRNAs) containing stem-loop structure, where mature miRNAs are embeded. Pri-miRNA is cleaved to mature miRNA by DICER LIKE1 (RNAse type III) (DCL1) accompanied with SERRATE (C2H2 zinc-finger protein) and HYPONASTIC LEAVES1 (HYL1) (dsRNA binding protein). HYL1 and SERRATE improve the efficiency of cleavage by DCL1. The zinc finger protein SERRATE has been described as an important element of the pri-miRNA processing machinery. SE interacts with other key miRNA biogenesis elements such as DCL1 and HYL1. The mutation in SERRATE leads to the increased levels of miRNA primary transcripts and diminished amount of mature miRNAs. Our laboratory shown that SE directly interacts with Cap Binding Complex (CBC) which consists of two proteins: CBP20 and CBP80. CBC recognises and binds to 5' end of new transcripts. Despite it, SERRATE also interacts with U1 snRNP complex and PRP40. It was shown, that PRP40 (which is auxiliary protein of U1snRNP complex) also associates with CTD of RNA Polymerase II (RNAPII).

In this project, we studied the role of PRP40 in the recruitment of spliceosome and microprocessor to RNAPII complex during transcription. We applied confocal microscopy methods to study a distribution of splicing factors: U1-70k, PRP40, SERARTE, U1snRNA and the proteins of microprocessor complex: HYL1 and DCL-1 in nuclei of Arabidopsis thaliana. By using statistical approach, we studied the colocalization rate between tested protein and active RNAPII (phosphorylated at serine 5 and serine 2 in CTD domain). The experiments performered in mutants (prp40, se-2, cbp20/80, hyl1) allowed us to assess the changes in the colocalization rate between microprocessor proteins, splicing factors and RNAPII and eluside the role of this proteins in the communication between RNAPII complex, spkiceosome and microprocessor.

185 Tissue-specific regulation of RISC loading by Hsp90 cochaperone Cyclophilin 40

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Gene silencing is triggered by the effector RNA-induced silencing complexes (RISCs) comprising small non-coding RNAs (sRNAs) and Argonaute (Ago) family proteins at the core. At the most downstream of sRNA biogenesis, during RISC formation, duplex precursors of many microRNAs (miRNAs) and short-interfering RNAs (siRNAs) are loaded to Ago proteins with the help of chaperone complexes containing Hsp90 and interacting cochaperones. It is not fully understood how RISC formation is regulated by Hsp90 cochaperones in tissues. In plants, an Hsp90 cochaperone Cyclophilin 40 (CYP40) activates miRNAs by facilitating the binding of duplex precursors to AGO1 during RISC formation (Iki et al., 2012). Although Cyp40 is well conserved among eukaryotes, the physiological roles and molecular functions of the animal orthologues have been elusive. We found the expression of *Cyp40* in an invertebrate model *Drosophila melanogaster* (*dCyp40*) is not ubiquitous but rather restricted in testes. Newly generated null allele of *dCyp40* showed male sterile phenotype, lacking mature sperms but negligible effect on germline stem cell maintenance and the early differentiation, suggesting essential roles during late spermatogenesis. Generally, in fly, miRNAs are loaded to Ago1 and function as Ago1-RISCs, while Ago2 recruits distinct miRNA species selected through well-established Ago sorting mechanisms. Ago2 also forms RISCs with endogenous (endo-)siRNAs and required for normal sperm maturation. Deep-sequencing and quantitative PCR analyses on sRNAs expressed in *dCyp40* mutant testes shows the selective downregulation of Ago2-sorted miRNAs, while endo-siRNAs were unaffected, suggesting that dCyp40 underpins miRNA-Ago2 loading in testes. Ago1-bound sRNA profile was also altered in the absence of *dCyp40*. Based on these results, we propose that animal Hsp90 cochaperones have regulatory potential for RISC formation to alter the repertoire of sRNAs in tissues as exemplified here with dCyp40 in testes.

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186 miR820-mediated epigenetic regulation in rice

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Epigenetic regulation has been implicated in plant development and stress responses. The underlying mechanisms of epigenetic regulation include DNA methylation, histone modification, and non-coding RNA-mediated regulation of gene expression. Of these, non-coding small RNAs, including microRNAs and small interfering RNAs, play a crucial role in negative regulation of gene expression at both transcriptional and posttranscriptional levels. microRNA820 is a small RNA produced from transcripts originated from a region inside CACTA DNA transposons in rice. It targets OsDRM2, which is involved in de novo DNA methylation of CG and non-CG sequences in the rice genome through a RNA-dependent DNA methylation mechanism to suppress transposon activity. Interestingly, both miR820 and OsDRM2 are down-regulated by drought stress treatment. To explore the function of miR820, transgenic rice plants over-expressing miR820 was generated. The transgenic plants exhibited drought-resistant phenotype compared with wild type plants. In addition, several transposable elements, including RIRE7, CACTA, and Tos17, were up-regulated in these transgenic plants. We also confirmed that those transposons were less-methylated in the miR820 over-expressing plants. These results might be due to the down-regulation of OsDRM2, which is responsible for the suppression of those transposable elements. Possible roles of this epigenetic regulation by miR820 and OsDRM2 as well as their agricultural impacts on drought stress resistance will be discussed.

187 The Arabidopsis SERRATE protein interacts with WW domain-containing DEAD box helicases.

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The SERRATE protein (SE) is an important subunit of the plant microRNA (miRNA) biogenesis machinery. Our studies on the Arabidopsis SE interactome shows a large number of proteins interacting with SE. Many of the proteins identified have not been previously described as SE interactors. Among these novel SE-associated factors we identified many DEAD box helicases. The involvement of helicases in processing of plant miRNA precursors have not yet been reported, however, two of them have been ascribed to miRNA biogenesis in animal cells: DDX5 unwinds the miRNA/miRNA* duplex and participates in the loading of a mature miRNA into the silencing complex in the case of let-7, while DDX17 influences the processing efficiency of the murine miR-132. In our SE interactome data among others DEAD box helicases we identified DRH1, RH46 and RH40 that belong to the DDX5/DBP2 subfamily. In contrast to the helicases involved in miRNA in animals, plant DRH1, RH46 and RH40 have the WW domain that can interact with the CTD domain of RNA polymerase II. Our results show that DRH1, RH46 and RH40 interact directly with SE. Moreover, we show that DRH1 interacts also with two other factors involved in miRNA processing in plants: TOUGH (TGH) and Not2b, which are important players in the regulation of miRNA biogenesis at the transcriptional level. Our results suggest that the helicases belonging to the DEAD-box family are involved in co-transcriptionally processing of miRNA in the plant cell.

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188 miR-29-CLIP identifies RNA processing factors as potential regulators of cutaneous repair

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Our previous data suggested an improvement of cutaneous repair upon downregulation of miR-29 family members in mouse model of wound healing *in vivo.*To better understand the mechanism of enhanced epidermal growth, we set off to identify endogenous targets of miR-29 in the skin using miR-CLIP method developed by Imig et al.[1] This method requires a probe comprising psoralen-biotin-modified precursor miR-29, which is processed by cellular Dicer, binds the target RNA, and incorporates to a functional RISC [2]. We found that covalently linked psoralen at adenine 41 and biotin at uridine 58 of pre-miR-29a sequence allows the release of functional miR-29a-3p. It in turn targets mRNAs with predicted 'seed' sequence complementarity in the 3'UTRs in HEK293T cells and in immortalized human keratinocytes. We proceeded with miR-29a-CLIP in HEK293T cells followed by the NGS sequencing and identified both new and previously reported mRNA targets of miR-29. While functional characterization of these mRNAs in cutaneous repair is ongoing, we would like to report a new group of miR-29 targets identified by miR-CLIP and comprising RNA processing factors. Among them, the splicing factor SFPQ, ATP-dependent RNA helicase DDX3X,and nucleolar RNA helicase DDX21have the highest SVR score of the miR-29 biding to the 'seed' matching sites in 3'UTRs. We predict that an increase in these proteins caused by a loss of miR-29 during wound healing is beneficial for cutaneous repair. It would be important to further investigate the role of RNA processing enzymes in tissue regeneration, especially if their levels can be modulated by miR mimics and anti-sense nucleotides.

[1]Imig J, Brunschweiger A, Brümmer A, Guennewig B, Mittal N, Kishore S, Tsikrika P, Gerber AP, Zavolan M, Hall J. miR-CLIP capture of a miRNA targetome uncovers a lincRNA H19-miR-106a interaction. *Nat Chem Biol.*2015

[2]Menzi M, Pradère U, Wang Y, Fischer M, Baumann F, Bigatti M, Hall J. Site-Specific Labeling of MicroRNA Precursors: A Structure-Activity Relationship Study. *Chembiochem.*2016

189 Decreased stability of the variant mRNA by microRNA targeting a heterozygous coding mutation site largely contributes to haploinsufficiency of tumor suppressor FOXL2

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Here, we show that a highly conserved somatic heterozygous missense mutation in the FOXL2 tumor suppressor gene (c.402C>G; p.C134W) contributes to allelic imbalance in adult-type granulosa cell tumours (AGCTs), where miR-1236 selectively binds and degrades the variant *FOXL2* mRNA. The level of the variant *FOXL2* mRNA is dramatically decreased and inversely correlated with miR-1236 levels in AGCT patients. Our present study provides another layer of mechanism for FOXL2-mediated pathogenesis where the highly conserved missense mutation can cause gene silencing, leading to development of AGCT.

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190 Evaluation of the diagnostic potential of microRNAs in hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) ist the most common type of primary liver cancer (85-90%). The standard methods for diagnosis of HCC are imaging and serum biomarkers: like alpha-fetoprotein (AFP), des-gamma-carboxy prothrombin (DCP/PIVKA-II) and AFP-L3%. These biomarkers, however, are lacking the sensitivity and specificity necessary for early detection of HCC, which is crucial for successful resection or treatment. Thus there is a high need for new biomarkers for the early diagnosis of HCC. Several publications have revealed that an expression level of microRNAs, small, non-coding RNA species released into the blood, can serve as an early marker for various diseases, including HCC. The goal of this study was to evaluate the diagnostic role of miRNAs in HCC, alone and in combination with known protein biomarkers.

Methods and Results

First, miRNA expression levels of 100 patient plasma samples (early and late HCC vs. chronic liver disease control group) were analysed and compared by next generation sequencing (NGS). In a second approach, small RNA was isolated from 60 patient plasma samples (early and late HCC vs control group), followed by analysis of expression levels of known miRNAs by qPCR. Finally, the results from the NGS and qPCR discovery approaches were combined for the selection of 26 miRNAs (including 2 novel miRNAs) whose expression differentiated between HCC and the control group. Selection criteria were p-value, area under the curve (AUC) and fold change for HCC vs. controls. Finally, a validation of those 26 candidates in new sets of 200 (training cohort) and 300 (validation cohort) plasma samples via qPCR was performed. MiR-21 and miR-423 could be confirmed in cohorts as possible single biomarkers for the diagnosis of HCC; miR-652 was found as putative single biomarker for early HCC in cirrhosis patients. The combination of miR-652 and protein biomarker PIVKA-II showed a slight improvement in the detection of early HCC.

Conclusion

Single miRNAs and a combination of miRNAs and protein PIVKA-II were confirmed as possible biomarker for the (early) diagnosis of HCC in plasma. However, a combination of several miRNA as biomarker signature did not improve the clinical performance of the best miRNA candidate miR-21.

191 The multifaceted population of small non coding RNAs deriving from plant tRNAs: biogenesis and functions

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Small non coding RNAs (sncRNA) deriving from tRNAs (tRFs), exist in all branches of life. Long (30-35 nt) and short (18-25 nt) RNA fragments, are mainly generated from mature tRNAs. In the model plant Arabidopsis, tRFs originate not only from nuclear- but also from organellar-encoded tRNAs and fluctuations in the tRFs population depending on tissues and stress conditions were observed [1]. We recently demonstrated that the Arabidopsis Dicer-like proteins are not major contributors in the biogenesis of tRFs. Rather, using *in vitro* and *in vivo* approaches, we demonstrated that the Arabidopsis RNases T2 are key players of both long and short tRFs production. Beyond plants, we showed that yeast or human RNase T2 can generate long and short tRFs, thus suggesting conserved features of these enzymes in eukaryotes [2].

Among Arabidopsis tRFs, two classes were identified. The first class concerns tRFs immunoprecipitated with Argonaute1, suggesting a role of these sncRNAs in RNA silencing. Strikingly, our novel data show that these tRFs are strongly enriched in nuclei. Interestingly, a few of them originate from organellar tRNAs and may represent part of a retrograde signaling pathway. In contrast, the second class contains abundant tRFs not or very poorly associated with AGO1. We now demonstrated that some of them are able to efficiently inhibit translation *in vitro* and that a few nucleotides within their sequences are important elements for protein synthesis inhibition. These tRFs can associate with polyribosomes and our data suggest that tRFs could act as general modulation factors of the translation process. Transfection of such tRFs into Arabidopsis protoplasts allowed us to perform *in vivo* live imaging and to show that they can be found in dynamic foci. The identification of the foci and the tRFs interactome, the next step of our studies, must bring new insights in the biology of sncRNAs deriving from tRNAs.

1 Cognat V et al. *Nucleic Acids Res.* **45**, 3460–3472.
2 Megel C, Hummel G et al. *Nucleic Acids Res.* **47**, 941-952.

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192 Dealing with pressure: Levels of snoRNA -derived fragments in cytoplasm and their association with ribosomes are dependent upon stress conditions but independent from snoRNA expression

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In recent years, a number of small RNA molecules derived from snoRNAs have been observed. Findings concerning the functions of sdRNAs in cells are limited primarily to their involvement in microRNA pathways. However, similar molecules have been observed in Saccharomyces cerevisiae, which is an organism lacking miRNA machinery. Considering our previous observations, we examined the subcellular localization of sdRNAs in yeast. Our findings reveal that both sdRNAs and their precursors, snoRNAs, are present in the cytoplasm at levels dependent upon stress conditions. Moreover, both sdRNAs and snoRNAs interact with translating ribosomes in a stress-dependent manner. As a consequence of binding to ribosomes, yeast sdRNAs exhibit inhibitory activity on translation. However, observed levels of sdRNAs and snoRNAs in the cytoplasm and their association with ribosomes suggest independent regulation of these molecules by yet unknown factors.

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193 Nuclear RNA export factor variant triggers Piwi–piRNA-mediated co-transcriptional silencing

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piRNAs are a class of small noncoding RNAs that associate with PIWI proteins and guide piRNA-induced silencing complexes (piRISCs) to target and repress TEs for the maintenance of genomic integrity. Among three PIWI proteins expressed in the *Drosophila*ovary, only Piwi is located in the nucleus of both germline cells and surrounding somatic cells. Within the nucleus, Piwi–piRISCs repress TEs transcriptionally, by spreading H3K9me3 marks and recruiting the linker histone H1 to form heterochromatin. Among factors identified as necessary for the effector phase of Piwi-mediated silencing, Panoramix (Panx; also known as Silencio) plays an essential role downstream of Piwi. Panx interacts with Piwi and promotes the deposition of H3K9me3 marks on target TE chromatin. However, it is unclear how Panx promotes the silencing of target TEs through the association with Piwi–piRISCs. Here, we identified Nxf2, a member of the nuclear RNA export factor (NXF) family highly expressed in the ovary, as a protein that forms a complex with Panx and Piwi. Nxf2 further associates with p15 (Nxt1), a co-adaptor for nuclear RNA export. Unlike Nxf1, the other NXF variant that plays a major role in mRNA export, Nxf2–p15 instead transcriptionally regulates TEs in the Piwi–piRNA pathway and also stabilises the protein level of Panx. The LRR domain of Nxf2 harbouring RNA binding activity is essential for recruitment of the Piwi–piRISC complex to target TEs. Notably, ectopic targeting of Nxf2 could initiate co-transcriptional repression of the target reporter gene in a manner independent of H3K9me3 marks or H1. These results suggest that Nxf2 is required to enforce the association of Piwi–piRISC complexes with the nascent transcript of target TEs and trigger co-transcriptional repression, prior to heterochromatin formation in the nuclear silencing pathway.

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194 microRNA degradation in human physiology and pathology

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MicroRNAs are key molecules in cancer biology and have potential as therapeutic tools. Overall, miRNA cellular levels are determined by the sum of two processes: biosynthesis, which generates new miRNA molecules, and decay, that clears old miRNAs. Very little is known about the degradation of microRNAs. Using tailored approach based on *in vivo*RNA labelling and high-throughput sequencing, we managed to precisely measure miRNA biosynthesis and decay rates in mammalian cells, and revealed that different pools of miRNAs with specific decay patterns exist. Therefore, unlike previously thought, not all miRNAs are stable molecules and clearance of miRNAs might be accelerated. Some endogenous transcripts can induce miRNA degradation through a mechanism known as TDMD, Target-Directed miRNA Degradation, which is characterized by a specific miRNA:target architecture and the accumulation of certain miRNA isoforms (isomiRs) with modifications at the miRNA 3′ end. To date, it is not known how many RNA transcripts capable of TDMD (TDMD-transcripts) exist and, more importantly, what their possible impact on human physiopathology is.We developed a web tool, called TDMD-finder, with a systematic annotation of TDMD-transcripts in Human and Mouse genomes. In our analysis, we identified approximately 4000 'high confidence' TDMD interactions and a subset of 1515 target:miRNA pairs (corresponding to 930 different transcripts), carrying miRNA degradation elements (MDE) significantly conserved at phylogenetic level (PC set). To investigate the implications of TDMD in human cancer, we provided an integrative analysis of qualitative and quantitative features of TDMD-transcripts and their cognate miRNAs in 21 different human cancers, using genetic information, copy number variations, transcriptional and miRNA expression datafromThe Cancer Genome Atlas repository (TCGA multiomic platform). We revealed a pervasive role of target-directed degradation mechanism and pinpointed new potential oncogenic events in human cancer.

195 The *Drosophila* dsRNA-binding protein Blanks exports siRNA precursors from the nucleus

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RNA interference targets aberrant transcripts via cognate sequences programmed via small interfering RNAs, which are derived from double-stranded RNA precursors. Several functional screens have identified *Drosophila* Blanks as a facilitator of RNAi, but its molecular function remained unknown, potentially because of its restricted expression pattern (testes and certain cell culture lines). We demonstrate that Blanks only aids in RNAi phenomena triggered by dsRNA of nuclear origin. Blanks binds dsRNA, shuttles between nucleus and cytoplasm and the abundance of certain endogenous siRNAs is reduced in Blanks mutant testes. Increased nuclear retention of Blanks also distorts the small RNA profile, but only if Blanks is competent for RNA binding. We propose that Blanks facilitates the export of dsRNA to the cytoplasm for further processing by the RNAi machinery.

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196 An aging-upregulated miRNA in arcuate nucleus of the hypothalamus repress ATG7 expression along the aging

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Hypothalamus is known to play a central role in control of aging. The roles of differentially expressed genes in hypothalamus along aging have been extensively investigated. miRNAs are regulators of broad spectra of cellular physiology. Age-regulated genes in hypothamus include dozens of miRNAs (ref), yet their function and regulatory mechanisms for age-associated genetic alterations are still not well defined. Here, we show that an age-up regulated miRNA in arcuate (ARC) nucleus of hypothlamus represses the expression of ATG7 and causes age-associated obesity. From small RNA seq analysis, we observed 14 hypothalamic miRNAs and 109 miRNAs are significantly upregulated and downregulated in hypothalamus. Among the age-up regulated miRNAs, we confirmed that age-upregulation of a miRNA in arcuate nucleus. To examine the role of this miRNA in aging, we overexpressed and inhibited the expression of this miRNA via stereotaxic injection of AAV viral construct in situ. Overexpression of the miRNA in ARC nucleus of young mouse (3month) resulted in increased fat accumulation, reduced energy expenditure, and impaired glucose homeostasis. In contrast, inhibition of this miRNA in ARC nucleus of aged mouse (18month) resulted in decreased fat accumulation, increased energy expenditure, and improved glucose homeostasis. We confirmed that this miRNA strongly down-regulates the protein level of ATG7 in ARC nucleus of hypothalamus. The recue of ATG7 level in the miRNA-overexpressed mouse resulted in noticeable recovery of metabolic control. Our results highlight age-associated hypothalamic miRNAs as one of the upstream key regulators of gene expression in ARC nucleus of aging hypothalamus and of the associated decline of metabolic control at the organismal level.

Keywords: Hypothalamus aging, hypothalamic miRNAs, ARC, age-associated obesity, autophagy.

197 MSM1 has root specific reduction in mature miRNA accumulation and also pri-miRNA accumulation

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Micro-RNAs are small, non-coding RNAs which regulate gene expression through repression of translation or direct cleavage of mRNAs. They play integral roles in the development of both plants and animals, and in response to both abiotic and biotic stresses in plants.

As miRNAs are of such widespread importance, they are subject to strict spatiotemporal regulation, to ensure that they are expressed at the right place and at the right time. This is reflected in their multi-step biogenesis pathway, which opens them to a number of points of regulation.

Presented here is a putative mutant in a miRNA biogenesis pathway provisionally called MicroRNA Stability Mutant 1 (MSM1), which was isolated from a forward mutagenesis screen sensitised to mutation in miRNA pathways. MSM1 appears to accumulate a subset of miRNAs less highly in the root, but not the shoot. The affected miRNA have a reduction at both the mature and pri-miRNA level. These differences are also reflected in root and shoot morphology, with MSM1 having significantly shorter roots than control plants.

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198 MicroRNA-convergent gene fusions in cancer

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Studies of fusion genes have focused on the formation of fusions that result in the production of hybrid proteins or, alternatively, on promoter-switching events that put a gene under the control of aberrant signals. However, gene fusions may also disrupt the transcriptional control of genes that are encoded in introns downstream of the breakpoint. By ignoring structural constraints of the transcribed fusions, we recently identified a largely unexplored function of fusion genes. Using breast cancer as an example, we showed that microRNA (miRNA) host genes are specifically enriched in fusion genes and that many different, low-frequency, 5' partners may deregulate the same miRNA irrespective of the coding potential of the fusion transcript. This led us to suggest that the concept of recurrence, defined by the rate of functionally important aberrations, should be revised to encompass convergent fusions that affect miRNAs independently of transcript structure and protein-coding potential. We have now proceeded to validate and expand these results by analysing tumour samples from a number of different tissues including e.g. bladder, kidney, and lung. By combining matched sequencing data for whole genomes, RNA and small RNA, we can address new hypotheses regarding the underlying regulatory networks and functional consequences of these fusions. We are also studying the impact on other types of intronically encoded RNAs such as small nucleolar RNAs (snoRNAs).

199 Regulatory role and biogenesis of miR-125a: a multifaceted liaison

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miR-125a, a vertebrate homolog of the first discovered microRNA lin-4, plays a well-conserved role in downregulating the pluripotency factor Lin28, thus promoting phase transitions in development and/or cell differentiation in nematodes, insects and mammals. Many other targets of miR-125a are involved in cell proliferation and the main effect of its transfection in cultured cells is the inhibition of cell proliferation. This evidence and the ubiquitous expression of miR-125a suggest that it could act as a regulatory switch for differentiation/proliferation with an impact on normal development and also on cancer as a tumor suppressor. In accordance with this hypothesis, miR-125a is downregulated in several types of tumors, including hepatocellular carcinoma (HCC), where it targets sirtuin-7, matrix metalloproteinase-11, VEGF-A, Pokemon, and c-Raf.

The regulatory role and biogenesis of miR-125a constitute a multifaceted liaison, since some miR-125a target proteins are in turn involved in the regulation of miR-125a expression, at transcriptional (NF-kB, Pokemon, hepatitis B virus X protein) and post-transcriptional level (Lin28). One example is a positive self-regulatory loop occurring in hepatocarcinoma cells, whereby miR-125a, by down-regulating TNFAIP3, reduces the inhibitory action on NF-kB, an activator of miR-125a promoter, thus strengthening its own transcriptional activation. Another example is a similar regulatory loop occurring between miR-125a and its target Pokemon, an oncogene for liver cancer: Pokemon has a repressive effect on miR-125a promoter, however miR-125a is able to reduce the transcriptional inhibitory effect of Pokemon by inhibiting Pokemon expression. Again, another regulatory loop has relevance in hepatits B virus infection: the virus, through HBx, is able to increase miR-125a expression, that in turn interferes with the production of virus surface antigen, thus balancing viral replication with that of the host. Finally, the conserved target of the miRNA, Lin28, was found to post-transcriptionally inhibit miR-125a by binding its precursor, thus preventing the formation of the mature miRNA.

The impact of these molecular pathways on physiological processes such as development, but also on pathological ones, such as cancer, is just at the beginning of elucidation.

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200 Quality control in snRNP biogenesis

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Small nuclear ribonucleoprotein particles (snRNPs) are essential components of a spliceosome that catalyzes the crucial step of the gene expression called pre-mRNA splicing. snRNPs consist of small non-coding RNA, Sm proteins, which form the Sm ring around a specific Sm-binding sequence and a set of the proteins specific for each snRNP. snRNPs create the catalytic core of the spliceosome and therefore have to be under strict quality control. Previously, was shown that Xrn1 exonuclease degrades snRNAs lacking the Sm ring and they are accumulated in P bodies. However, the molecular mechanism of how the cells discriminate between mature and immature particles is still mostly unknown. In this work, we focus on molecular mechanisms that detect defective snRNAs in the cytoplasm. We observed that LSm1 protein bind the snRNAs after disruption of the Sm ring assembly. Further, we depleted the SmB/B' protein and observed localization of snRNPs in the P bodies, however, if we also depleted the LSm1 protein we lost the accumulation of defective snRNPs in P bodies. Together we found a new possible role of LSm1 protein in the localization of defective snRNPs in P bodies.

201 MicroRNA-184 - An Ally in Calcium Signalling in the Skin

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The skin provides a protective barrier against environmental, mechanical and microbial assault throughout life. Extracellular calcium (Ca^{2+}) regulates the behavior of keratinocytes, the cells that form the epidermal layer of the skin. Calcium-dependent keratinocyte differentiation has been linked to microRNAs (miRNAs), small non-coding RNA molecules that attenuate gene output through mechanisms that culminate in target mRNA degradation. However, the impact of store-operated calcium entry (SOCE) on keratinocyte miRNA expression has received little attention. Here, I present our recent findings showing a relationship between SOCE and the induction of miRNA-184 (miR-184) in keratinocyte differentiation and migration. Levels of miR-184, barely detectable in untreated cells, rose by about 30-fold after keratinocytes were exposure to 1.5 mM Ca^{2+} for 5 days. Pharmacologic and genetic inhibitors of SOCE abrogated Ca^{2+}-dependent miR-184 induction by 70%. Further, modulation of miR-184 levels using an exogenous miR-184 mimic or inhibitor enabled us to elucidate roles for miR-184 in keratinocyte differentiation. These include induction of involucrin, an important component of differentiating keratinocytes, elevation of p21 cyclin-dependent kinase (CDK) inhibitor and enhancement of DNA damage, as evidenced by higher levels of γH2AX, a marker of DNA double strand breaks. In addition, I will present our data implicating miR-184 in keratinocyte migration. We observed a 50-fold increase in miR-184 upon wounding keratinocyte monolayers. This occurred under routine low Ca^{2+} culture conditions, suggesting that high extracellular $Ca^{2+}$is not an obligatory requirement for SOCE-dependent miR-184 induction. The induction of miR-184 in wounded monolayers was completely abolished in the presence of pharmacologic SOCE inhibitors. Transfection of keratinocytes with a miR-184 mimic stimulated migration in scratch assays, whereas the converse was observed a miR-184 inhibitor was used. Together, these findings suggest miR-184 functions downstream of SOCE to promote differentiation and migration of epidermal keratinocytes.

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202 Target-Directed miRNA degradation in breast cancer

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MicroRNAs (miRNAs) are an evolutionary conserved class of small (18- 25nt) non-coding RNAs that function in post-transcriptional gene silencing by interacting with target RNAs. Levels of miRNAs are critical for their functions: the higher the number of available miRNA molecules, the higher the effects on target genes. A novel mechanism in control of miRNA levels has been described, which involves specific transcripts able to interact with miRNAs and then induce miRNA degradation. This mechanism, called Target-Directed miRNA degradation (TDMD), has been recently shown to function in vertebrates with endogenous transcripts, but its implications in human physiopathology are still unknown. We have previously shown that Serpine1 transcript uses TDMD to control levels and activity of two related miRNAs, miR-30b/c, in murine fibroblasts during serum response. We found that Serpine1 transcript is expressed at high level also in human cancer, in primary breast tumors and cancer cell lines of the Triple Negative subtype (TNBC); while miRNAs of the miR-30 family are frequently deregulated in cancer (including those of the breast), and known to exert a tumor- suppressive role. Focusing on the Serpine1:miR30b/c pair as model, we have applied molecular and genetic (CRISPR/Cas9) approaches to manipulate TDMD mechanism directly and investigate the effects afforded by miRNA degradation. Our preliminary results suggest that TDMD is actually used by cancer cells to keep low the activity of miRNAs low and provide selective advantage in various cancer phenotypes.

203 miR-32 promotes oncogenic Myc-induced prostate tumor growth *in vivo*.

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The androgen receptor (AR) signaling pathway is central to the emergence of castration-resistant prostate cancer (CRPC). We have previously identified androgen-regulated microRNAs (miRNAs) that may contribute to the development of CRPC. By expression analyses, we found miR-32 to be an androgen-regulated miRNA differentially expressed in CRPC compared to benign prostatic hyperplasia (BPH). *In vitro*, miR-32 is able to provide a significant growth advantage to LNCaP cells by reducing apoptosis.

To study how increased miR-32 expression contributes to prostate cancer formation and/or progression *in vivo*, we have established transgenic mice expressing miR-32 specifically in the prostate epithelium post-puberty. Expression of miR-32 transgene increases replicative activity in prostate epithelium. To study the effects of miR-32 overexpression in prostate cancer, the miR-32 mice were cross-bred with mice overexpressing oncogenic Myc in the prostate. We found that the prostates of Myc-overexpressing mice were significantly larger when miR-32 transgene was present. We also noted histological changes and an increase in Ki-67 staining by immunohistochemistry, indicating increased replicative activity, by miR-32 overexpression in the Myc-induced tumors. Microarray analysis performed to the tumor-containing prostates at 6 months of age revealed several genes with miR-32-induced expression changes, affecting known secretory proteins (*SPINK1*, *SPINK5*, *MSMB* and *SRGN*) and proteins involved in cell metabolism, proliferation and invasion (*SLC38A5*, *PDK4*, *FABP4, SEMA3A, ANGPTL1, DMKN* and *ALDH1A2*).

Our data show that miR-32 is able to affect replication potential of prostate epithelium and prostate tumor development by affecting several cancer relevant pathways.

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204 Does a site matter? The role of an alternative polyadenylation on microRNA-21 biogenesis

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MicroRNAs (miRNAs) are a group of evolutionarily conserved, small (20-23nt) non-coding RNAs, which play an essential role in many cellular processes, mainly via regulation of translation and influencing mRNA stability. Biogenesis and maturation of miRNA are very complex processes and can be regulated on many steps by wide group of factors. miR-21 is one of the best known and well-studied miRNA. It plays a crucial role in development, inflammation, cardiovascular diseases and cancer. The gene coding for miRNA-21 is located in the intronic region of the VMP1 gene. Moreover, pre-miR-21 is found just around 600 nucleotides downstream of the main polyadenylation signal site (PAS) for VMP1 gene, what may play an important role in its biogenesis. Alternative polyadenylation (APA) is RNA-processing mechanism that generates different 3′ ends of different RNAs. The main importance of APA is generation of numerous mature alternative transcripts having different regulatory potential or different function. In our study we monitored the efficiency of miR-21 biogenesis and activity of APA in VMP1. As a model we use COS7 cells transfected with VMP1 polyadenylation site-specific antisense oligonucleotide blocker. To observe the effect of accumulation of pri-miRNA and the mature form of miRNA after blocking of targeted PAS we used the cells with DROSHA/DGCR8 depletion. As a result we detected accumulation of pri-miRNA and consequently higher level of mature form of miR-21in oligonucleotides-treated cells. These results suggest that APA of VMP1 plays an important role in biogenesis of miR-21. Disturbances of miR-21 biogenesis in many disease stages would be connected with efficiency of alternative polyadenylation.

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205 Discovery and preliminary function study of tRNA -derived small RNA

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MicroRNAs (miRNAs) are a class of 19-25nts, evolutionarily conserved small RNAs which involved in a variety of life processes. Identification of the novel miRNA is important for elucidating the function of miRNAs. In this study, we identified high expressed miRNAs with miRNA chips in non-small cell lung cancer H1299 and H460 cells, then verified by qPCR and Northern Blot. Bioinformatic analysis and dual luciferase reporter assays were used to validate the targets of high expressed miRNAs. Results showed that miR-4454 was found highly expressed in H1299 and H460 cells. As miR-4454 is a newly discovered miRNA, which reported only existing in the human cells by sequencing, we further verified it existence in cells by Northern Blot. We found surprisingly that the 20nt mature miR-4454 did not exist in cells, but a 70nt hybridization band was detected. We found that the 70nt fragment is tRNAHis but not miR-4454 precursor (pre-mir-4454) by RNA sequencing. We also found that the sequence of 3'end of tRNAHis is almost exactly the same with the miR-4454, which led to a false positive result in miRNA chips and qPCR test. Our further experiments demonstrated that Dicer, the key factor in miRNA biogenesis, could cleave tRNAHis 3'end to generate a 16nt small RNA which could play functions similar to the miRNAs and inhibit the expression of CNKSR2, a predicted target gene of miR-4454. Our results suggested that lots of miRNAs in the miRNA database (miRBase) might be pseudo-miRNAs because these miRNAs are obtained by second generation sequencing or bioinformatic prediction, and most of them did not undergo a rigorous experimental verification. In conclusion, we found that miR-4454 was not existed in lung cancer cells but a novel 16nt small RNA which derived from the cleavge of 3'end of tRNAHis by Dicer enzyme, this kind of tRNA-derived small RNA could play functions similar to the miRNAs.

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206 microRNA within the endogenous spliceosome of breast cancer cells acts on novel nuclear targets

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Pre-mRNA splicing and microRNAs (miRNAs) function in regulating gene expression, alteration in which is associated with human diseases including cancer. Most studies of miRNA regulation focus on their role in the cytoplasm, however, recent studies reported the presence of miRNAs in the nucleus, yet their roles there remain to be elucidated. We showed a crosstalk between miRNAs biogenesis and splicing, and identified a subset of pre-miRNA fragments and mature miRNAs sequences within the endogenous spliceosome, including known cancer-associated miRNAs. We compared the profile of spliceosomal miRNAs by RNA-Seq of breast cell-lines representing three levels of cancer progression: the non-malignant MCF-10A, the intermediate level MCF-7, and the highly malignant MDA-MB-231 cells. We found that the fraction of mature miRNAs increases with tumorigenesis, from 20% in MCF-10A to 60% in MDA-MB-231 cells. Notably, we identified in the spliceosomal fraction miR-7704 that is complementary to sequences of the first exon of the long non-coding RNA (lincRNA) called HOXD Antisense Growth-Associated Long Non-Coding RNA (HAGLR) which plays a role in the development and progression of multiple cancers. We showed that miR-7704 negatively regulates the expression of HAGLR. Specifically, the level of miR-7704 decreases with the malignancy of the analyzed breast cell lines, while the level of HAGLR increases. We manipulated the levels of miR-7704, demonstrating that downregulation of miR-7704 by Anti-miR technology increased the level of HAGLR, while its overexpression decreased it. We further analyzed the 20 most highly expressed spliceosomal miRNAs, comparing their expression trend in cancer. We found that 35% of them show an opposite trend of expression with respect to that measured in the cellular counterpart of these miRNA in breast cancer. Specifically, miR-100, miR-30a and members of the let-7 family (7f-2, 7i, 7f1, 7g) show decreased abundance with increased malignancy, while, the level of these spliceosomal miRNAs actually increased with malignancy. Also, the level of spliceosomal miR-1246 decreased with malignancy, while it is increased in the cytoplasm. These findings suggest novel roles within the endogenous spliceosome for the above miRNAs, likely regulating different targets than their cytoplasmic ones. These miRNAs might serve as indicators for tumorigenesis.

207 "Non-coding RNA expression profiles and sequence-specificity of transcripts in plasma and extracellular vesiclesEVs in acute lymphoblastic leukemia survivors with the history of anthracycline treatment "

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Introduction of anthracycline based therapy significantly improved cancer patients survival, which for acute lymphoblastic leukemia (ALL) is now higher than 80%. Although very effective, anthracyclines are not specific against cancer cells, thus exert damaging side-effects on healthy non-cancerous cells - in particular in the cardiovascular system, resulting in increased risk of heart disease even many years after treatment. Mechanisms of this toxic action involve generation of double strand breaks in DNA with impaired potential of repair. We hypothesized that the damage caused by doxorubicin in cells that survived anticancer therapy may influence intercellular signaling and thus could be reflected in levels of circulating small non-coding RNAs (sncRNA).

Libraries of sncRNA prepared from RNA extracted from extracellular vesicles (EVs) and total plasma of 60 adult ALL survivors treated with doxorubicin and the same number of healthy controls, were sequenced on Illumina NextSeq500. miRNA expression (based on mirBase 22.1) was quantified via bwa-mem and miRDeep2 software. sncRNA expression was estimated via the kallisto software (and custom index based on RNAcentral database). All statistical analyses were performed in R environment with packages: edgeR (differential expression), RbiomirsGS (KEGG enrichment), topGO (GO-term enrichment), msa (multiple sequence alignment) as well as with custom R code. Results were considered significant under FDR<0.05.

We identified over 200 (in plasma) and 40 (in EVs) miRNAs, whereas over 60 (in plasma) and almost 30 (in EVs) other sncRNAs were differentially expressed between patients and controls. The targets of differentially expressed miRNAs are associated with cardiomyopathy and interventricular septum size. Additionally, we detected over 90 miRNAs and 11 sncRNAs with altered proportion between plasma and Evs in ALL group when compared to controls. This suggests that anthracycline treatment might affect RNA export to extracellular compartment. We found specific patterns of the sequence within transcripts that are enriched or depleted in plasma and exosomes. Additionally, motifs enriched in transcripts differentially divided between EVs and plasma were studied. This study has been supported by a grant of National Science Centre in Poland (SONATA 2015/17/D/NZ7/02165).

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208 Convergent and Divergent Evolution of microRNAs

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MicroRNAs (miRNAs) are endogenously expressed small noncoding RNAs. Metazoan miRNAs and their target genes often interplay to compose complex regulatory networks. Given the complexity of these networks, it is intriguing to investigate how miRNAs originate and evolve. Our longstanding interests are to decipher the evolutionary principles of miRNAs and their target sites. Previously, my collaborators proposed a birth-and-death model of miRNA evolution which suggests most newly emerged miRNAs are evolutionarily transient and have a high birth rate followed by a high death rate. We also found that once a new miRNA survives its initial stage, many adaptive mutations are required to drive that miRNA to develop function.

Furthermore, we found variation in miRNA target sites is associated with increased gene expression variation in human populations. Nevertheless, it yet remains unclear what factors affect the survival and function development of a new miRNA or target site. Recently, we found that the genomic clustering helps new miRNAs survive and develop functions in animals. We put forward a "functional coadaptation" model which describes how natural selection has driven miRNAs in the same cluster to target the same or functionally related genes although those miRNAs have independent origins. Our results advance our understanding of the mechanisms and evolutionary driving force of miRNA clustering and expand our view of the regulatory functions of clustered miRNAs. We also show that miRNA duplication accelerates the recruitment of functional new targets even if the paralogous miRNAs maintain the same seeds. Collectively, our discoveries indicate that (1) clustering drives miRNAs of different origins to evolve convergently towards similar function and that (2) functional diversification following miRNA duplications influences the evolution of new target sites.

209 The miRNA529c in *Marchantia polymorpha* liverwort reproductive development

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In angiosperms, the phase change from vegetative to reproductive growth involves the suppression of the squamosa promoter-binding-protein-like (SPL) class of transcription factors, which is carried out by the specific microRNAs (miRNAs/miRs) miR156/529. Non-vascular land plants also undergo a phase transition to the reproductive state but knowledge regarding the control mechanisms is limited. We investigated the transition to reproduction in the liverwort *Marchantia polymorpha*, focusing on the role of miR529c. Initially, we established *mir529c* null mutants using CRISPR/Cas9. In the absence of environmental condition (far-red enriched light) that is usually needed to induce the reproductive stage, thalli of these mutants produced gametes without constructing neither antheridiophore nor archegoniophore possibly under the condition that target transcription factor is expressed constitutively. Next, we designed the miR529-resistant Mp*SPL2* transgene and transformed the wild type to see the regulation from the other side.

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210 Spatial differences of miRNA genes expression in the early stages of colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer worldwide. Most of available datasets from high-throughput experiments, aimed to describe more thoroughly molecular classification of CRC, contain data on mRNA expression and presence of DNA polymorphisms. However, molecular classification of cancer is likely affected by intra-tumour heterogeneity. Specifically, little is known about an intra-tumour heterogeneity in microRNA content and concentration. Mature miRNAs, act as endogenous suppressors through binding RISC to 3' untranslated regions of target mRNAs. miRNA molecules can act either as tumour suppressors (tsmiRs) or tumour promoters (oncomiRs) but always via their target mRNAs. Intratumour heterogeneity may contribute to failure of cancer therapy because of pre-existing or emerging clones of drug-resistant cancer cells. The profile of miRNAs may reflect the actual stage of the tumour and should be useful in prediction of the disease course. In addition, miRNAs could be potential targets for antisense therapy.

Samples from 12 patients suffering from early stage colorectal cancer were obtained during surgery of primary tumour. We sampled 3 distinct regions of each tumour (central region - C, half the radius - R and periphery - P) and healthy mucosa (M). Overall we used 44 samples for miRNA profiling in a single NextSeq run. Differential expression (DE) was performed using Bioconductor edgeR, dexus and ordinalNet packages in the R environment.

We identified 1748 miRNA transcripts for which at least 5 reads were present. The most abundant transcript was miR-143-3p with overall read count 19,364,818 (13.16% of reads). DE analysis reveal miR-1224-5 as the most down regulated (logFC -6.43) transcript between C and M. Additionally, we determined the expression gradient across the tumours.

As expected, each fragment of the tumour has differentially expressed genes compared to healthy mucosa and the biggest difference is between C and M. We can see as well that most miRNAs are down regulated at the C location. We confirmed statistically significant intratumour heterogeneity at the level of miRNome between C and R.

211 hAgo2 modulates Dicer expression via a miRNA-independent mechanism

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MicroRNAs are a class of short RNAs that play important roles in regulating cellular gene expression. Alterations in miRNA expression can strongly influence cellular physiology. Numerous proteins including Dicer and hAgo2 take part in the regulation of gene expression.

Overexpression of hAgo2 suppressed Dicer expression at the levels of both protein and mRNA while the reduction in hAgo2 expression enhanced Dicer expression. hAgo2 protein did not suppress Dicer promoter activity supporting that hAgo2 protein probably regulates Dicer expression at the post-transcriptional level. hAgo2 protein was showed to inhibit the Dicer 3'-UTR reporter activity. Post-transcriptionally inhibition of Dicer by miRNA (let-7, mir-103/107) had been previously reported. However, hAgo2 still suppressed Dicer expression in the cells depleted of these miRNAs. Moreover, the reporter activities of Dicer mRNA 3'-UTR with the mutations of the miRNA binding sites were still suppressed by hAgo2. Therefore, in addition to a miRNA-dependent pathway, hAgo2 can also modulate Dicer expression through a miRNA-independent mechanism.

Previous reports have demonstrated that RNA binding protein AUF1 represses dicer expression. Suppression of Dicer expression by AUF1 requiring hAgo2 was demonstrated in this study. Interactions of hAgo2 and AUF1(s) proteins were demonstrated by the co-immunoprecipitation assay. As expected, hAgo2 could not suppress the Dicer mRNA 3'-UTR reporter with a mutation in the potential AUF1-binding site. Thus, downregulation of Dicer expression through the 3'-UTR requires both hAgo2 and AUF1.

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212 Characterizing RNA Aptamers as tools to dissect the distinct molecular functions of HSF1

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The Heat Shock Response is a highly conserved protective mechanism that is regulated at the transcriptional level by transcription factors called Heat Shock Factors (HSFs). When activated by high temperatures or stress, HSFs strongly induce the expression of Heat Shock genes (HS genes), that encode Heat Shock Proteins (HSPs). Of the family of HSFs in mammals, HSF1 is the functional homolog of the single HSF in yeast and the fruit fly. HSF1 consists of at least three functional domains; the DNA binding domain (DBD), Trimerization domain (TD) and the Trans-Activation domain (TAD) which function to coordinate HSF1's ability to bind to its target DNA elements and trans-activate HS genes under HS conditions. To elucidate the mechanistic roles of each domain in living cells, I am using the novel approach of blocking domains with RNA aptamers. RNA aptamers are short, single stranded RNAs that bind specifically and with high affinity to their selected target, whether it is a protein domain, transcription factor or small molecule.

We have thousands of selected candidate aptamers for HSF1, however, we needed to identify those that have domain specific binding in order to test a domain specific effect. Initially, to identify the binding affinities of the selected RNA Aptamers, we used Electrophoretic Mobility Shift Assays (EMSA). It appears that the initial selection yielded many high affinity (Kd = 4-200 nM) aptamers, however this method of characterization does identify where the aptamers bind HSF1. Using a higher resolution, biochemical, UV crosslinking approach I have identified that many of the selected aptamers bind to the DBD-TD portion of HSF1. To test whether these aptamers inhibit DNA binding, I performed competition assays with a Heat Shock Element (HSE) and found at least one candidate aptamer that inhibits the DNA binding capacity of HSF1.

I am currently expressing a few selected inhibitory aptamers in mammalian cells to identify the extent to which they inhibit HSF1 induced transcription in vivo. We will measure the primary effects of aptamer expression on genome-wide transcription using Precision Run-On sequencing (PRO-seq) which allows for base-pair resolution mapping of actively transcribing RNA Pol II.

213 An RNA Polymerase III Roadblock controls expression of the MEP3 gene and yeast entry into stationary phase.

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Transcriptional roadblocks occur when a DNA binding protein is bound to the DNA and prevents an RNA Polymerase from transcribing through it. These roadblocks have been shown to promote transcription termination in synergy with the NNS termination pathway (Roy et al. Genome Res.2016). However, the role of roadblocks in other biological processes is not well understood. Here, we show that the presence of RNA Polymerase III at the tN(GUU)P tDNA locus prevents pervasive antisense transcription, which emanates from a retrotransposon, through the *MEP3* gene. *MEP3* encodes an ammonium permease that controls the entry of *S.cerevisiae* into stationary phase and is located immediately downstream from the tN(GUU)P tDNA locus. Inhibiting RNA Polymerase III recruitment from the tN(GUU)P locus, either using the anchor away technique or by deletion of the tDNA gene results in antisense transcription through the *MEP3* gene body, causing an inhibition of *MEP3* expression. Reduced expression of *MEP3* due to the loss of the RNA Pol III roadblock results in a defect in the transition from log phase growth to stationary phase growth identical to that detected in a *MEP3* knockout strain. We further show that expression of the sense/antisense MEP3 pair is inversely correlated, and that the deleterious effects of the antisense transcript can be rescued by insertion of a transcriptional terminator. Overall, these data suggest that Pol III transcriptional roadblocks may play a role in preventing pervasive transcription to help safeguard the cell. Additionally, we show here that this mechanism for Pol III transcriptional roadblocks may be conserved between various species.

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214 Predicting Rho-dependent termination of transcription at genome scale

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Bacterial transcription termination proceeds via two main mechanisms triggered either by simple, well-conserved (intrinsic) nucleic acid motifs or by the motor protein Rho. Intrinsic terminators are easily detected upon computational scans of genomes and many specimens have been studied experimentally. By contrast, only a few of the diversiform Rho dependent terminators have been characterized in detail and their molecular determinants remain elusive. To tackle this issue we have developed a new strategy combining the biochemical characterization of a large training set of genomic sequences with the multivariate modeling of the resulting Rho-dependent termination "response" using Orthogonal Projections to Latent Structures Discriminant Analysis [OPLS-DA] and a set of customized explanatory variables (aka sequence descriptors). We show that this approach is successful at predicting Rho-dependent termination with a success rate of ~85% (based on internal cross-validation). Using our OPLS-DA model to scan the genomes of E. coli and Salmonella, we found many more Rho-dependent termination sites than anticipated from transcriptomics analyses, suggesting that layers of Rho-dependent regulation have yet to be analyzed. We will discuss the lax consensus features underlined by the sequence descriptors successfully used for modeling as well as the practical utility of our computational model to predict Rho-dependent termination at the genome scale and across the bacterial kingdom.

215 Induction kinetics of plant stress-related genes is related to their memory status under repeated drought conditions.

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Plants, as sessile organisms, have evolved to react to rapid, recurring changes in their local environment. To make these reactions most efficient, plants can store information about past environmental stimulus and use it to adjust physiological or developmental responses to a future stimulus. Such a 'priming' of organismic responses to environmental stimuli results in phenotypic plasticity which improves fittness under future stressful conditions. At the cellular level, stress response reflects changes in expression of stress-related genes while increased tolerance, acquired as a result of priming, corresponds to modified gene responses. Generally, priming is a process closely linked to transcription. Therefore, stress-memory is often suggested a "transcriptional" memory. The molecular mechanisms of stress memory and, particularly, transcriptional memory, are poorly understood in plants. We show that the induction of stress-related genes expression has different kinetics in case of 'trainable' and 'non-trainanble' genes.

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216 A role of TFIIIC factor in control of tRNA gene transcription

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Transcription of tRNA genes by RNA polymerase III requires the assembly of multisubunit transcription factors TFIIIC and TFIIIB into a preinitiation complex. TFIIIC plays a primary role by recognizing the internal promoter elements in tRNA genes and by facilitating the assembly of TFIIIB upstream of the transcription start site. TFIIIB complex, consisting of the Brf1, TBP and Bdp1 subunits, is considered the initiation factor which is able to recruit the polymerase.

Here, we explored the mechanism by which TFIIIC recruits TFIIIB onto TATA-less tRNA genes in yeast Saccharomyces cerevisiae. This mechanism appears to involve a stepwise series of intricate protein-DNA and protein-protein interactions, some of which are regulated by environmental cues. Under repressive conditions, TFIIIC is tightly associated with tDNA. Two subunits of TFIIIB, Brf1 and Bdp1, are bound separately to TFIIIC and do not interact with each other. Transcription initiation, induced by an environmental signal, leads to dissociation of TFIIIC from tDNA, which is correlated with the release of Brf1 and Bdp1 from TFIIIC and their subsequent transfer to tDNA. Concurrently an increase of Brf1-Bdp1 association is observed which is consistent with the formation of TFIIIB complex on tDNA.

In summary, TFIIIC-mediated recruitment of TFIIIB to tDNA is promoted by the signal for transcription activation. Nevertheless, under repressive conditions, TFIIIC acts as a transcription inhibitor by sequestering subunits of TFIIIB and preventing their recruitment to the transcription start site. Therefore, depending on environmental conditions, TFIIIC may activate or repress transcription of tRNA genes.

217 Regulation of tRNA transcription involves interaction with translation elongation machinery

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Maf1 is a general and global negative effector of RNA polymerase III conserved in eukaryotes. The deletion of yeast gene encoding Maf1 (*maf1*-delta) leads to increased level of tRNA, although transcription of individual tRNA genes is elevated to various extends. The *maf1*-delta mutation resulted in the temperature sensitive (*ts*) growth on non-fermentable carbon sources and in the decrease of translational readthrough at nonsense codons. Both, the *ts* and antisuppression phenotypes of *maf1*-deltaΔwere compensated by increased dosage of TEF2 gene, encoding translation elongation factor eEF1-α. TEF2 overdose has no effect on Pol III association with chromatin and transcription efficiency of tRNA genes but specifically changed patterns of tRNA precursors. Direct measurements of the rate of protein synthesis show that TEF2 overdose results in decreased translation efficiency in *maf1*-deltaΔcells. Additionally, phenotype of *maf1*-delta can be overcome by treatment of cells with cycloheximide. In conclusion, we suggest that growth phenotype of *maf1*-deltaΔcan be suppressed by the inhibition of translation elongation owing to the facilitated codon adaptation to differential tRNA pools generated in the absence of Pol III regulation by Maf1.

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218 The catalytic role of the Integrator Complex Endonuclease in cleaving nascent mRNAs to attenuate transcription

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The Integrator complex (INT) is a 14 subunit group of proteins involved in transcriptional regulation. INT has been shown to be essential for the 3' end formation of both UsnRNA and eRNA. Biosynthesis of both of these RNAs is intimately dependent on the endonucleolytic function of the INT subunit 11(IntS11), which is a paralog of CPSF73 – the RNA nuclease required for cleavage and polyadenylation. More recently, others and we have shown that INT also associates with paused RNAPII at the 5' end of a diverse set of mRNA-encoding genes with an enrichment for those involved in immediate early response (IEG) pathways. While the IntS11 catalytic function as an endonuclease is defined in UsnRNA and eRNA production, the importance of IntS11 cleavage activity in regulating mRNA transcription is less understood.

Using a combination of molecular and biochemical approaches coupled with functional genomics, we show that the Integrator complex is recruited to the 5′ ends of many Drosophila protein-coding genes. The common feature of these genes is that they are expressed to very low levels normally despite having robust recruitment of RNAPII to their promoter region. Using RNA-seq, we find that depletion of IntS11 leads to de-attenuation of these INT-occupied genes resulting in their profound upregulation. PRO-seq analysis reveals that depletion of IntS11 results in significant release of RNAPII from the promoter proximal region. We find using triptolide, which inhibits initiation, that genes attenuated by Integrator have remarkably short RNAPII promoter half -lives reflecting a high degree of promoter-proximal termination. Importantly, we developed an RNAi-rescue strategy to re-express either wild-type IntS11 or an IntS11 mutant that cannot cleave RNA (E203Q) in S2 cells. Using this approach, we definitely have found that INT-mediated attenuation is completely dependent on IntS11 endonuclease function. Our work suggests a unifying and evolutionarily parsimonious model of Integrator function in which the Integrator complex is broadly used to drive RNAPII termination across the transcriptome and control nascent RNA fates.

219 tRNA transcription regulation in LPS-activated macrophages

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Macrophages are professional phagocytic cells of the innate immune system. They have many biological functions, such as antigen presentation, target cell cytotoxicity, phagocytosis and regulation of inflammation. Activation of macrophages with lipopolisacharydes (LPS), a major component of the outer membrane of most Gram-negative bacteria, induces rapid transcriptional changes and, within a few hours, transcription of several hundred genes is altered. LPS engage the Toll-like receptor 4 (TLR4) and induce various signalling pathways, including mitogen-activated protein kinases (MAPK), and activate several transcription factors such as nuclear factor (NF)-kappaB (NF-κB).

We have previously shown that treatment of macrophages with LPS induces RNA polymerase III (Pol III) activity and that NF-κB is involved in this process. However, inhibition of the NF-κB pathway only partially precludes Pol III activation upon LPS treatment, and this suggests that other signalling pathways are involved in the activation of Pol III in these conditions.

MAP kinases have been previously shown to act on Pol III mainly through modulation of the levels of TFIIIB transcription factor components. While our results show that MAP kinases are involved in Pol III upregulation upon LPS treatment, we have found that this is not a result of increased levels of Pol III machinery. Instead, we propose that MAP kinases act directly on the Pol III transcription apparatus or via chromatin modifications at tRNA genes.

Furthermore, LPS treatment activates the target of rapamycin (TOR) kinase, which is known to activate Pol III via phosphorylation and inactivation of its negative regulator, Maf1. Consistently, we observe Maf1 phosphorylation upon LPS treatment, and, therefore, we propose that this protein is also implicated in Pol III regulation in macrophages.

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220 Sequential regulation of RNases III and G expression largely contributes to H-NS-mediated pathogenicity of *Salmonella* Typhimurium

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Endoribonucleases play an important role in posttranscriptional regulation of gene expression in bacteria. Among them, RNase G (Rng) participates in degradation of a subset of mRNAs in *Escherichia coli*. However, its pathophysiological role remains largely uncharacterized. Here, we show that RNase G affects expression levels of H-NS, which were strongly associated with the pathogenicity of *S.* Typhimurium cells in host environments. Our results demonstrated that 5'-UTR of *hns* mRNA is directly cleaved by RNase G, leading to decreased stability of the mRNA. We further show that RNase III, the expression level of which is up-regulated in host environments, down regulates RNase G expression. We propose that RNase G and III-mediated modulation of *Salmonella* pathogenicity island 1 type III secretion system involves H-NS as a key factor in *S.* Typhimurium.

221 Expanding nucleotide recoding strategies to study RNA population dynamics: TimeLapse-seq with 6-thioguanosine

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RNA sequencing (RNA-seq) is a powerful tool to provide a snapshot of the transcriptome by measuring the relative abundance of RNA species in a biological sample, however any temporal information (e.g. rate of RNA transcription and decay) is lost. We and others have recently developed mutational mapping based strategies to bridge this shortcoming by building on extensive work on metabolic labeling of newly made RNA as well as chemical strategies of nucleotide recoding. We established TimeLapse-seq, where the hydrogen bonding pattern of s^4U-residues are recoded to the hydrogen bonding pattern of cytidine allowing T-to-C mutations to identify new transcripts transcriptome wide. So far TimeLapse-seq and related RNA-seq mapping strategies have been limited to the metabolic label 4-thiouridine (s^4U). Here we report the first use of nucleotide recoding under TimeLapse-seq conditions to a second metabolic label, 6-thioguanosine (s^6G). Under the same conditions established for s^4U, we are able to recode s^6G to a 2-aminoadenosine analogue using RNA-friendly oxidative nucleophilic-aromatic substitution leading to G-to-A mutations that identify new transcripts. We present the first application of s^6G recoding to reveal RNA population dynamics and discuss the opportunities a second recodeable nucleotide provides to study the dynamics of RNA metabolism.

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222 Understanding the fidelity mechanism of HIVRT using computer simulations

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The fidelity of DNA polymerase has been the subject of many studies. HIV reverse transcriptase (HIV RT), a member of the DNA polymerase family serves as a model system to study high-fidelity polymerase due to its extensive structural and kinetic characterization. We study the fidelity mechanism of HIVRT using all-atom molecular dynamics (MD) simulations and compare our findings with experiments. Long time scale dynamics of the processes are accessed by Milestoning method. Computing the kinetics and thermodynamics of the transitions between functional states along the polymerase pathway for a matching nucleotide in comparison to a mismatching nucleotide provides valuable insights into the fidelity mechanism. We showed that a large conformational change occurring on a millisecond timescale locks the correct nucleotide at the active site but promotes the release of a mismatched nucleotide. We extended our study to metal ion binding and product release steps. MD simulations explain why the catalytic Mg^{2+} binds only to the closed state and not to the open state, an observation consistent with crystal structures. The initial binding of Mg^{2+} to the catalytic site is mediated by water molecules in the open state. Further tightening of Mg^{2+} occurs at the closed state. Protonation of D185 at the closed state triggers the release of catalytic Mg^{2+} from the site. This poses an explanation for the rapid exchange rates observed in stopped-flow kinetic measurements. Simulations show no evidence for a third metal ion in correct nucleotide incorporation. In contrast, the poor alignment of mismatching nucleotide attracts an additional Mg^{2+} ion. A third metal ion is also observed after chemistry for the mismatching addition. Our simulations allowed predicting the rate of product release for a match and a mismatching nucleotide. The difference in the electrostatic environments between the matching and mismatching nucleotide results differences in product release rates. The product release found to be slower in a mismatching nucleotide while the process is rapid in the synthesis of matching nucleotide. Our results show excellent agreement with the stopped-flow kinetic measurements on product release providing a physics-based explanation to the differences in product release rates in the mechanism of HIVRT.

223 Structural snapshots of actively transcribing influenza polymerase

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Influenza virus RNA-dependent RNA polymerase uses unique mechanisms to transcribe its single-stranded genomic vRNA into mRNA. The polymerase is initially bound to a promoter comprising the partially base-paired 3′ and 5′ extremities of the vRNA. A short, capped primer, ′cap-snatched′ from a nascent host polymerase II transcript, is directed towards the polymerase active site to initiate RNA synthesis. Here we present structural snapshots, determined by X-ray crystallography and cryo-electron microscopy, of actively initiating influenza polymerase as it transitions towards processive elongation. Unexpected conformational changes unblock the active site cavity to allow establishment of a nine base-pair template-product RNA duplex before the strands separate into distinct exit channels. Concomitantly, as the template translocates, the promoter base-pairs are broken and the template entry region is remodelled. These structures reveal new details of the influenza polymerase active site that will aid optimisation of nucleoside analogue or other compounds that directly inhibit viral RNA synthesis.

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224 The C-terminal domain of RNA Polymerase II contacts nascent transcripts

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The large subunit of RNA polymerase II (RNAPII) has two major domains; a catalytic N-terminal domain (NTD) and regulatory C-terminal domain (CTD). The CTD is comprised of multiple copies of a heptad repeat (26 in yeast) that forms a binding platform for the association of transcription factors, RNA processing and packing factors with the transcribing polymerase. Recent analyses have shown that the CTD can form a phase separated region that clusters pre-initiation and initiating RNAPII.

Here we have separately mapped the locations of the NTD and CTD regions RNAPII by UV crosslinking to the nascent transcripts. To achieve this a protease cleavage site was introduced between the NTD and CTD. The intact protein was crosslinked in actively growing cells and purified RNAPII was cleaved in vitro. The NTD or CTD regions were recovered separately and associated RNA fragments were identified by sequencing.

The 5' regions genes were depleted in CTD interactions, perhaps reflecting CTD sequestration. Relative enrichment of the CTD was seen at 3' splice sites, and at spliced exon-exon junctions. Strong enrichment for CTD binding was also seen upstream of mRNA cleavage and polyadenylation sites.

Applying machine learning and hidden Markoff modeling identified two discrete CTD phosphorylation states that specifically corelated with CTD-RNA association in introns and p(A) proximal regions.

Mapping the precise crosslinking sites in the CTD by mass-spectrometry revealed a preference for RNA crosslinking adjacent to phosphorylated heptads, particularly with Ser2 and Ser7 phosphorylation.

We conclude that the CTD of RNAPII contacts the nascent transcript and speculate that this facilitates binding and/or off-loading by CTD-bound RNA processing factors.

225 The *Drosophila* 7SK snRNP complex is specifically required for synaptic growth and function of motoneurons.

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The 7SK snRNP is a ribonucleoprotein complex composed of the abundant non-coding nuclear RNA 7SK, the RNA binding proteins Methylphosphate Capping Enzyme (MePCE), La-related protein 7 (LARP7) and Hexamethylene bis-acetamide-inducible (HEXIM). In higher eukaryotes, the 7SK complex plays a major role in preventing premature entry of paused Pol II into the elongation phase by sequestering the positive transcription elongation factor (P-TEFb). Intriguingly, despite this general function, larp7 loss of function in human is viable but produces several defects including restricted growth and intellectual disability, also known as the Alazami syndrome. Currently, it is unclear how the loss of function of the 7SK complex gives rise to this specific syndrome. The aim of my PhD was to dissect the function of this complex in development. I used the fly model to generate mutants of several 7SK complex subunits using CRISPR/Cas9. I find that the knockout of LARP7 or the 7SK RNA in *Drosophila* does not affect viability but alters fly locomotion. Consistently, alteration of the 7SK complex specifically reduces axonal growth at neuromuscular junctions (NMJ). Using CRISPR/Cas9 mediated eGFP knock-in, I demonstrate that LARP7 is enriched in a few subtypes of motoneurons and acts autonomously in these cells to promote axonal growth. Interestingly, decreasing the level of the P-TEFb complex partially restores axonal growth and locomotion indicating that the 7SK complex regulates growth via a transcriptional function. Transcriptomic analysis of mutant motoneurons revealed that the 7SK complex regulates genes containing high GC content at the promoter as well as long introns. Electrophysiology experiments by Duch lab in these mutants showed interesting functional changes in the motoneurons. Altogether, our work adds insights into the specificity of the 7SK snRNP complex in promoting specific functions in multicellular organisms

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226 Depletion of Abundant RNAs in Eukaryote, Blood Samples, and Bacteria Increases Sensitivity of Next Generation Sequencing-Based Transcriptome Profiling.

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A challenge in whole-transcriptome sequencing is the large dynamic range of transcript expression within a total RNA sample. Highly expressed transcripts with minimal biological interest can dominate readouts, masking detection of more informative lower abundant transcripts. Here, we present a method to enrich for RNAs of interest by eliminating abundant, typically unwanted, RNAs before sequencing. This method is based on hybridization of probes to the target RNA and subsequent enzymatic degradation of the bound RNAs and the probes.

We optimized this method to remove abundant RNAs (such as ribosomal RNA) from human, mouse, rat, and bacterial total RNA samples. We also expanded the probe design to remove adult, fetal and embryonic hemoglobin transcripts from multiple derivate blood samples for which globin can constitute up to 70% of total mRNA transcripts.

Using strand-specific RNA sequencing we measured depletion efficiency, library complexity, transcript coverage and transcript expression before and after depletion across a wide range of samples and RNA input amounts. We achieved high depletion efficiency (up to 99.9 % rRNA depletion) with minimal off target effects. We detect high number of transcripts, with even coverage across the transcript length and retention of transcript complexity even at the lowest inputs. The method works efficiently in low input and highly degraded total RNA including that from formalin-fixed-paraffin-embedded (FFPE) samples.

We conclude that the reduction of abundant transcripts for RNA-Seq studies significantly increases the ability to detect true biological variations that could not be detected in non-depleted samples. The method described here is a reliable and simple solution that greatly improves sensitivity in transcriptome RNA-Seq studies. Furthermore, it is amenable to high throughput sample preparation and robotic automation for easy implementation in a clinical setting.

227 CDK11 binds replication dependent histones mRNAs and regulates their expression.

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Expression of canonical, replication-dependent histones (RDH) is highly regulated during the cell cycle echoing their main role during cell division and epigenetic inheritance. RDH genes produce the only non-polyadenylated transcripts and for their correct expression recruit a battery of alternative 3' end processing factors. Exploiting metaplots, positional heat maps and computational methods, we decipher CDK11 binding along RDH mRNA and DNA identifying it as key player in the molecular regulation of RDH biogenesis.

Applying ChIP-seq, we described CDK11 binding to RDH gene body that triggers RNAPII C-terminal repeat domain phosphorylation on the serine2 (Ser2) residues. This Ser2 phosphorylation is required for RNAPII to continue transcription elongation on RDH genes. Using nucleotide resolution crosslinking immunoprecipitation (iCLIP), we identified CDK11 binding along RDH transcripts with accumulation upstream of stem loop, an RNA structure formed at the 3' end of RDH transcripts. This RNA-CDK11 interaction is complementary to the DNA-CDK11 interaction on the RDH and both are required for the correct expression of RDH. Thus, CDK11 knockdown exhibits a strong decrease in RDH expression measured by RNA-seq and a small proportion of unprocessed RNAs read-through after the stem-loop. CDK11 stem loop binding is required to recruit RDH processing factors that will cleave the transcripts just downstream of the stem loop. Therefore in the absence of CDK11, this process becomes deficient.

CDK11 is an essential gene for growth of several cancers and is of potential clinical importance as a druggable player to improve cancer prognosis by yet unknown molecular mechanism.

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228 Marchantia polymorpha *SPL* gene family members as an archetype of plant specific transcription factors family controlling the timing of developmental phase transition

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The *SPL* genes play crucial roles during the key stages of plant development in the representatives of simple forms like mosses and much more complex angiosperms. Their essential role is to control the transition from the juvenile to adult phase and from the vegetative to reproductive phase. At present there are no published data about the function of the SPL transcription factors from liverworts, one of the oldest living land plants. Interestingly, in our studies concerning the identification of genes involved in the development of sex-organs in the dioecious liverwort *Pellia endiviifolia* we have found, that one member of Pellia *SPL* gene family is female-specifically expressed, moreover its expression is additionally correlated with archegonia development. Lack of molecular tools for *P.endiviifolia* genetic manipulation prompted us to undertake detailed functional studies on SPL genes from the model liverwort, *Marchantia polymorpha*. In comparison to the *SPL* gene families from other plants studied to date, where the number of genes is higher than 10, the Marchantia genome encodes only four *SPL* genes. What is more, a large number of SPL-family members is regulated by microRNA: miR156 and, in some species, miR529. Also in Marchantia the two representatives, Mp*SPL1* and Mp*SPL2*, are posttranscriptionally regulated by two miRNA molecules. These data suggest that from all studied to date land plants, *M.polymorpha* posses the simplest set of SPL transcription factors which on the plant SPL phylogenetic tree resembles the major groups recognized in other plant species. Therefore we hypothesize, that the liverwort *SPL* gene family may reflect an ancient archetype which in the course of evolution could give rise to all other *SPL* gene families of vascular plants. The functional analyses of *SPL* genes from liverwort *M.polymorpha* will foster substantial insights into the genetic basis for variation in plant life history traits including body architecture and phase change evolution.

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229 PHF3 binds RNA polymerase II via the SPOC domain and regulates neuronal gene transcription

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Transcription is tightly coordinated by different regulators that associate with RNA polymerase II (Pol II). Heptapeptide repeats within the C-terminal domain (CTD) of the largest subunit of Pol II are differentially phosphorylated during transcription to govern dynamic binding of regulatory factors. Here, we identify the SPOC domain within the human PHD-finger protein 3 (PHF3) as a new Pol II CTD-binding domain and show by X-ray structure analysis that two positively charged patches on the SPOC surface anchor phospho-groups on Serine-2 from adjacent CTD repeats. We demonstrate that PHF3 tracks with Pol II across the length of genes in human cells. PHF3 and its SPOC domain repress the transcription of a subset of target genes, particularly neuronal genes. PHF3 knock-out in mouse embryonic stem cells impairs neuronal differentiation due to untimely derepression of key neuronal genes, including the transcription factors Ascl1, Pouf32, and Sox2. Our study establishes the SPOC domain as a new reader of the Pol II CTD, and PHF3 as a regulator of neural gene expression that directly binds to the Pol II CTD via its SPOC domain.

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230 Nuclear proximity drives gene expression covariances in single mammalian cells to enable translational co-folding

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Studies into gene co-expression patterns in single cells could yield important new regulatory and functional insights, yet such studies have until now been limited by the confounding effects of cell differentiation and the cell cycle. Here, we apply a tailored experimental design that eliminates these confounders by generating a highly homogeneous non-dynamic cell population of over 350 individual cells. Surprisingly, highly quantitative single-cell sequencing of this population using Smart-seq2 yields a statistically robust gene expression covariance network with more than 80,000 significantly covarying gene pairs, exceeding stochastic gene expression, and outlining known and novel gene interaction. Using a covariance enrichment approach we provide the first evidence that miRNAs induce transcriptome-wide covariances, and compare the relative importance of nuclear organization, transcriptional and post-transcriptional regulation in defining gene expression covariances. We find that nuclear organization has the greatest impact, and that genes encoding physically interacting proteins specifically tend to covary, lending evidence to the importance of co-translational folding. Finally, we show that we can successfully predict gene function and regulation, as well as linkage between individual regulators and functions using gene covariance information. In summary, we give a proof-of-principle that single-cell sequencing can be applied to a highly homogeneous cell population to simultaneously study upstream gene regulation and the downstream functional implications of that regulation.

231 RNA polymerase I elongation is determined by the structure of the nascent pre-rRNA and torsion in the rDNA

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The basic mechanism of RNA polymerase (RNAP) transcription is conserved across all domains of life, but functional and structural differences have evolved to provide regulatory advantages. Analyses of RNAPII distribution by high-resolution methods, including ChIP-exo, NET-seq and CRAC, have revealed notably uneven polymerase distribution across transcription units. These complex patterns are very difficult to interpret due to high complexity of sources of variation in signals. In this work, we used a relatively simple system, RNAPI, to understand basic features of eukaryotic transcription.

RNAPI transcribes the pre-rRNA from a single, multicopy, nucleosome-free locus. Due to the high abundance of mature rRNAs, stringent purification is needed to reliably identify association with the nascent transcripts in genome-wide analyses, therefore we used CRAC protocol (UV cross-linking and analysis of cDNA). Mapping of RNAPI at nucleotide resolution using CRAC revealed a 5' bias for RNAPI along the transcription unit accompanied by a striking uneven pattern of local polymerase occupancy with an apparently regular distribution of peaks and troughs.

We identified two features of nascent transcript that were correlated with the profile of RNAPI: G+C-content with the transcription bubble and folding of nascent RNA immediately adjacent to the polymerase. High G+C-content of RNA:DNA hybrid within the transcription bubble correlated with slower transcription elongation. Strong structure in the nascent RNA correlated with faster transcription elongation. We envisaged that this functioned by limiting RNAPI backtracking, and confirmed this effect by *in vitro* transcription assays. Combing the effects of G+C-content and folding of nascent RNA improved the fit of sequence features to RNAPI density, but did not explain the 5' bias. To address this, we developed a mathematical model for RNAPI that combined the effects of DNA torsion under transcriptional stress with folding energy. This showed how stochastic RNAPI loading can give rise to the regular RNAPI spacing seen in Miller chromatin spreads, and could largely reconstitute our experimental data.

This work has established links between *in vitro* measurements and the output of *in vivo* experiments of RNAPI profiling. Understanding of this process is an important first step to modeling the more complex system of RNAPII transcription.

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232 The Integrator complex cleaves nascent mRNAs to attenuate transcription

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The transition of RNA polymerase II (RNAPII) from initiation to productive elongation is regulated at many metazoan genes. Once RNAPII has transcribed a 20-60 nucleotide (nt) long RNA, it can stably pause, awaiting signals for release into the gene body. Alternatively, RNAPII can turn over via promoter-proximal termination mechanisms that remain poorly characterized. Here, we used genome-scale RNAi screening, transcriptomics, and focused mechanistic studies to reveal that the Integrator complex attenuates transcription of many *Drosophila*genes, including the inducible Metallothionein A (MtnA) gene. The RNA endonuclease activity of Integrator has a well-established role in generating the 3′ends of uridine-rich small nuclear RNAs (snRNAs) and we show that Integrator is analogously recruited to the 5′ ends of protein-coding genes where it cleaves nascent mRNAs to trigger transcription termination. However, unlike at snRNA gene loci, Integrator-mediated termination potently represses protein-coding gene expression, while still enabling RNAPII engagement of promoters and future gene activation. In total, this work reveals that Integrator broadly catalyzes RNAPII termination across the transcriptome, thereby suggesting a unifying and evolutionarily parsimonious model of Integrator function.

233 Dynamics of transcription factor binding to DNA

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Pioneer transcription factors have the ability to bind DNA even when it is wrapped around histones. This allows them to act at the very beginning of a cascade of events that leads to the recruitment of further factors and eventually changes the gene expression program of the whole cell. This is important to maintain cell identity, execute differentiation programs, and reprogram cells into a pluripotent state.

The interaction between transcription factors and their DNA target sequence has been well understood on a molecular level, owing to the availability of high resolution structures. Despite this, ChIP-Seq data demonstrates that the majority of DNA recognition sites are not occupied in the genome. This discrepancy between *in vitro* and *in vivo* data is not well understood and our knowledge about the binding site selection process remains very limited. While DNA binding assays typically utilise DNA of up to 30 bp, the human genome consists of 3 billion bp. Assays using short DNA fragments give only little information about the dynamics of the binding process, however, reconstituting the *in vivo* situation in a test tube is challenging.

Here, we use a binding assay that utilises long 30 kbp stretches of DNA which are immobilised on both ends by optical tweezers. With this method, we can examine the dynamics of DNA binding and cooperative behaviour of purified, fluorescently labelled pioneer factors such as human Klf4 or Drosophila Zelda. The use of larger DNA more resembles the situation inside the nucleus and can give unique insight into the binding site selection process which cannot be achieved with traditional methods. As a result, we will be able to create a model of how pioneer factors can recognise the correct binding site inside the human genome to initiate gene activation.

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234 Transcription factors regulating activities of transcriptional and translational apparatus during growth transition in *Escherichia coli*

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In the process of *Escherichia coli* K-12 growth from exponential phase to stationary, marked alteration takes place in the pattern of overall genome expression through modulation of both parts of the transcriptional and translational apparatus. In transcription, the sigma subunit with promoter recognition properties is replaced from the growth-related factor RpoD by the stationary-phase-specific factor RpoS. The unused RpoD is stored by binding with the anti-sigma factor Rsd. In translation, the functional 70S ribosome is converted to inactive 100S dimers through binding with the ribosome modulation factor (RMF). Up to the present time, the regulatory mechanisms of expression of these two critical proteins, Rsd and RMF, have remained totally unsolved. In this study, attempts were made to identify the whole set of transcription factors involved in transcription regulation of the *rsd* and *rmf* genes using the newly developed promoter-specific transcription factor (PS-TF) screening system. In the first screening, 74 candidate TFs with binding activity to both of the *rsd* and *rmf* promoters were selected from a total of 194 purified TFs. After 6 cycles of screening, we selected 5 stress response TFs, ArcA, McbR, RcdA, SdiA, and SlyA, for detailed analysis *in vitro* and *in vivo* of their regulatory roles. Results indicated that both *rsd* and *rmf* promoters are repressed by ArcA and activated by McbR, RcdA, SdiA, and SlyA. We propose the involvement of a number of TFs in simultaneous and coordinated regulation of the transcriptional and translational apparatus. By using genomic SELEX (gSELEX) screening, each of the five TFs was found to regulate not only the *rsd* and *rmf* genes but also a variety of genes for growth and survival.

235 Prebiotic peptide bond formation by the proto-ribosome: a missing link between RNA and protein dominated world

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High resolution structures of ribosomes from various organisms determined by us and elsewhere, reveal that the peptidyl transferase center (PTC) is located in the core of a pseudo symmetrical rRNA sub-region, within the otherwise asymmetrical particle. The nucleotide sequence of this region is highly conserved among all domains of life, hinting that its key role was maintained throughout evolution. Hence, it may imply that it is a remnant of an early origin of life entity and its pseudo 2-fold symmetry may imply a dimeric origin. These characteristics, in addition to the findings that RNA can create itself and can possess catalytic activities led to the "proto-ribosome" concept. Namely that this entity represents the origin of the ribosome, and perhaps also the origin of life, which existed in the RNA dominated world and is the missing link to the protein dominated contemporary world.

During contemporary protein synthesis, peptide bond formation is performed in the large ribosomal subunit (LSU) at the PTC, which consists solely of ribosomal RNA(rRNA). The PTC hosts the 3' tRNA ends of both the A-tRNA and the P-tRNA, at proper stereochemistry that provides the geometrical requirements for substrate assisted catalysis. Isolated LSU can catalyze peptide bond formation in vitro without the presence of the small ribosomal subunit (SSU). Moreover, isolated LSU can catalyze peptide bond formation of the fragment reaction, that consists of only the 3'ends of the tRNA molecules, namely the amino acylated CCA and puromycin, a 3'end tRNA analog. This reaction is routinely used to assay the peptidyl transferase activity of the entire ribosome as well as of the LSU.

The proto-ribosome hypothesis states that there was an RNA entity, capable of self-folding, dimerizing, accommodating substrates, catalyzing chemical reactions like peptide bond formation and releasing the product. Thus, we first designed several constructs resembling this conserved region and tested their ability to dimerize. Some of them were found to homo dimerize and we monitored their ability of forming peptide bond. On this way we achieved few constructs that could perform the peptide bond reaction. The formation of the peptide bond was monitored using MALDI.

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236 Post-synthetic approach to the preparation of 17-merRNA fragments (Sc ASL tRNA Glu) containing mcm^{5}U / ncm^{5}U/ cm^{5}U and labeled with Cy5 at the 5'-end

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Over the last decade, significant progress has been made towards an understanding of biosynthesis pathways of different modified/hypermodified nucleosides located in tRNA molecules, especially often in the anticodon arm domain.[1] Of particular interest is the recent discovery that the Elongator complex catalyzes the post-transcriptional modification of tRNA by attaching the carboxymethyl function (cm^5) to the base of uridine in the tRNA wobble position to obtain cm^{5}U-modified unit.[2] For the further study of the pathway leading to the installation of the final mcm^{5}U/mcm^{5}s^{2}U tRNA modifications, chemically synthesized appropriately modified oligoribonucleotides may serve as useful models.

Herein, we present our results on the chemical preparation of a set of modified 17-mer RNA fragments with the sequence of anticodon stem and loop of *Saccharomyces* tRNA^{Glu} arm (containing mcm^{5}U/ncm^{5}U/cm^{5}U units at the site related to 34 tRNA wobble position) by the use of post-synthetic transformation of the precursor oligoribonucleotide bearing mcm^{5}U modification and additionally labeled with fluorescent Cy5 marker at the 5'-end.

The precursor 17-mer RNA, containing 5-methoxycarbonylmethyluridine (mcm^{5}U-RNA) and Cy5, was synthesized manually according to the modified protocol of the solid phase phosphoramidite method of oligoribonucleotide synthesis. Subsequently, various conditions for post-synthetic deprotection of the mcm^{5}U-RNA allowed to preserve the ester function of modification (mcm^{5}U-RNA) or convert the modified unit into an amide or carboxylic acid, leading to ncm^{5}U-RNA and cm^{5}U-RNA. Fully deprotected RNA samples were purified by RP-HPLC and IE-HPLC and analyzed by MALDI-TOF and ESI mass spectrometry.

References:

1. M. Helm, J.D. Alfonzo, Chem Biol., 2014 21(2),174;
2. Ting-Yu Lin, N.H. Abbassi, et al. Nature Commun., 2019, 10, 625;

237 Synthetic Anti-CRISPR Nucleic Acid Inhibitors of CRISPR-Cas9

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Clustered regularly interspaced short palindromic repeat (CRISPR) RNAs and their associated effector (Cas) enzymes are facile research tools and promising candidates for drug development. However, CRISPR-Cas enzymes can produce unwanted 'off-target' gene editing. For therapeutic use, editing at unintended targets or in non-target tissues could lead to dangerous side-effects. Drug-like molecules that can inactivate CRISPR-Cas enzymes could help facilitate safer therapeutic development. Based on the requirement of Cas proteins to bind guide RNA cofactors and target DNA substrates, we rationally designed synthetic nucleic acid-based inhibitors of *Streptococcus pyogenes* (*Sp*) Cas9. Initial designs targeting the CRISPR RNA guide sequence (anti-guide), repeat sequence (anti-tracr), or protospacer adjacent motif (PAM)-interaction domain (anti-PAM) of *Sp*Cas9 only bound with moderate affinity. However, coupling anti-PAM and anti-tracr modules together was synergistic and resulted in low nanomolar binding affinity rivaling that of natural anti-CRISPR proteins. These inhibitors efficiently inhibited Cas9 DNA cleavage activity. Incorporating chemically-modified nucleotides that enhance RNA hybridization resulted in greater inhibition and dose-dependent suppression of gene editing in human cells. These novel nucleic acids provide a platform for rational design of CRISPR-Cas enzyme inhibitors that should translate to other CRISPR effector enzymes and enable better control over CRISPR-based applications.

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238 Translationally Active mRNAs Suitable for Fluorescent Labeling in Living Cells

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The 7-methylguanosine (m7G) cap structure is a unique feature present at the 5' ends of messenger RNAs (mRNAs), and it can be subjected to extensive modifications, resulting in alterations to mRNA properties (e.g. translatability, susceptibility to degradation). It also can provide molecular tools to study mRNA metabolism. We developed new mRNA 5' cap analogues that enable the site-specific labeling of RNA at the 5' end using strain-promoted azide-alkyne cycloaddition (SPAAC) without disrupting the basic function of mRNA in protein biosynthesis. Some of these azide-functionalized compounds are equipped with additional modifications to augment mRNA properties. The application of these tools was demonstrated by labeling translationally active mRNAs in living cells.

239 Development of Capturing Technique for 5′-Diphosphate RNA

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In eukaryotes, the 5' end of mRNA is protected by 7-methylguanosine triphosphate (m^7G) cap. Beside its protective role against RNA degradation, the m^7G cap is recognised by numerous biomolecules, e.g. by the translation initiation factor [1]. Recently, it was found that also bacteria possess capped RNA. The nicotinamide adenine dinucleotide (NAD) was found to be attached to 5' end of some regulatory small RNAs (sRNA) and sRNA-like 5'-terminal fragments of certain mRNAs [2]. Since also coenzyme A was found to be covalently attached to some RNAs in prokaryotes, the possibility that there are many other forms of 5' end of RNA with specific role, is very high.

Recently, the 5' diphosphate termini of RNA from reoviruses was identified as RIG-I-like receptor agonist [3]. The RIG-I-like receptors (retinoic acid-inducible gene I) are mammalian cells mechanisms to detect and defend themselves from invading viruses. The aim of this work is to develop a methodology for capturing 5'-diphosphate capped RNA. The bimetallic chelates known for their affinity towards pyrophosphorylated peptides [4] might be used also for capturing 5'-diphosphorylated RNA. In this work, we performed the synthesis of a zinc chelate and studied its affinity towards RNA by selecttive capture of 5'-diphosphate capped RNA confirmed through radioactive 2D-electrophoresis on a polyacrylamide gel. Such a method will allow us to develop new sensors for certain viral infections.

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References:

1. Rhoads, R. E. *J. Biol. Chem.* **2009**, *284*, 16711-16715.
2. Cahová, H.; Winz, M.-L.; Höfer, K.; Nübel, G.; Jäschke, A. *Nature*. **2015**, *519*, 374-377.
3. Goubau, D; Schlee, M. et al. *Nature*, **2014**, *514*, 372-375.
4. Conway, J. H.; Fiedler, D. *Angew. Chem. Int. Ed*. **2015**, *54*, 3941-3945.

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240 Sequence selective recognition of double-stranded RNA by cationic nucleobase and backbone-modified peptide nucleic acids

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The important roles that non-coding double-stranded RNAs (dsRNA) play in biology and development of disease makes them attractive targets for molecular recognition. However, designing of small molecules that selectively recognize RNA has been a challenging and involved process because RNA helix presents little opportunity for shape-selective recognition. We discovered that cationic nucleobase- and backbone-modified peptide nucleic acids (PNA) bind with high sequence selectivity and low nanomolar affinity to dsRNA via major groove triple helix formation under physiological conditions. Most interestingly, the modified PNAs exhibited unique RNA selectivity and had up to two orders of magnitude higher affinity for the dsRNAs than for the same dsDNA sequences. Recent studies showed that the deep and narrow major groove of RNA presented a better steric fit and hydrogen bonding arrangements for the PNA ligands than the wider major groove of DNA. Conjugation of PNA with short lysine peptides further enhanced binding affinity and cellular uptake of PNA. PNAs carrying M and Lys modifications were efficiently taken up by cells, while the unmodified PNA showed little uptake.

Recently, we found that nucleobase- and backbone-modified PNAs recognized dsRNA sequences present in biologically relevant RNA, such as mRNAs and microRNAs, with high affinity under physiological conditions. In collaboration with Prof. Naoki Sugimoto's group at FIBER, Kobe, Japan, we demonstrated that triplex-forming PNA suppressed translation by forming a highly stable and sequence selective triple helix with a dsRNA region in the 5´UTR of long mRNA both in vitro and in cells. Triple helix formation was also able to inhibit maturation of pre-microRNA hairpins. Taken together our results suggest that the cationic modified PNAs may be promising compounds for modulating the function of biologically relevant dsRNA in live cells. Given the infancy of our understanding of the various roles that RNA plays in gene regulation, it is conceivable that triplex-forming PNAs may become valuable tools for studying biologically relevant dsRNA in live cells. The current presentation will discuss our most recent results on sequence selective recognition of complex biological dsRNA molecules and potential applications of this recognition in biomedical research and biotechnology.

241 The comparison of the contributions of *E. coli* ProQ and FinO proteins to the annealing of FinP sRNA to 5' -UTR of *traJ* mRNA

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Small RNAs (sRNAs) participate in bacterial adaptation to environmental changes and in the control of cellular homeostasis by regulating the translation or stability of selected mRNAs. To perform this function, sRNAs bind fully or partly complementary sequences in regulated mRNAs, in which they are often assisted by RNA-binding proteins. One such protein is FinO, which is encoded on F-like plasmids as part of FinP/FinO fertility inhibition system. FinP is an antisense RNA complementary to the 5'UTR of traJ mRNA. It requires FinO protein for protection from degradation by RNase E, so that FinP could base pair with RNA target, ultimately leading to occlusion of ribosome binding site and repression of traJ translation. Another such protein is chromosome-encoded ProQ protein, which N-terminal domain displays structural homology with FinO. Recently it has been shown that ProQ interacts with numerous regulatory RNAs in E. coli and S. enterica.

In this study, we compared the binding of FinO and ProQ proteins to FinP sRNA and 5'-UTR of traJ mRNA to better understand how these proteins are involved in the interactions between sRNAs and complementary mRNAs. The free RNAs and their complexes were separated using electrophoretic mobility shift assay under non-denaturing conditions. We analyzed the annealing of FinP sRNA to traJ mRNA in the absence or presence of either ProQ or FinO. The results suggest that both FinO and ProQ facilitate the base pairing of these RNA molecules. In future studies we plan to compare the role of FinO and ProQ proteins in the pairing of other known sRNAs and their mRNA targets. We expect that the results of these studies will help to better understand the differences in RNA binding and matchmaking properties of these proteins, which may help explain their functions in E.coli cells.

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242 Exploration of RNA sequences in pre-miRNA affecting the efficiency of Dicer cleavage reactions by the small molecules binding

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Non-coding RNAs have been involved in development, differentiation, and many other biological processes, and their diverse roles provide opportunities targeting and modulating the functions with small molecules. Our laboratory has been developing small molecules that bind to specific structures of DNA and RNA and applying them as tools to regulate their functions. However, designing small molecules targeting particular RNA structures remains a challenge, partly due to limited understanding in the small molecule-RNA interactions. The improved knowledge of the small molecule-RNA interactions should advance the new design of small molecules targeting RNA.

MicroRNA (miRNA) is one of the functional non-coding RNAs, which is produced from the precursor (pre-miRNA) by enzyme Dicer. The miRNAs are loaded onto AGO2 to form an RNA-induced silencing complex (RISC) and suppress translation of target mRNAs. Therefore, small molecules binding to a specific pre-miRNA and affecting its cleavage by Dicer can be tools to modulate the expression of the target proteins. In our previous work, we demonstrated that our designed small molecules bind to pre-miRNAs at their target sequences and inhibit the cleavage by Dicer.

In this study, we explored RNA sequences in pre-miRNA affecting the efficiency of Dicer cleavage reactions upon binding of small molecule. We synthesized a pre-miR136 mutant library with six randomized nucleotide bases at the stem region and used them as substrates for the Dicer cleavage reaction. The reaction products obtained in the presence or absence of our small molecules were sequenced using a next generation sequencer (NGS) to examine how the cleavage reactions of 4096 sequences in the library were affected. From the sequencing results, we found that one of our ligands NCD (Naphthyridine Carbamate Dimer) altered the cleavage efficiency of some pre-miR-136 mutants by Dicer.

243 Towards unravelling the secrets of thio-effect in messenger RNA cap

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The 5′ end of eukaryotic mRNAs is co-transcriptionally modified by attachment of a positively charged nucleoside - 7-methylguanosine - connected to the first transcribed nucleotide through a 5′,5′-triphosphate bridge (m^7GpppNpN...). The structure, called 5′ cap, protects transcript from premature degradation by exonucleases and is specifically recognized by several proteins and enzymes involved in regulation of cellular processes related to gene expression, including mRNA maturation, nuclear export, initiation of translation, and mRNA turnover. Chemical modification of cap has provided access to messenger RNAs with increased cellular stability and superior translational properties that could benefit the mRNA-based therapies. The prime examples are β-phosphorothioate derivatives of dinucleotide cap structure, particularly β-S-ARCA, that could serve as primers for in vitro transcription. Despite the proven potential of β-S-ARCA-capped transcripts in augmenting mRNA expression in vivo (phase I of clinical trials for melanoma and breast cancer vaccination completed), the molecular basis for the beneficial 'thio-effect' remained unclear.

Here, we used X-ray crystallography, NMR spectroscopy, and microscale thermophoresis (MST) to investigate the consequences of the O-to-S substitution within cap for its interaction with translation initiation factor 4E. Using closely related β-selenophosphate analogs and anomalous X-Ray scattering we unambiguously assigned absolute configuration of asymmetric phosphorus atom in both diastereomers. An insight from 11 original X-Ray structures of eIF4E in complexes with β-modified cap analogs allowed us to identify the contacts between β-sulfur atom and positively charged amino acids as key interactions crucial for complex stabilization and determinant of ligand conformation. We observed two distinct alignment of cap's triphosphate bridge that correlate with β-phosphorus stereochemistry. One of them was observed earlier for dinucleotides (m^7GpppA/m^7GpppG) in crystal structures and now confirmed by us for trinucleotide m^7GpppApG using X-Ray crystallography and NMR chemical shift perturbation mapping, while the other one - characteristic for (S_p)-β-S isomers - has never been observed in mammalian eIF4E complexes. Finally, thermodynamic characterization of the complexes by MST verified that the effect observed for di- and trinucleotides is also relevant for longer mRNA containing β-phosphorothioate within cap.

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244 Fast and unbiased purification of RNA-protein complexes after UV cross-linking

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Post-transcriptional regulation of gene expression in cells is facilitated by formation of RNA-protein complexes (RNPs). While many methods to study eukaryotic (m)RNPs rely on purification of poly-adenylated RNA, other important regulatory RNA classes or bacterial mRNA could not be investigated at the same depth. To overcome this limitation, we developed PTex[1] (Phenol Toluol extraction), a novel and unbiased method for the purification of UV cross-linked RNPs in living cells.

PTex is a fast (3-4 hrs) low-tech protocol: applying biphasic organic extractions, its purification principle is solely based on physicochemical properties of cross-linked RNPs and thus does not require a distinct nucleic acid sequence, tag or antibody. This enables us now for the first time to interrogate RNA-protein interactions system-wide and beyond poly(A)-RNA from a variety of species and source material. We will present high-throughput screens for RNA-binding proteins from human HEK293 cells and bacteria. Next, we will demonstrate how PTex can be used to purify individual RBPs from mouse tissue or cell culture and how PTex can simplify existing complex workflows such as CLIP-Seq.

[1]Erika C Urdaneta et al. Purification of cross-linked RNA-protein complexes by phenol-toluol extraction. *Nature Communications*, 10(1):990, Mar 2019

245 Revealing genome-wide RNA structure-function relationships *in vivo*

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RNA structure is intimately connected to RNA function. The structure of RNA can differ radically *in vitro* versus *in vivo* owing to the physico-chemical environment including temperature, ionic conditions, and growth conditions. We developed the Structure-seq method that combines chemical probing of RNA structure with high-throughput sequencing, thus allowing characterization of RNA secondary structure genome-wide and *in vivo*. Our application of Structure-seq to the model plant Arabidopsis revealed significant correlations between DMS reactivity, transcript processing, and gene function [1]. Currently, we are applying Structure-seq to rice seedlings and bacteria to assess genome-wide changes in RNA structure caused by various stressors including temperature and nutrient starvation [2] and thereby evaluate the hypothesis that structure changes play significant roles in post-transcriptional regulation of gene expression. We are also developing additional chemical probes to query the base-pairing status of all four nucleotides *in vivo* [3, 4]. I will present our latest findings on these topics.

1. Ding, Y., Tang, Y., Kwok, C. K., Zhang, Y., Bevilacqua, P. C. & Assmann, S. M. (2014). *In vivo* genome-wide profiling of RNA secondary structure reveals novel regulatory features. *Nature* **505**, 696-700.

2. Su, Z., Tang, Y., Ritchey, L. E., Tack, D. C., Zhu, M., Bevilacqua, P. C. & Assmann, S. M. (2018). Genome-wide RNA structurome reprogramming by acute heat shock globally regulates mRNA abundance. *Proc Natl Acad Sci* **115**, 12170-12175.

3. Mitchell, D., 3rd, Ritchey, L. E., Park, H., Babitzke, P., Assmann, S. M. & Bevilacqua, P. C. (2018). Glyoxals as *in vivo* RNA structural probes of guanine base-pairing. *RNA* **24**, 114-124.

4. Mitchell, D., Renda, A. J., Douds, C. A., Babitzke, P., Assmann, S. M. & Bevilacqua, P. C. (2019). In vivo RNA structural probing of uracil and guanine base-pairing by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). *RNA* **25**, 147-157.

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246 Exploring the diversity of stable Lentiviral Producer Cell Pools by Single Cell RNA expression analysis

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Stable cell lines for the production of lentiviral vectors for gene therapy represent an attractive alternative to widely-used transient transfection systems due to a simplified manufacturing process and potential for operation in high cell density bioreactors. Stable transfection results in a polyclonal pool with a wide range of productivities, screening clones individually to find the high producers is labour intensive and without being able to measure the diversity of the pool it is difficult to know how many clones to screen.

Using the 10x Genomics single cell RNA sequencing platform, we have been able to describe the diversity within a stable pool for the first time, allowing assessment of different conditions on pool dispersion and predictions of top clone titre based on how many clones are printed. We have analysed induced and uninduced stable pools, different payloads, as well the final clone. By correlating vector genome RNA expression and infectious titre data from the top clones to those of the stable pool we were able to determine that vector genome RNA is a good predictor of high titre clones and could estimate how many cells we would have to print to get a clone in the top 1%. One of the challenges was to capture the different spliced isoforms of the transfer vector, of which only the full-length transcript gets packaged and is therefore useful for titre prediction. Since the standard 10x Genomics technology cannot detect differentially spliced isoforms, by using longer sequencing reads we managed to capture and quantify two different isoforms of the transfer vector genome.

In summary, we have demonstrated that stable pools have, in fact, cells with a wide spread of productivity, however the amount of work needed to be able to pick the best, most productive cell is substantial. We hope in the future to use this information to establish a faster, more efficient stable cell line development process, and to gain knowledge about the stable pool RNA expression profile at different time points.

247 PSM: a novel technology for monitoring translation in living cells

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Our perspective of protein synthesis has been changing: from a simple mechanical translation of codons, to a sophisticated regulatory system that controls where, when, how much and which proteins are synthesized in the cell, affecting gene expression and transcription regulation. Simultaneously, regulatory elements of protein synthesis are emerging as innovative druggable targets, despite their house-keeping roles. This convergence reveals new opportunities for drug discovery in areas as varied as cancer, viral infections, fibrosis and neurodevelopmental disorders.

Anima's novel technology termed Protein Synthesis Monitoring (PSM) is based on the introduction of labelled tRNAs as FRET pairs into the cell. During the process of polypeptide elongation, these donor and acceptor labeled tRNAs come in close vicinity, thus generating a FRET signal enabling us to monitor, directly from the ribosomes in living cells, the process of protein synthesis. The intensity of the FRET signal correlates with the number of ribosomes containing the tRNA FRET pair, providing a real-time, live-cell assay for monitoring protein synthesis. PSM can monitor overall protein synthesis, using bulk tRNA, or the synthesis of a specific protein, using one or more selected tRNA pairs enriched in the protein's sequence.

Here we describe the application of our PSM technology as a high-throughput screening platform, supporting both primary and secondary screens. Our current focus is on three protein-synthesis related diseases: fibrosis (overproduction of collagen type I), viral infections (hijacking of the cellular protein synthesis apparatus), and oncology (overproduction of the undruggable oncogene Myc). We will describe the technology, the underlying bioinformatics of tRNA pairs, and applications in drug discovery and high content screening.

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248 Characterization of translation initiation using ultrahigh-throughput droplet-based microfluidic screening

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In prokaryotes, translation initiation is mainly regulated by mechanisms modulating the accessibility of the Ribosome Binding Site (RBS) [1]. RBS masking/demasking can be orchestrated by factors acting in *cis* (e.g. riboswitches) or in *trans* (e.g. small regulatory RNAs) [2]. These mechanisms may involve long stretches of sequences and, therefore, identifying key residues and dissecting the process at work may require analyzing a large number of mutants through time-consuming and labor-intensive experiments. Droplet-based microfluidics is extremely appealing for such analyses as it allows to produce and manipulate picoliter volume individual water-in-oil droplets [3] which permit to characterize a large number of variants in parallel. In fact, with this technology, as few as 100 microliters of IVTT mixture allows for producing up to 5 million droplets at a rate of several thousands of droplets per second. Upon gene expression and droplet sorting, the use of Next Generation Sequencing (NGS) allows for analyzing the whole screening output in a rapid and semi-automated way. Therefore, this technology, should make possible to characterize translation initiation mechanisms at the molecular level in a fast, cost effective and efficient way.

Here, I will present how using droplet-based microfluidic screening in tandem with NGS allowed us to explore the whole sequence space of a 9 nucleotides-long randomized region (~ 260,000 different sequences) for its capacity to recruit the ribosome and initiate translation. Thus, we characterized the sequence landscape able to support translation initiation in prokaryotes. This experiment now opens the way to the characterization of more complex regulatory mechanisms controlling translation initiation.

[1] Shine and Dalgarno.; The 3′-Terminal Sequence of Escherichia coli 16S Ribosomal RNA: Complementarity to Nonsense Triplets and Ribosome Binding Sites. PNAS **1974**, 71, 1342-1346.

[2] Wagner and Romby; Small RNAs in Bacteria and Archaea: Who They Are, What They Do, and How They Do It. Advances in Genetique **2015**, 90, 133-208.

[3] Autour and Ryckelynck.; Ultrahigh-Throughput Improvement and Discovery of Enzymes Using Droplet-Based Microfluidic Screening. Micromachines **2017,** 8, 128.

249 Building A Better Transcriptome

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Short-read sequencing technology continues to dominate the RNA research field for interrogating transcriptome landscapes. Due to fragmentation of full-length molecules, heavy computational tools are needed to estimate the transcriptional landscape; however, they fail to accurately recapitulate the transcriptome. Recently, we employed a long-read cDNA sequencing approach using Oxford Nanopore Technology (ONT) to evaluate isoforms at the single cell level. We have also improved the current long-read technology by developing a rolling circle amplification (RCA), which dramatically increases the base accuracy. This method has been further adapted as a PCR free method and includes a depletion strategy to eliminate abundant unwanted transcripts, making it easier to identify never before seen transcripts. By combining high-throughput, accuracy, and the ability to sequence transcripts from end-to-end, our method is a valuable tool for the RNA research community. From single cells to bulk RNA studies we have established a toolset necessary for creating better, more precise transcriptomes.

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250 Novel Approaches to Dramatically Reduce Impurities in RNA Produced *in vitro* by T7 RNA Polymerase

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T7 RNA polymerase *in vitro* transcription is the most common method to synthesize large quantities of RNA longer than 30-50 nt. However, the transcription pool is often contaminated with undesired, longer products. The required gel purification is low yield, time consuming and not precise. It is often impossible to separate n from n-i or n+i products and the most abundant band is not always the desired (n) product. In a recent study, we showed that >90% of these long RNA products are double stranded and are generated via diverse and distributive *cis* primer extension of the nascent RNA. Other studies have shown that *in vitro* synthesized RNA triggers the innate immune response, complicating therapeutic applications. To overcome the purity issue of *in vitro* transcription, we are engineering novel transcription platforms for T7 RNA polymerase that dramatically increase the correct RNA product. The results show that since longer products are reduced significantly, the correct product yield is improved when compared to conventional synthesis methods. By overcoming the issue of purity during synthesis, we achieve a transcription pool of relatively monodisperse, correct RNA products. We expect this to be a key development for the RNA therapeutics industry and for any RNA researchers who require high yields of pure RNA at low cost.

251 Engineering of an aptamer-gRNA for multiplexed gene editing

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CRISPR-Cas9 has led to great advances in gene editing for a broad spectrum of applications. To further the utility of Cas9, there have been efforts to achieve temporal control over its nuclease activity. While different approaches have focused on regulation of Cas9 or single guide RNA (sgRNA)-regulated CRISPR interference, none of the reported methods enable stringent and multiplexed control of the nuclease activity in bacteria. We developed novel RNA linkers to combine theophylline- and 3-methylxanthine-binding aptamers with the sgRNA, enabling small molecule-dependent editing in *Escherichia coli*. These activatable sgRNAs enable orthogonal, temporal and post-transcriptional control of *in vivo* gene editing. Further, they reduce the death of host cells caused by cuts in the genome, a major limitation of CRISPR-mediated bacterial recombineering. Temporal control of the sgRNA also increases library coverage and decreases bias in multiplex CRISPR/Cas9 editing experiments.

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252 Targeted FLEXequi RNA-panels for customized high resolution RNAseq applications

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RNA-panels are a crucial method for the selective analysis of marker gene expression. However, when transcripts with a broad range of abundancy are targeted, RNA-panels share the same limitations with standard RNA-seq that rare transcripts receive only a minor proportion of sequencing reads when compared to the high abundant transcripts. The possibility to sequence rare transcripts with the same reliability as high abundant transcripts does not only support result validity and reduces sequencing costs but also expands the number of putative candidates suitable for an RNA-panel. Within the project ceRNA-Psych (FFG Bridge 853294) which addresses the question of a putative relevance of competitive endogenous genes in neuropsychiatric disorders, Lexogen developed a targeted FLEXnorm RNA-seq panel applicable on patient peripheral blood samples, with high specificity of ≥95% and tuneable target specific enrichment factors.

The starting point for the panel development were neuropsychiatric disorder associated competitive endogenous gene candidates which had been selected based on findings from the 1000 genome project and gene expression analysis of brain tissue. To identify transcribed regions and verify expression homology in PBMCs, exemplary patient (n=2) and control samples (n=2) were sequenced first with high read depth (>40 Mio read per sample) using whole transcriptome sequencing (Corall from Lexogen). The subsequent FLEXequi panel was developed based on these sequencing data and included (1) detected pseudogenes, (2) differential expressed genes and (3) a selection of genes described in literature as differential expressed in neuropsychiatric disorders. To equalise target concentrations a FLEX specific blend method was developed and characterized for specificity and equalization with retrograde projection. The panel was used to analyse the targeted expression patterns in the blood samples from patients and controls.

253 "FOREST" reveals quantitative RNP interactome landscape using gene-annotated and structured probe libraries

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Comprehensive identification of functional RNA motif is necessary to understand the precise mechanisms of post transcriptional regulations. Biochemical and computational analyses have discovered structured RNA motifs throughout genome, but the relations to functions (e.g. natural aptamers, riboswitches and ribozymes) remain largely unknown.

Here, we developed FOREST (Folded RNA element profiling with structural motif library), a massively parallel assay system with a large-scale RNA library that contains various RNA structural motifs. Since the motif library is generated from scalable and designable oligo DNA pools, FOREST can comprehensively analyze functions of various structural motifs defined by users. All structural motifs in the library are attached to RNA 25 mer barcodes, therefore custom DNA microarray can quantify the amount of functional RNA by barcode-based hybridization. This platform provides RT-free and PCR-free direct quantification to massively evaluate the function of highly-structured RNA motifs.

As a demonstration, we first designed the terminal loop motif library of 1869 human pre-miRNAs and analyzed the RNA-protein (RNP) interaction with SNRPA protein. The results showed that FOREST successfully assessed the binding affinity with quantitative score and identified the critical sequence and structural features. Then, we expanded the library to the stem-loop motifs of human 5'UTR and HIV-1 RNA. Using them, we discovered functional RNA elements that interact with endogenous EIF3-complexes and regulate translation in cell.

Finally, we asked whether FOREST can massively and quantitatively evaluate the interaction of a higher-order RNA structure-binding protein. Using anti-G-quadruplex antibody, we confirmed that FOREST detected G-quadruplex-forming RNA motifs with high binding score. In addition, elucidating the landscape of G-quadruplex structure from human pre-miRNA and 5'UTR RNA motifs, we also found a wide variety of non-canonical G-quadruplex structures that cannot be explained by known sequence-based rules.

Using FOREST assay system we could elucidate quantitative RNP interactome landscapes with structural and genomic information. FOREST will serve as a versatile platform to analyze the function of sequence-structure-motif at an unprecedented scale, exploring the new regulatory layer orchestrated by functional RNA structures.

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254 Fluorescent turn-on probes for the development of binding and hydrolytic activity assays for mRNA cap-recognising proteins

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m7G cap is an unusual nucleotide structure present at the 5' end of all eukaryotic mRNAs, consisting of 7-methylguanosine linked by 5'5'-triphosphate bridge with the first transcribed nucleotide. Cap specifically interacts with numerous nuclear and cytoplasmic proteins, thereby participating in many important biological processes essential for cell growth and function. An example is eIF4E protein, which is crucial for mRNA translation under normal conditions and if overexpressed leads to malignant transformation of cells [1]. Cap degradation by DcpS enzyme is another therapeutically relevant process, since inhibitors of DcpS enzyme are potential drugs in SMA treatment [2]. To provide small molecular probes to study eIF4E, DcpS and other biologically important cap-recognizing proteins we synthesized a series of cap-derived fluorescent probes. The mononucleotide cap analogs labelled with different fluorescent tags were obtained in CuAAC reaction between 7-methylguanine nucleotides containing 3-butynyl-C-phosphonate moiety and fluorescent tag azides [3]. We next performed spectroscopic studies to determine the influence of conjugation with 7-methylguanosine on the fluorescent properties of the dyes. We observed that while for pyrene derivatives the fluorescence is strongly quenched by 7-methylguanosine, it remains unchanged for other tested dyes. Pyrene fluorescence changes upon binding by eIF4E or hydrolysis by DcpS were used to develop binding- or activity-based assays for evaluating the affinity of chemically modified cap analogs to the proteins. To optimize the probes structure, eight new pyrene-labelled probes were synthesized and studied for their specificity to eIF4E and DcpS. Finally, using optimized probes we developed assays for fast and efficient screening and binding constants determination for eIF4E and DcpS, as well as activity assay for DcpS-catalysed hydrolysis. The utility of new assays was validated on a previously characterized libraries of eIF4E and DcpS inhibitors [4].

References: [1] Lazaris-Karatzas, A. et al., Nature 1990, 345, 544. [2] Singh, J., et al. ASC Chem. Biol. 2008, 3, 711. [3] Wanat, P., et al. Org. Lett. 2015, 17, 3062. [4] Walczak, S., et al. Chem. Sci. 2017, 8, 260.

255 Ribosome profiling reveals new weakly translated ORFs overlapping annotated genes in *Escherichia coli* LF82

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Introduction

Due to the DNA triplet code, an overlap of two or more potential protein-coding genes at the same DNA locus is possible. In contrast to viruses, the existence of large overlapping regions (>30 amino acids) of protein-coding genes in bacteria is not generally accepted due to an information constraint which is associated with overlapping genes. Here we report the detection of several ORFs fully or partially overlapping with annotated genes in the Crohn´s disease-associated bacterium *Escherichia coli* LF82, which were identified by transcriptome and translatome sequencing (ribosome profiling).

Methods

For transcriptome and translatome analysis, whole mRNA as well as mRNA fragments covered by ribosomes were sequenced (Illumina Hiseq). Reads were processed and mapped to the reference genome using Bowtie2 before they were visualized in the genome browser Artemis. Translated ORFs located antisense to annotated genes were detected using the REPARATION algorithm. All reads were normalized to sequencing depth and ORF length (RPKM: "reads per kilobase per million mapped reads") and the translatability of the identified ORFs was determined by calculating the "ribosome coverage value" (RCV).

Results

Under the conditions tested over a hundred new ORFs partially or fully overlapping antisense with annotated genes were predicted. The overlapping genes identified were on average shorter than the predicted annotated genes and showed a lower RPKM of the transcriptome and translatome, indicating only weak expression. Nevertheless, high RCVs suggest that these ORFs are indeed translated.

Conclusion

Ribosome profiling lead to the identification of many actively translated overlapping ORFs in *Escherichia coli* LF82 by high-throughput sequencing, supporting the hypothesis that overlapping, protein-encoding genes are more common in prokaryotes than has been expected so far.

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256 Mapping microRNA regulatory networks with AGO2 eCLIP-seq in the human hematopoietic hierarchy

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MicroRNAs (miRNAs) are critical post-transcriptional regulators of molecular networks that act by targeting mRNAs to modulate protein expression. Understanding the cell-type specific gene regulatory networks governed by miRNAs requires experimental identification of miRNA-mRNA target interactions in purified cell populations. Protocols based on cross-linking immunoprecipitation (CLIP) of Argonaute-2 (AGO2) have been developed to generate and enrich for chimeras between miRNAs and their mRNA target sites, which can then be identified by high-throughput sequencing. These methods are dependent on the specificity of AGO2 immunoprecipitation and efficiencies of chimera formation and sequencing library generation, currently restricting their application to cells and tissues for which ample sample material is available.

We have previously identified miRNAs that are differentially expressed across the human hematopoietic stem/progenitor cell (HSPC) hierarchy and identified the mechanisms of how several of these govern stemness states. In order to enable generation of lineage-specific miRNA-mRNA maps in immunophenotypically defined cell populations of the human cord blood (CB) hematopoietic hierarchy, we have developed a modified AGO2 CLIP procedure based on our enhanced CLIP (eCLIP) protocol. We applied this method to human CB-derived $CD34^+$ HSPCs and identified over 100,000 unique miRNA-mRNA interactions for over 800 distinct miRNAs. Attesting to the specificity of our method, the majority of miRNA target sites mapped to mRNA coding and 3' untranslated regions. Among the most highly enriched miRNAs were miR-142 and miR-126, critical for HSPC formation and maintenance. We also identified targets of miRNAs with previously unknown roles in HSPC homeostasis. Together, we describe a novel AGO2 eCLIP-seq approach to identify miRNA targets from small input cell numbers. In combination with our miRNA expression datasets across purified human CB cell populations, we anticipate that our method will yield fundamental insights into the miRNA-dependent regulatory networks controlling human hematopoietic lineage specification.

257 Development of lipid nanoparticles for the mRNA-mediated cancer immunotherapy

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Background/Introduction: There have been lots of interests modulating immune system to cure or prevent various disease. As a new class of vaccine, mRNA have great advantages in terms of safety, easy storability, and ability to induce broad immune response. The immune response is stimulated once specific antigens are presented by antigen presenting cells (APCs) (e.g. dendritic cells (DCs), macrophages) by delivering mRNA encoding target antigen into the APCs. Depending on the target antigens, mRNA vaccine system can be utilized either as a prophylactic and therapeutic vaccine for pandemic and cancer, respectively. To deliver mRNA effectively to the APCs, specific delivery system is needed. Among various delivery system, lipid nanoparticles (LNPs) have shown great potential in the field of cancer immunotherapy. Several studies have reported that even though LNPs can induce antigen specific immune response to some extent, adjuvants are still needed for the efficient immunostimulatory effect of mRNA vaccines. In this study, we developed adjuvant-incorporated LNPs as an mRNA delivery system for cancer immunotherapy.

Methods: LNPs having adjuvants and mRNAs were prepared by rapidly mixing ethanol phase containing cholesterol, cationic lipid, DSPC, and adjuvant Pam3CKK and aqueous phase having mRNA followed by dialysed against 1X PBS. The volume ratio of ethanol and aqueous phase was 1:1 and weight ratio of mRNA to cationic lipid was 10:1. The mRNA loading amount into LNPs was measured by using a ribogreen RNA staining dye.

Results: The hydrodynamic size of LNPs was around 100 nm measured by using dynamic light scattering (DLS). The mRNA expression efficiency was verified *in vivo* by using luciferase mRNA as a model gene. Three hours after i.m. injection of different amount of LNPs, considerable luciferase gene expression was measured with 10 ug of mRNA injection. In addition, the adjuvant-encapsulated LNP showed great potency in the induction of proinflammatory cytokines compared to LNP without adjuvants.

References

· Ugur Sahin et al. mRNA-based therapeutics — developing a new class of drugs, Nature Reviews Drug Discovery 13, 759–780 (2014)..

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258 Towards a post-PCR world: direct counting of microRNAs from single cells

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Cell-to-cell heterogeneity is always present in a cell population, and bulk observations may not always represent all behaviors of a single cell. Therefore, analyzing single cells permits discovery of mechanisms not seen when studying a bulk population of cells. Current techniques for single- cell analysis usually lack adequate sensitivity and quantitative accuracy for rare or hard-to-amplify species. However, there is a new technique that promises to bypass these challenges: an amplification-free kinetic fingerprinting approach for digital single-molecule detection, called Single-Molecule Recognition through Equilibrium Poisson Sampling (SiMREPS). This method allows for highly specific, sensitive, and rapid detection of molecular species, but has not been tested on single cells and will require optimization and coupling with other methods, such as single-cell extraction and lysis. In this study, we investigate the detection of miRNA panels in single cells by combining microfluidics and single-molecule microscopy. Our end goal is to develop a platform for parallel detection of multiple microRNA (miRNA) biomarkers, to be able to detect their abundance in single cells to help better understand human health and predict the likelihood of human disease, including cancer progression.

259 Transcriptome-wide *in vitro* system reveals intrinsic RNA-binding specificities of the *Drosophila* helicase maleless and the Dosage Compensation Complex

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The dosage compensation complex (DCC) of *Drosophila melanogaster*is a paradigmatic, multi-enzyme chromatin regulator, which doubles the transcriptional output from the single male X chromosome to match the combined output of the two X's in females. The assembly of two long non-coding RNAs roX1/roX2 with five male-specific-lethal (MSL) proteins is crucial for DCC function. Amongst the DCC subunits, the DExH helicase maleless (MLE) is best-characterized for its interaction with roX RNAs. Structural studies revealed surprising specificity of MLE for uridines, rationalizing the conservation of uridine-rich motifs in critical roX substrates. Exposing these motifs by MLE is hypothesized to recruit MSL proteins and to assemble the DCC. However, how roX RNAs are specifically integrated into the complex remains elusive.

In order to define RNA-binding principles of DCC subunits, we developed vitRIP-Seq, a versatile *in vitro* transcriptome-binding assay. This assay allows to determine the intrinsic RNA-binding specificity of purified proteins in context of the full transcriptome, without the impact of extrinsic cellular factors. We subjected MLE to vitRIP-Seq and observed that it not only binds to both roX RNAs with high affinity, but also to a number of coding and non-coding RNAs with a bias towards the 3' end. These RNAs are enriched in poly-uridine stretches, resembling the nucleobase specificity observed in the crystal structure. vitRIP-Seq of the reconstituted DCC core complex lacking the helicase subunit revealed promiscuous binding to multiple RNA species, including roX1, and no preference for roX2. In presence of MLE and ATP, however, the DCC specifically enriches roX2 from the transcriptome. Within the core complex, we identified the MSL1/MSL2 module to be essential for recruiting and binding roX2-loaded MLE. The data suggest that MLE's intrinsic RNA-binding specificity coupled with its unwinding activity catalyze selective association of roX2 with the DCC. We conclude that vitRIP-Seq is a widely applicable tool to comprehensively study the intrinsic RNA-binding specificity of individual proteins or complexes in a transcriptome-wide setup.

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260 Single-cell transcriptomics analysis reveals changes in alternative polyadenylation during cell cycle progression

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Single-cell transcriptomics has revolutionized the way we can study the dynamics of gene regulation and its impact in cell proliferation and differentiation. However, the lack of accurate methods to quantify gene isoforms in individual cells has limited our understanding of the role of post-transcriptional regulatory processes such as splicing and alternative polyadenylation (APA) in these processes.

In this work, we have we have developed a new method to quantify APA events in individual cells using Drop-seq data. Using this method, we have estimated the abundance of thousands of 3' end isoforms in HEK293 cells. Additionally, we have developed a new method to computationally sort the cells along the cell cycle. With this method, we have been able to recover the oscillating patterns of known cell cycle markers and studied the expression dynamics of individual 3' isoforms during cell cycle progression. Our preliminary results show that many oscillating genes use specific 3' isoforms in different cell cycle phases. Together, these results suggest that the choice of 3'UTR could be related to the observed changes in expression levels of oscillating genes.

261 scSLAM-seq reveals core features of transcription dynamics in single cells

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Single cell RNA sequencing (scRNA-seq) has highlighted the important role of intercellular heterogeneity that contributes to phenotype variability in both health and disease. However, current scRNA-seq approaches only provide a snapshot of gene expression and convey little information on the true temporal dynamics and the stochastic nature of transcription. A further key limitation is that the RNA profile of each individual cell can only be analyzed once. Here, we introduce single cell thiol-(SH)-linked alkylation of RNA for metabolic labeling sequencing (scSLAM-seq), which integrates metabolic RNA labeling, biochemical nucleoside conversion and scRNA-seq to directly record transcriptional activity by differentiating new from old RNA for thousands of genes per single cell. We demonstrate that scSLAM-seq allows to perform full-length scRNA-seq and quantifying transcripts using unique molecular identifiers. scSLAM-seq recovers the earliest virus-induced changes in cytomegalovirus (CMV) infection and directly enables dose-response analyses. It depicts "on-off" switches and transcriptional bursting kinetics with extensive gene-specific differences that correlate with promoter-intrinsic features (Tbp-TATA-box interactions and DNA methylation). Gene and not cell-specific features thus explain the heterogeneity in transcriptomes between individual cells and the transcriptional response to perturbations. scSLAM-seq is compatible with other scRNA-seq applications and will greatly improve the sensitivity of the respective approaches to decipher the molecular mechanisms with major implications for developmental biology, infection and cancer.

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262 Nanopores allow direct sequencing of RNA strands, giving full-length reads with low bias, and provides a means of measuring transcript poly(A) tail lengths.

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Ribonucleic acid sequencing provides the ability to monitor the RNAs present in a given sample. This enables the detection of the presence and nucleotide sequence of viruses, or to build a picture of how active transcriptional processes are changing - information that is useful for understanding the status and function of a sample. Oxford Nanopore's sequencing platform is the only available technology that can sequence RNA molecules directly, rather than depending on reverse transcription and PCR. There are several potential advantages of this approach over other RNA-seq strategies, including the absence of amplification and reverse transcription biases, the ability to detect nucleotide analogues and the ability to generate full-length, strand-specific RNA sequences. Here we demonstrate the ability of an array of nanopores to sequence RNA directly, with the additional potential of multiplexing samples. We designed four barcoded adapters (rBC01-04) for the Direct RNA sequencing kit which are incorporated at the beginning of the read and the samples were sequenced both independently and mixed together in equimolar proportions. We can correctly identify the individual barcodes with high confidence (> 99%), and we recover roughly the correct proportion of reads from the mixed prep with high confidence and a low proportion of unclassified reads. Direct RNA sequencing also enables poly(A) tail length estimation using *nanopolish* software. To demonstrate this, we generated samples with known tail lengths and found good correspondence between estimated and expected lengths. We then compared the per-transcript median tail lengths of the *Saccharomyces cerevisiae* ccr4Δ mutant and wild-type strains. The distribution of significant differences lies above zero with a median of 7.42, indicating and upwards shift in the poly(A) tail lengths as expected for this mutant strain. Direct RNA sequencing is a completely new way of analyzing the sequence of RNA samples and it will improve the ease and speed of RNA analysis, while yielding richer biological information.

263 Full-length Nascent RNA Sequencing on Nanopore Devices to Measure Co-Transcriptional Splicing

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In recent years, it has become clear that the majority of RNA splicing occurs co-transcriptionally. The interconnection of splicing with transcription means that regulation of transcription elongation can influence splicing patterns in addition to transcript abundance. As a first step towards investigating the relationship of splicing with transcription, we sought to develop a precise and direct method to detect the position of RNA Polymerase II with respect to the frequency of splicing. To this end, we combine ultra-long read nanopore sequencing with a modified Precision Run-On Sequencing (PRO-seq) protocol, which yields highly pure nascent RNA from active polymerases genome-wide. Our results corroborate widespread co-transcriptional splicing in human and fly. Association of RNA transcript splicing states with published polymerase transcription rates allow extrapolation of splicing kinetics at any gene with sufficient sequencing depth. Our calculations show that splicing occurs rapidly, with many introns becoming spliced within seconds of 3' splice site exposure. Using this method and data generated from multiplexed Oxford Nanopore Technologies Minion sequencing runs, we are able to measure splicing rates genome-wide in Drosophila melanogaster and show that our assay can detect a global shut-down of splicing after treatment with splicing inhibitor Pladienolide-B.

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264 Establishing a high-throughput NMR screening protocol to assess the binding capabilities of second messenger binding riboswitches.

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Riboswitches are non-coding RNAs binding a specific second-messenger, modulating transcriptional or translational efficiency of their corresponding genes. These regulation mechanisms are found in a wide variety of bacteria and are often associated with lifestyle changes like biofilm formation and transition into anaerobic metabolism. These lifestyle changes are for example required for the pathogenesis of *Vibrio cholerae* and *Clostridium difficile.* Novel small molecules, which bind to these riboswitches, could provide new tools to target these germs. Herein we investigate the binding of multiple RNA constructs from this riboswitch class to a library of 106 fluorine labeled fragments. Our high throughput experiment design allows screening of all fragments on a specific RNA sample in as little as 3-4 days including RNA preparation. Utilizing ^{19}F-1D and ^{19}F-CPMG experiments binding or non-binding can be confirmed by chemical shift perturbation and modulation of the relaxation behavior. The initial screening phase yielded several binding fragments. Some fragments were capable to distinguish riboswitches even in cases of similar secondary structure.

265 RNP-MaP: Mapping protein interaction networks on any RNA in living cells

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RNA-protein complexes (RNPs), which are tied together by interactions between RNA and RNA-binding proteins (RBPs), are critical in many biological regulatory networks. RBPs rarely act alone, but understanding how multiple RBPs coordinate on an RNA is currently difficult without extensive prior knowledge of the constituents of an RNP. We have developed a strategy, called RNP mapping by mutational profiling (RNP-MaP), to comprehensively identify protein-binding sites and to characterize protein interaction networks on an RNA in a single, straightforward experiment. RNP-MaP combines live-cell chemical probing, selective for RNA-protein interactions, with a simple sequencing readout to locate protein interaction sites within any RNA and with single nucleotide resolution. Moreover, RNP-MaP enables detection of simultaneous binding events by multiple proteins within single RNA molecules and reveals where proteins cooperate to form functional interaction networks. We have used RNP-MaP to interrogate non-coding RNAs ranging in length from 100 to 20,000 nucleotides. RNP-MaP accurately identifies all major protein binding sites on the U1 small nuclear RNA and highlights interactions between proteins that form the RNP. RNP-MaP also reveals the overlapping RNP networks in two structurally-related, but sequence-divergent RNAs: RNase P and RMRP. Finally, we applied RNP-MaP to explore how RNP networks interface with conserved sequences within the XIST long non-coding RNA that are critical for X chromosome dosage compensation, and we identify novel functional motifs in XIST that regulate RNA stability and localization. RNP-MaP will be widely useful for discovery and mechanistic analysis of protein interaction networks across any RNA of interest.

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266 The yeast scavenger decapping enzyme DcpS hydrolyzes guanosine caps *in vitro* and facilitates enzymatic mRNA recapping with modified GTP.

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The 5’ ends of mature eukaryotic mRNAs are co-transcriptionally modified with a m^7G cap structure via a unique 5’-5’ triphosphate linkage. In the course of RNA turnover, this cap is removed by decapping enzymes from either of two families of hydrolases: the NUDIX family, which release m^7G diphosphate and leave a ligatable 5’ monophosphate, and the Histidine triad (HIT) family, which release m^7G monophosphate and leave a 5’ diphosphate. The 5’ diphosphate end can be recapped using Vaccinia virus capping enzyme, which enables the application of selective tagging of previously capped RNA with modified GTP analogs. Here we measure the decapping activity of the *Saccharomyces cerevisiae* HIT family pyrophosphatase, DcpS, on 25mer RNAs with 30 different cap structures, including Cap1. We demonstrate that yDcpS *in vitro* can decap RNA of a length up to at least 1.4 kb, rendering the 5’ end of the RNA enzymatically “recappable”. Finally, we describe proof-of-principle experiments in which m^7G capped RNAs are decapped and recapped with desthiobiotin-modified GTP. This approach provides a facile method for the enrichment of eukaryotic mRNA 5’ ends for sequencing applications.

267 Tools for splice modulation drug discovery: Spinach Splice Sensor Platform

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RNA splicing plays a central role in the generation of proteome diversity and in gene regulation. Splicing affects cellular processes, such as cell-fate and differentiation, acquisition of tissue-identity, and organ development. Consequently, defects in splicing are linked to many diseases, including spinal muscular atrophy, Duchenne muscular dystrophy, Parkinson's disease, and several types of cancer. Despite the significance of splicing in these diseases, drug discovery efforts targeting them are far and few. A critical bottleneck for such efforts is the lack of robust high-throughput assay tools to monitor endogenous spliced RNAs in the cell. Even though there are excellent tools such as RT-qPCR and RNA-seq to study RNA splicing, they are cost-prohibitive at this scale and are not readily adaptable for high-throughput screening (HTS) due to their complex and time-consuming methodology. While splice mini-gene offers the advantage of higher throughput, it lacks the ability to monitor the endogenous target RNA due its artificial design. Thus, there is an unmet need for simple and robust HTS-ready assay tools to monitor RNA splicing.

Addressing this need for robust assays for splice modulation drug discovery, Lucerna has developed an easy-to-use, HTS-ready splice sensor platform that can specifically detect any splicing event of interest. We have developed splice sensors against a variety of splicing targets, including pyruvate kinase isoforms M1 (PKM1) and M2 (PKM2), ciRS-7 circular RNA, and splicing targets involved in neurodegenerative diseases. The splice sensor platform displays rapid response times (<30 min), high selectivity (300-fold specific fluorescence), excellent sensitivity (can detect 10% change in splicing), and extended readout window (16h). In addition, the splice sensor platform can significantly reduce the false positives and error rates compared to other competitor assays due to its simpler homogenous workflow. In summary, the Spinach splice sensor platform offers robust, homogenous, and customizable assays for high-throughput splice modulating drug discovery.

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268 Brain-APEX-seq: spatial proximity-based capture of local transcriptomes in neurons from the mammalian brain

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APEX-seq is a novel method for RNA sequencing based on spatial proximity to the peroxidase enzyme APEX2. By targeting APEX2 to distinct subcellular locales inside neurons from the mammalian brain, we will produce a nanometer-resolution spatial map of subcellular transcriptomes that may reveal unique dynamics of gene expression at synapses and uncover new connections between neuronal activity and RNA metabolism/localization. This new method may provide the much-needed spatial specificity for the study of different aspect of RNA species in the mammalian central system, including local translation, epitranscriptome, RNA transport system, etc. The findings may lead to the discovery of new molecular substrates regulating neuronal activity and behaviors, neural circuit wiring/miswiring, dynamics of synaptic growth/trimming, and various types of brain disorders.

269 Systematic Evaluation and Optimization on the Experimental Steps in RNA G-quadruplex Structure Sequencing

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Guanine (G)-rich sequences in RNA can fold into stacks of G-quartets, and assembly into secondary structure called RNA G-quadruplex (rG4). To map the rG4s in a high-throughput and transcriptome-wide manner, we have previously developed RNA G-quadruplex Structure Sequencing (rG4-seq). Yet, its current limitations to further utilities are i) high RNA input needed, ii) long preparation time required, and iii) lack of a robust experimental pipeline. Here, we evaluate and optimize the 5 key steps involved (See scheme). Overall, we have lowered the RNA input and shortened the preparation time. This improved method can be applied to the structurome studies and other biological applications that require cDNA library preparation in a more efficient manner.

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270 Genetic interactions of *PRP45* alleles highlight the dependence of splicing on H2A.Z

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Posttranslational modifications and remodeling of nucleosomes contribute an important but incompletely understood aspect of gene expression, which affects all stages of transcription and transcript processing, including splicing. Prp45 is a protein with high proportion of predicted unstructured regions, which forms part of the basic core of spliceosome through the catalytic cycle. The protein adopts an extended conformation, spanning the whole spliceosomal core and interacting extensively with Prp46 and Prp8. Prp45 also contacts U2 snRNP proteins (Hsh155) as well as RES and NTC complex factors. Metazoan orthologs of Prp45 were found associated with components of the elongating RNA PolII complex as well as with sequence specific transcription factors and their co-regulators.

We have shown previously that Prp45 aids the recruitment of 2nd step helicase Prp22 to spliceosome and that C-terminally truncated Prp45 (*prp45*(1-169)), while still capable of supporting splicing, causes defects in both splicing steps. Co-transcriptional recruitment of U2 snRNP, U5 snRNP and NTC is impaired, demonstrating that Prp45 is also important for early stages of spliceosome assembly. Remarkably, truncated Prp45 slows down the recruitment of branch point-dependent factors (U2 snRNP) but does not affect U1 snRNP dissociation.

Prp45 truncation leads to pre-mRNA accumulation but has only mild effect on mRNA levels. Here, we show that the effect of Prp45 truncation can be graded, suggesting that the protein mediates multiple splicing factor-association events. We examined the splicing phenotypes of *prp45* alleles and their dependency on the components *in trans* (such as chromatin factors) and *in cis* (splicing sequences). Detailed analysis of the genetic interaction network of *prp45*(1-169) revealed spliceosomal components, chromatin remodelers and effectors of chromatin modifications, including factors which affect H2B ubiquitination and H3 acetylation and methylation. In agreement with related studies, H2A.Z-encoding *htz1*, SWR, INO80, and SAGA complex components represented prominent interactors. *prp45* interactions with *htz1* were stronger than the interactions with H2A.Z remodeling complexes tested and depended on the degree of Prp45 truncation. Our results illustrate the importance of chromatin environment in the splicing cycle of *Saccharomyces cerevisiae*.

271 The RNA-binding protein Hfq is important for ribosome biogenesis and affects translation fidelity

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Hfq is the bacterial member of the Sm family of RNA binding proteins and is mostly known for promoting sRNA-mRNA interactions. However, numerous sRNAs do not depend on Hfq for its mode of action and Hfq is even dispensable for riboregulation in many bacteria, suggesting other function(s) for this RNA-binding protein.

In this work we show that the widely-conserved RNA chaperone Hfq plays a critical role in rRNA processing and ribosome assembly in *Escherichia coli* (Andrade et al, EMBO J 2018). Hfq binds the 17S rRNA precursor and facilitates its correct processing and folding to mature 16S rRNA. For the first time, Hfq is shown to be a novel biogenesis factor in bacteria, which associates with immature pre-30S particles but not with mature 30S subunits. As consequence, inactivation of Hfq results in the decrease of the pool of mature 70S ribosomes which correlates with a reduced volume of translation.

Using a set of point mutants in the different RNA binding surfaces of Hfq, we found that only mutations in residues in the distal face of Hfq reduce ribosome levels in contrast with mutations in the proximal and rim surfaces which govern interactions with the sRNAs. Our results suggest that Hfq-dependent regulation of ribosomes is independent of its function as sRNA-regulator. In addition, the Δ*hfq* mutant showed a substantial increase in frameshifting, aberrant initiation from alternative start codons, and stop-codon readthrough, indicating that the accuracy of translation in Hfq-depleted background is severely compromised.

Overall, we demonstrate that Hfq globally affects the quality of protein synthesis. Inactivation of Hfq compromises both translation efficiency and fidelity, both features of aberrantly assembled ribosomes. Our work expands the functions of the Sm-like protein Hfq beyond its function in small RNA-mediated regulation and unveils a novel role of Hfq as crucial in ribosome biogenesis and translation.

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272 Co-transcriptional splicing modulates gene expression

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Splicing and transcription occur concurrently during the synthesis of eukaryotic pre-messenger RNA. We previously developed single molecule nascent RNA sequencing methods (Single Molecule Intron Tracking; SMIT) to monitor the progression of the splicing reaction relative to position of RNA polymerase II (Pol II)[1]. For 87 endogenous yeast genes, spliced mRNA was observed when Pol II is just downstream of each intron, suggesting that splicing occurs rapidly as Pol II transcribes past the intron, and we identified gene-specific differences in splicing. Here, we have constructed a novel, modular HA-YFP reporter gene that allows us to quantify the contribution of intronic features to observed gene-specific differences in splicing. Substitution of the consensus yeast GUAUGU 5' splice site (SS) sequence with a minor variant (GUAcGU) results in a significant decrease in spliced mRNA and protein synthesis. Further testing of all possible variants of the consensus splice site sequence revealed significant differences in gene output. Using SMIT, we find that the splice site substitutions impair co-transcriptional splicing and our results suggest that post-transcriptional splicing cannot compensate for co-transcriptional defects. Together, these data support a model wherein transcription and splicing cooperate within a "window of opportunity" to tune gene expression.

1. Carrillo-Oesterreich, F., Herzel, L., Straube, K., Hujer, K., Howard, J., Neugebauer, K.M., 2016. Splicing of Nascent RNA Coincides with Intron Exit from RNA Polymerase II. Cell 165, 372-381.

273 The mRNA export receptor NXF1 coordinates transcriptional dynamics, alternative polyadenylation and mRNA export

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Alternative polyadenylation (APA) produces mRNA isoforms with different 3'UTR lengths. Previous studies indicated that 3' end processing and mRNA export are intertwined in gene regulation. Here, we show that mRNA export factors generally facilitate usage of distal cleavage and polyadenylation sites (PASs), leading to long 3'UTR isoform expression. By focusing on the export receptor NXF1, which exhibits the most potent effect on APA in this study, we reveal several gene features that impact NXF1-dependent APA, including 3'UTR size, gene size and AT content. Surprisingly, NXF1 downregulation results in RNAP II accumulation at the 3' end of genes, correlating with its role in APA regulation. Moreover, NXF1 cooperates with CFI-68 to facilitate nuclear export of long 3'UTR isoform with UGUA motifs. Together, our work reveals important roles of NXF1 in coordinating transcriptional dynamics, 3' end processing, and nuclear export of long 3'UTR transcripts, implicating NXF1 as a nexus of gene regulation.

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274 Tracking mRNA isoform fate during neural differentiation

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Temporal regulation of transcription and alternative pre-mRNA splicing play a major role in cellular differentiation. Rates of transcription and steady state levels of mRNA are poorly correlated with protein abundance in vertebrate cells and the mechanisms responsible for this discrepancy are not fully understood. Alternative splicing has long been known to expand the protein coding capacity of the cell and can attenuate gene expression by producing substrates for nonsense mediated decay (NMD). The extent to which alternative splicing influences other types of post-transcriptional gene regulation is less well known. The central nervous system expresses a staggering array of developmentally regulated alternative mRNA isoforms. To interrogate the cytoplasmic fate of these alternative mRNAs and determine alternative sequence features responsible for alternative fates, we use a cellular fractionation and high throughput sequencing approach (Frac-seq) for quantification of isoform-specific mRNA recruitment to polyribosomes in embryonic stem cells and early neural progenitor cells. We find thousands of events that result in isoform-specific polysome association, primarily events that alter the untranslated regions of transcripts, supporting the hypothesis that nuclear processing of a message influences the translational control of that message. We also find that NMD substrates are strongly enriched in the 80S fraction in embryonic stem cells and their polyribosome association increases in neural progenitor cells, suggesting an attenuation of nonsense mediated decay in neural stem cells compared to embryonic stem cells.

275 Genetic compensation triggered by mutant mRNA degradation

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The increasing number of mutants in various model organisms showing no obvious phenotype has renewed interest into how organisms adapt to gene loss. Genetic robustness, or the ability of an organism to maintain fitness in the presence of mutations, can be achieved via protein feedback loops. Recent evidence suggests that organisms may also respond to mutations by upregulating related gene(s) independently of protein feedback loops, a phenomenon called transcriptional adaptation. However, the prevalence of transcriptional adaptation and its underlying molecular mechanisms are unknown. Here, we develop and analyze several models of transcriptional adaptation in zebrafish embryos and mouse cell lines that we first show are not caused by loss of protein function. We find that the increase in transcript levels is due to enhanced transcription, and observe a correlation between mutant mRNA decay and transcriptional upregulation of related genes. To assess the role of mutant mRNA degradation in triggering transcriptional adaptation, we use genetic and pharmacological approaches and find that mRNA degradation is indeed required for this process. Notably, uncapped RNAs, themselves subjected to rapid degradation, can also induce transcriptional adaptation. Next, we generate alleles that fail to transcribe the mutated gene and find that they do not display transcriptional adaptation, and exhibit more severe phenotypes than alleles displaying mutant mRNA decay do. Transcriptome analysis of the mouse cell line models reveals the upregulation of a significant proportion of the genes that exhibit sequence similarity with the mutated gene's mRNA, suggesting a scenario whereby mRNA degradation products induce transcription through a sequence dependent mechanism. Further mechanistic analyses revealed RNA decay factors-dependent chromatin remodeling and antisense RNAs repression to be associated with the response. These and other results expand the role of the mRNA surveillance and degradation machinery in buffering against mutations by triggering the transcriptional upregulation of related genes. Besides implications for our understanding of disease-causing mutations, our findings will help the design of mutant alleles with minimal compensation due to transcriptional adaptation.

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276 ALYREF links 3'-end processing to nuclear export of non-polyadenylated mRNAs

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The RNA-binding protein ALYREF plays key roles in nuclear export and also 3'-end processing of polyadenylated mRNAs, but whether such regulation also extends to non-polyadenylated RNAs is unknown. Replication-dependent (RD)-histone mRNAs are not polyadenylated, but instead end in a stem-loop (SL) structure. Here, we demonstrate that ALYREF prevalently binds a region next to the SL on RD-histone mRNAs. SL-binding protein (SLBP) directly interacts with ALYREF and promotes its recruitment. ALYREF promotes histone pre-mRNA 3'-end processing by facilitating U7- snRNP recruitment through physical interaction with the U7- snRNP-specific component Lsm11. Furthermore, ALYREF, together with other components of the TREX complex, enhances histone mRNA export. Moreover, we show that 3'-end processing promotes ALYREF recruitment and histone mRNA export. Together, our results point to an important role of ALYREF in coordinating 3'-end processing and nuclear export of non-polyadenylated mRNAs.

277 Selective roles of vertebrate PCF11 in premature and full-length transcript termination

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The pervasive nature of RNA polymerase II (Pol II) transcription requires efficient termination. A key player in this process is the cleavage and polyadenylation (CPA) factor PCF11, which directly binds to the Pol II C-terminal domain and dismantles elongating Pol II from DNA *in vitro*. We demonstrate that PCF11-mediated termination is essential for vertebrate development. A range of genomic analyses, including: mNET-seq, 3' mRNA-seq, chromatin RNA-seq and ChIP-seq, reveals that PCF11 enhances transcription termination and stimulates early polyadenylation genome-wide. PCF11 binds preferentially between closely spaced genes, where it prevents transcriptional interference and consequent gene downregulation. Notably, PCF11 is sub-stoichiometric to the CPA complex. Low levels of PCF11 are maintained by an auto-regulatory mechanism involving premature termination of its own transcript, and are important for normal development. Both in human cell culture and during zebrafish development, PCF11 selectively attenuates the expression of other transcriptional regulators by premature CPA and termination.

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278 Withdrawn

279 DXO1 links nuclear gene expression and chloroplast function in *Arabidopsis thaliana*.

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The DXO family of proteins is involved in eukaryotic mRNA 5'-end quality control, removal of non-canonical NAD^+ cap and maturation of fungal rRNA precursors. In this work, we characterize DXO1, the *Arabidopsis thaliana* DXO homolog. We demonstrate that the biochemical properties of DXO1 associated with mRNA 5'-end quality control are severely affected by plant-specific modification within the active site. DXO1 does have strong deNADding activity *in vitro*, although its physiological function remains to be characterized. However, enzymatic DXO1 activities are not its major function *in vivo*, since strong morphological and molecular aberrations observed upon DXO1 knockout in plants can be reverted by the full-length protein regardless of the presence of a functional active site. In turn, the plant-specific extended N-terminal fragment of DXO1 is crucial for its function *in vitro* and *in vivo*. In addition, DXO1 knockdown leads to the accumulation of RNA quality control siRNAs that are characteristic of *Arabidopsis* RNA degradation mutants, but the contribution of DXO1 to this effect is probably indirect. Instead, DXO1 has a strong impact on chloroplast-localized processes. We propose that RNA turnover in *dxo1* plants is altered as a result of retrograde chloroplast signaling that modulates gene expression.

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280 Global secondary-structure formation as a major constraint on CRISPR RNA biogenesis

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CRISPR-Cas systems represent RNA-directed immune systems whose nucleases have been harnessed as powerful tools for genome editing. One unique feature of these immune systems is that multiple guiding CRISPR RNAs (crRNAs) are encoded within individual CRISPR arrays as alternating invader-targeting spacers and intervening repeats. The arrays are transcribed as pre-crRNAs that undergo processing into individual crRNAs. The crRNAs then complex with Cas nucleases to carry out the immune function of the systems. To-date, the different mechanisms of crRNA processing have been well-established and can involve different Cas proteins and host factors. However, little is known about how the sequence of the array impacts the resulting abundance and targeting activities of the processed crRNAs. The knowledge gap is particularly important, as spacers can be virtually any sequence and natural arrays can encode hundreds of spacers.

Here, we used the Type V-A system in *Francisella novicida* and its characteristic Cas12a nuclease as a model to interrogate factors influencing crRNA biogenesis, abundance, and targeting activity. By applying computational analyses and varying experimental assays in cell-free systems, bacteria, and mammalian cells, we found that the outcomes of crRNA biogenesis are principally shaped by the global secondary structure of the pre-crRNA. First, the predicted global secondary structure of the transcribed array strongly correlated with the abundance of individual crRNAs processed from both native and synthetic arrays. Second, stabile misfolding of a characteristic loop within the repeat was responsible for diminished crRNA abundance and poor targeting activity. Third, any stable hairpins flanking the array caused incomplete processing, diminished abundance of the adjacent crRNA, and also poor targeting activity mediated by this crRNA. Finally, native terminal repeats consistently harbored mutations that buffered against flanking sequences and prevent generation of an unintended crRNA. Our study thus revealed the multifaceted role of secondary structure in crRNA biogenesis, with broad implications for immunity against foreign invaders and the use of CRISPR arrays for multiplexing with CRISPR technologies.

281 RNA Atlas: a nucleotide resolution map of the human transcriptome

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Technological advances in RNA expression profiling revealed that the human genome is pervasively transcribed, generating an unexpectedly complex transcriptome consisting of various classes of RNA molecules and a huge isoform diversity. Many of these RNAs show high tissue specificity, with some being expressed in only one or few cell types. While numerous large-scale RNA-sequencing studies have been performed, samples involved are often complex tissues, masking transcripts expressed in low-frequent cell populations, and sequencing methods typically focus on one class of RNA transcripts.

We assembled the most comprehensive human transcriptome across an extensive cohort of human samples, consisting of 160 different normal cell types, 45 tissues and 93 cancer cell lines. For each sample, strand-specific total RNA, poly-A RNA and small RNA libraries were generated and sequenced using Illumina technology, yielding a total of 65 billion reads. The generated transcriptome includes matching expression profiles of lncRNAs, protein coding genes, circRNA and miRNAs across all samples. By integrating our gene models with independent evidence of active transcription from the FANTOM5 CAGE data and chromatin states maps from the Roadmap Epigenomics Consortium, we defined a stringent set of 50235 gene loci, of which 19668 are novel. From these loci, 37140 circRNAs were expressed. Count data from poly-A and total RNA sequencing libraries were combined to reveal the polyadenylation status of each transcript in each sample. While a small fraction of novel genes was predicted to have coding potential, the majority of novel genes were non-coding, single exonic, and highly enriched for non-polyadenylated transcripts. Interestingly, a subset of genes showed variable polyadenylation status across samples, mainly driven by alternative isoform usage. Biological information content of each RNA biotype was assessed by evaluating RNA expression - sample ontology associations and complex tissue deconvolution. Furthermore, we exploited the availability of intron reads from the total RNA sequencing data to assess the regulatory potential of miRNAs, lncRNAs and circRNAs at the transcriptional and post-transcriptional level. Taken together, the RNA atlas serves as a unique resource for further studies on the function, organization and regulation of the different layers of the human transcriptome.

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282 Co-transcriptional splicing in mammalian erythroblasts

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Splicing of pre-mRNAs occurs co-transcriptionally across multiple cell types and species. Previously, our lab has developed single molecule nascent RNA sequencing methods - including single molecule intron tracking (SMIT) and long read sequencing of nascent RNA - to study co-transcriptional splicing in budding and fission yeasts[1,2]. Using these methods, we were able to estimate the kinetics of single intron removal in both yeasts by relating the 3'end of nascent RNA (position of RNA Polymerase II) to progress of the splicing reaction. In comparison to yeast, mammalian genes are much more complex - on average they contain eight long introns surrounded by short exons. In order to determine the extent to which splicing occurs co-transcriptionally in mammalian pre-mRNAs, and how fast this splicing could occur, we have now adapted our single-molecule nascent RNA sequencing methods for use in a mammalian system. We have chosen murine erythroid leukemia (MEL) cells as a model system, as they can be easily differentiated in vitro, and they express a subset of erythroid-specific genes at high levels. We isolated nascent, chromatin-associated RNAs from MEL cells before and after induction of terminal erythroid differentiation and performed long read sequencing on the Pacific Biosciences Sequel platform. By focusing our analysis on a set of nascent RNAs that are highly expressed, we obtained sufficient read depth to visualize the progression of transcription and splicing across single, full-length transcripts. We see that for the majority of introns, splicing is completed very soon after the intron is fully transcribed. Thus, we conclude that highly expressed pre-mRNAs in MEL cells are spliced co-transcriptionally, and that the mammalian spliceosome can assemble and act rapidly on this set of pre-mRNAs. In combination with other nascent RNA-seq methods, this work provides a high-resolution description of mammalian gene expression.

1. Oesterreich, F.C., Herzel, L., Straube, K., Hujer, K., Howard, J., Neugebauer, K.M., 2016. Splicing of Nascent RNA Coincides with Intron Exit from RNA Polymerase II. Cell 165, 372-381.

2. Herzel, L., Straube, K., Neugebauer, K.M., 2018. Long-read sequencing of nascent RNA reveals coupling among RNA processing events. Genome Res 28, 1008-1019.

283 Transcriptome-wide analysis of translation-dependent mRNA decayduring T cell and macrophage activation reveals a global effect of translation on mRNA stability and a potential role of UTRs in its regulation

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Post-transcriptional control is crucial for regulating protein expression, both basally and in response to extracellular cues. Proper signal transduction requires tight control of both response induction and termination. While much work has gone into understanding how mRNAs are translationally upregulated to boost protein expression during signaling, much less is known about how such responses are terminated. One way protein expression might be attenuated following translational activation is by targeting mRNAs to translation-dependent degradation (TDD), thus making any increase in protein expression self-limiting. The extent to which TDD is a general mechanism for limiting protein expression is currently unknown.

Here we describe a comprehensive analysis of basal and signal-dependent gene expression in primary mouse T cells and macrophages. In order to measure the impact of TDD in regulating gene expression, we performed RNA-Seq, PAS-Seq and ribosome profiling to monitor RNA levels, 3'UTR length, translational efficiency, and RNA degradation transcriptome-wide, both before and after activation of T cells and macrophages. Our data surprisingly indicate that most unstable mRNAs are decayedto some extent in a translation-dependent manner, both in resting and activated cells. Further, we observe a general negative correlation between mRNA stability and ribosome density – that is, mRNAs occupied by fewer ribosomes are apparently more stable than mRNAs occupied by more ribosomes. Interestingly,the extent of TDD is inversely correlated to the length of the 5'UTR and 3'UTR but not to that of the coding sequence. These unexpected observations highlight the strong interconnection that exists between mRNA translation and decay.

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284 U1 snRNP-dependent suppression of premature polyadenylation in plants: searching for the mechanism

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Regulation of pre-mRNA processing in eukaryotes provides important checkpoints to modulate gene expression during development and in response to environmental cues. Once transcription has been initiated, the nascent transcripts undergo a series of processing steps on their way to mature mRNAs. In particular, pre-mRNA splicing and polyadenylation are highly regulated, giving rise to different mRNA isoforms from the same transcripts through alternative splicing and alternative polyadenylation. Although one to one stoichiometry of all spliceosomal snRNPs is needed for spliceosome activity, in the nucleus U1 snRNP is present in a large excess over all other snRNPs. This points towards additional functions of U1 snRNP beyond splicing. Indeed, it has been shown in human cells that U1 snRNP is also important for inhibition of premature polyadenylation sites. This U1 snRNP activity is known as telescripting. We described a similar mechanism in A. thaliana while investigating the role of introns in miRNA genes. We found that the active 5'ss inhibits utilization of the nearest downstream polyadenylation site. To further investigate the interplay between U1 snRNP and the polyadenylation machinery we performed co-immunoprecipitation (co-IP) using antibodies against U1-70K - the core protein of U1 snRNP, and we identified CFIS2, one of two plant orthologues of human CFIm25, and the orthologue of human CFIm68. CFIm25 and CFI68 are both subunits of the human Cleavage Factor I polyadenylation complex (CFI), the UGUA-dependent enhancer of mRNA 3'processing. Additionally, molecular analyses of the knockout mutant of A. thaliana CFIS2 revealed changes in polyadenylation as well as in splicing of many transcripts, suggesting reciprocal relations between splicing and polyadenylation. Moreover, we observed no direct interaction between U1-70K and each subunit of the CFI complex but instead we discovered that eIF4A-III interacts with both U1-70K and CFIS2, and mediates the crosstalk between splicing and polyadenylation machineries. eIF4A-III from A. thaliana, apart from being described as a functional component of the Exon Junction Complex, is an important factor for abiotic stress adaptation. Our newly identified interactions shed light on the crosstalk between splicing and 3'end processing, and its function as a driving force behind the stress response.

285 Y14 Participates in DNA Damage Response and Repair via Interaction With the Non-homologous End Joining Complex

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Y14 is a core factor of the exon junction complex (EJC); it not only participates in post-splicing RNA processing events but also regulates pre-mRNA splicing. We recently found that DNA damage signals accumulated in Y14-depleted cells and also in *Rbm8a* (Y14) haplodeficient mouse neocortex. Since DNA repair deficiency threatens genomic stability, we explored the potential role of Y14 in DNA damage response/repair. First, Y14 depletion downregulated the expression of several DNA damage response (DDR) factors and induced R-loops, both of which likely lead to DNA damage accumulation. Second, using immunoprecipitation coupled with mass spectrometry, we found several DDR factors as potential Y14-interacting partners. Further results confirmed that Y14 interacts with the non-homologous end joining (NHEJ) complex as well as several DDR factors in an ATM-dependent manner. Y14 co-fractionated with Ku in chromatin-enriched fractions and further accumulated on chromatin upon DNA damage. Y14 knockdown delayed recruitment of DDR factors to DNA damage sites as well as the formation of gH2AX foci. Intriguingly, Y14 depletion also reduced ubiquitination of Ku and hence impaired its recycling. Accordingly, Y14 depletion compromised the efficiency of DNA end-joining (*iScience*, accepted). Since Y14 interacts with the NHEJ factors in an RNA-dependent manner, we attempt to identify whether specific RNA is involved. In conclusion, we report that Y14 likely plays a direct and EJC-independent role in DNA damage repair via its interaction with the DNA damage repair factors.

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286 Molecular dissection of lncRNA activities: ANRIL as a Model System

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The increased knowledge on the long non-coding RNA (lncRNA ≥ 200-nts lacking obvious coding regions) and their involvement in global genome regulation push the lncRNA-disease interconnection to a promising and timely field of research. Due to flexible 2D structures and multiple protein binding sites, lncRNAs carry intrinsic features conferring a wide regulatory potential. Although lncRNAs are being extensively studied, the field is still suffering from a lack of knowledge on their structure/function relationships at the molecular and cellular level.

The team focuses on the characterization of the lncRNA named ANRIL (Antisense Non-coding RNA in the INK4 Locus). ANRIL is an ideal study model since it is associated to human diseases and is likely implicated in regulating the fine-tuning of gene expression via the recruitment of nucleoprotein complexes.

Until now, ANRIL functions have only been superficially explored at the molecular level. ANRIL belongs to the 9p21 locus also called CDKN2A/B. The rate of expression of ANRIL transcripts predisposes to cardiovascular diseases and cancer. ANRIL via a -cis regulatory activity recruits the polycomb repressor complex PRC1 and PRC2 that are involved in the transcriptional repression of the 9p21 locus. Beside its well documented -cis activity, ANRIL is also suspected to modulate gene expression on distal (-trans) genomic loci. Our work tends to demonstrate that ANRIL isoforms affect selectively and directly the fine-tuning of the expression of several genes involved in pathologies. Indeed, Chromatin Isolation by RNA Precipitation followed by high-throughput sequencing (ChIRP-seq) experiments showed that ANRIL contacts various genomic loci. Also, conditional ANRIL expression experiments followed by transcriptome analysis identified genes and pathways that are selectively regulated by ANRIL in an isoform specific manner. Furthermore, RNA chromatin extraction experiments showed that some ANRIL sub-domains are enriched in the DNA/Chromatin fraction suggesting that they contain features allowing the appropriate RNA chromatin recognition and/or promoting ANRIL -trans activity. We expect that the conjugation of cellular, biochemical and structural approaches we are currently deploying will allow us to generate a clearer vision of ANRIL activity and its molecular links to diseases.

287 SyntDB - a database of human lncRNA syntologs across primate species

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Long non-coding RNAs (lncRNAs) are typically defined as transcripts of more than 200 nucleotides that do not get translated into proteins. Recent studies indicate that they participate in a wide range of cellular processes. Still only a small fraction of lncRNAs have been characterized functionally and evolutionary conservation studies may be of great importance in pinpointing molecules bearing crucial biological roles. We built a set of human lncRNAs expressed across hundreds of human tissues and cell lines and identified their syntologs (lncRNAs expressed in syntenic genomic regions) across 11 primate species, including four great apes. The analysis was done with our custom pipeline, which exploits genome alignments and RNA-Seq driven predictions when looking for syntenic transcripts between compared species. This helped us track evolutionary dynamics of human non-coding transcripts and select highly conserved lncRNAs with highest regulatory potential.

The data we obtained is made available in a newly developed online database, dubbed SyntDB. The resource stores more than 78 000 of expressed human lncRNAs and up to 18 000 lncRNAs for each of non human primates. It bears a number of browse, search and visualization options, along with download section and statistical summaries for transcriptomes. Our database is easy to use and browse thanks to database design, on the fly visualizations and extensive use of modern web stack technologies. Also all the data available for download in forms convenient to reproduction and reuse.

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288 Characterizing the expression and function of the circRNA Cdr1as and its regulatory network in mouse primary neurons

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Cdr1as is a highly expressed brain circRNA, relevant for synaptic neurotransmission and normal brain function. The molecular mechanism explaining this function is not understood. It has been hypothesized that Cdr1as expression is regulated by a complex network including miR-7, miR-671 and lncRNA Cyrano, but the exact interplay among them is unknown.

Here, we characterize and quantify expression and localization of Cdr1as and Cyrano in mouse primary cortical neurons from wildtype and Cdr1as-mutant animals using single molecule imaging techniques. In addition, we analyzed the changes in Cdr1as and Cyrano upon perturbation of miR-7 expression.

We show that both Cdr1as and Cyrano are broadly expressed in cortical neurons (soma and neurites), suggesting a possible role in transport of miR-7 to subcellular locations within neurons. By analyzing Cdr1as after miR-7 overexpression, we showed that Cdr1as and Cyrano are being downregulated and Cdr1as localized only to somas. Also specific neuronal gene changes are ocurring. Suggesting a negative regulation of the circRNA and Cyrano when exposed to high concentration of miR-7 together with possible specific regulatory mechanisms. An additional layer of complexity to Cdr1as/miR-7/miR-671/Cyrano network.

289 Sex-specific development in haplodiploid honeybee is controlled by the female-embryo-specific activation of thousands of intronic lncRNAs

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Embryonic development depends on a highly coordinated shift in transcription programs known as the maternal-to-zygotic transition, and sex chromosome silencing occurs during early embryonic development. Here, we applied a single-embryo RNA-seq approach to characterize the embryonic transcriptome dynamics in haploid males vs. diploid females of the happlodiploid insect honeybee (*Apis mellifera*). We observed typical zygotic genome activation (ZGA) occurred in three major waves specifically in female honeybee embryos; haploid genome activation was much weaker and occurred later. Strikingly, we also observed three waves of transcriptional activation for thousands of long noncoding transcripts (lncRNA), 73% of which are transcribed from intronic regions and 65% were specific to female honeybee embryos. These findings support a model in which introns encode thousands of lncRNAs that are expressed in a diploid-embryo-specific and ZGA-triggered manner that function to silence gene expression from the entire set of extra chromosomes in the females of these haplodiploid animals.

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290 Defining the dynamics of TERRA molecules in living cancer cells

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Telomeric repeat-containing RNAs TERRA are expressed in several organisms and play key roles in telomere biology, including regulation of heterochromatin formation and control of DNA replication. TERRA also exerts extratelomeric functions by regulating gene expression through interaction with specific TERRA-binding sites within the genome. In addition, in mouse embryonic stem cells (mESCs) TERRA has been shown to promote somatic homologous X-chromosome pairing, which is required for proper X chromosome inactivation (XCI). Accordingly, TERRA transcripts localize to the X and Y chromosomes

in mESCs and human embryonic stem cells. These findings indicate that TERRA molecules undergo complex dynamics within the nucleus. Nevertheless, little is known about the dynamics and function of TERRA expressed from a single telomere in cancer cells.

We have developed a live-cell imaging assay based on the MS2-GFP system in order to image single-telomere TERRA transcripts in human cancer cells. To this aim, we generated clones containing MS2 sequences integrated at a single telomere. By expressing a MS2-GFP fusion protein which specifically recognizes MS2 RNA sequences, we observed that MS2-tagged TERRA transcripts form discrete foci within the nucleus. In particular, confocal live-cell imaging analyses revealed the formation of two populations of TERRA-MS2-GFP foci: TERRA RNA single molecules, which freely diffuse within the nucleoplasm, and TERRA RNA clusters. Simultaneous time lapse confocal imaging of TERRA particles and telomeres showed that TERRA clusters transiently localize with chromosome ends. Interestingly, depletion of MS2-tagged TERRA transcripts by antisense oligonucleotides resulted in the induction of DNA damage as detected by an increased number of cells with γH2AX foci at telomeres and elsewhere in the genome. These findings suggest that single-telomere TERRA transcripts participate in the maintenance of genome integrity in cancer cells. We will use this approach to investigate in detail the dynamics of TERRA transcripts in cancer cells.

291 Are neuronal cytoplasmic lncRNAs associated with polysomes and translated?

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Many long non-coding RNAs (lncRNAs), like mRNAs, are capped, spliced and polyadenylated. However, lncRNAs exhibit far more tissue and developmental-stage specific expression than mRNAs. They are particularly enriched in the nervous system and testes. Several lncRNAs have been found to contribute to neuronal differentiation and lncRNA mis-regulation has been implicated in neurological disorders (e.g BACE1-AS, BC200). Whilst the nuclear functions of lncRNAs have been extensively studied, less is known about the role of lncRNAs in the cytoplasm, even though many are localized there.

LncRNAs contain many small Open Reading Frames (smORFs), i.e. in-frame start and stop codons. Many cytoplasmic lncRNAs have been shown to interact with translation machinery and a small proportion of lncRNA-smORFs show evidence of translation, resulting in the synthesis of small peptides (yeast, *D.melanogaster* and mouse). However, these translation events remain controversial and their biological function poorly understood.

To determine the biological importance of these potential lncRNA-ribosome interactions we have characterized lncRNAs and their potential translation during neuronal differentiation of SH-SY5Y cells. Polysome profiling shows a global reduction of translation upon differentiation. Interestingly, we found that ribosomal protein mRNAs are specifically depleted from polysomes upon differentiation, during this translational repression. qRT-PCR of these sucrose gradient fractions indicates that several neuronal lncRNAs are associated with 80S and small polysome complexes.

To globally detect lncRNA-translation machinery interactions we have performed Poly-Ribo-Seq. This has identified the presence of three lncRNA populations in the cytoplasm: a) Translated lncRNAs, b) Polysome associated non-translated lncRNAs, and c) Cytosolic lncRNAs, not associated with polysomes. We have found 178 lncRNAs whose expression is upregulated during differentiation, 70% of which are associated with polysome complexes e.g. LINC01116. Overall, we discover lncRNAs with significant ribosome profiling signal and triplet periodicity, indicating that they are translated. Poly-Ribo-Seq analysis also reveals differences in the ribosome occupancy and polysome association of these lncRNAs upon differentiation. We are currently investigating the potential role of these lncRNAs, and their translational machinery interactions, in neuronal differentiation.

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292 Neuronal circular RNAs in a mouse model of autism spectrum disorders

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Circular RNAs (circRNAs) represent a class of endogenous noncoding RNAs characterized by a covalently closed loop structure, resulting from a backsplicing reaction. Recent studies have shown their implication in post-transcriptional regulation of gene expression through several mechanisms as miRNA and protein binding, and mRNA trapping. circRNAs are highly enriched in the nervous system, as highlighted by RNAseq analyses in Drosophila and mammals. In the brain their expression is controlled during neuronal development and respond to synaptic activation, suggesting a role of circRNAs in the regulation of synaptic activity during development (Chen and Schuman, 2016). The Autism Spectrum Disorders (ASDs) are developmental disorders characterized by impairments in social interactions and communication, and by repetitive and stereotyped behaviors and interests. It has been hypothesized that the deregulation of the activity-dependent signaling network at the synapses could represent the key molecular component of this pathology (Ebert and Greenberg, 2013). In order to elucidate a possible role of circRNAs in autism, by next generation sequencing analyses of the circular transcriptome, we explored circRNA expression profile of the hippocampus of the BTBR T+tf/J (BTBR) mouse model for ASDs. Selected circRNAs candidates, differentially expressed in ASD , have been identified and further characterized. Our study suggests a possible role of circRNAs in the context of ASDs. Further analysis will allow us to better elucidate biological functions of BTBR altered circRNA, both in physiological and pathological conditions.

293 Identification and characterisation of circular RNAs by multiple displacement amplification

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Circular RNAs (circRNAs) are the newly discovered class of long non-coding RNA having a potential role in gene regulation by miRNA sponging, transcriptional regulation and translational ability across eukaryotes. However, owing to its less expression than linear mRNA, high depth sequencing reads become mandatory for identification which is a major hurdle often augmented by the newly evolving computational pipeline developed specifically for a particular set of organisms. This presentation deals with an experimental procedure by using multiple displacement amplification (MDA) of RNase R treated, RNase H minus Reverse Transcriptase (RT) derived cDNA for circRNA identification in Oryza sativa and Nicotiana benthamiana. The MDA generated amplicons were restriction digested, cloned and sequenced or used for next generation sequencing (NGS) and analysed by DCC pipeline for genome-wide circRNA identification. In order to augment the sensitivity to detect the lowered level expressed circRNAs, the method is in the process of further fine-tuning by depleting ribosomal RNA. Further, selected circRNAs were validated by divergent PCR followed by Sanger sequencing and/or restriction digestion and northern hybridisation. Identified circRNAs are characterised for its expression status over corresponding mRNA, genome location, conservation against reported circRNAs, etc. which will be discussed in the presentation. Putative functional properties like miRNA sponging ability and thereby transcriptional regulation and biogenesis by alternative backsplicing are also explored in our dataset. Our method is proved to be working in identifying circRNAs from different organisms (with or without having genome information) tested and found to be efficient and cost-effective in finding circRNAs from much less sequencing reads.

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294 Relationship between human centromeric satellite I noncoding RNA and m6A RNA modification

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Centromere is the important region for chromosome segregation. Human centromeres consist of repetitive sequences, and noncoding RNA (ncRNA) called as satellite I ncRNA is transcribed from these regions. Depletion of this RNA caused defects in chromosome segregation, suggesting that satellite I ncRNA has a role in this step (Ideue et al, 2014). Several proteins associate with this ncRNA and function as a ribonucleprotein complex to regulate chromosome segregation (Cho et al, 2018).

Here, we analyzed proteins interacting with satellite I ncRNA at interphase. Pulldown and Immuno-precipitation experiments revealed that WTAP, a component of the m6A RNA modification complex associates with satellite I ncRNA. m6A RNA modification is widely observed on celluer RNAs and regulates their localization, stability and translational efficiency. We found that IMP3/IGF2BP3, which was reported as a reader of m6A RNA modification, also interacts with satellite I ncRNA. Interestingly, depletion of satellite I ncRNA caused relocalization of WTAP from the nucleus to the cytoplasm at interphase. Based on these results, relationship between centromeric ncRNA and m6A RNA modification will be discussed.

295 A natural antisense long non-coding RNA overlapping the UGT76E12 gene of Arabidopsis thaliana regulates its expression by influencing local chromatin state in cis

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In recent years, eukaryotic non-coding RNAs were found to play an important role in a variety of processes ranging from gene expression regulation to translation. In plants, they have been shown to be involved in a wide range of biological processes including flowering time regulation and root development, and hormone and stress responses. Natural antisense long non-coding RNAs (lncNATs), a sub-type of lncRNAs that are transcribed from the antisense DNA strand of a protein-coding gene, have been revealed to influence development in various plant species. Using the model organism Arabidopsis thaliana, we investigated the regulatory potential of an lncNAT which overlaps the UDP-glycosyltransferase 76E12 gene (UGT76E12). Basal expression of UGT76E12 and its lncNAT (lncNAT-UGT76E12) is developmentally regulated and mostly localized in the root. Additionally, the expression of UGT76E12 is strongly induced in response to salt stress and to necrotrophic fungal infection. Ectopic overexpression of lncNAT-UGT76E12 did not affect sense gene expression, however; a knock out line of lncNAT-UGT76E12 (nat-ugt76e12ko) showed reduced steady-state levels of UGT76E12 mRNA and decreased accumulation of transcripts of the same gene in response to stress. The absence of differences in UGT76E12 mRNA stability between WT and nat-ugt76e12 lines and the analogous localization of promoter activity in reporter gene lines of UGT76E12 and lncNAT-UGT76E12, suggest a cis regulatory mechanism mediated by lncNAT-UGT76E12. Furthermore, chromatin immune precipitation (ChIP) and DNA methylation analysis indicate an altered chromatin state in nat-ugt76e12 compared to the WT confirming the predicted cis regulatory role of lncNAT-UGT76E12 on UGT76E12 expression. Additionally, the expression levels of UGT76E11, the closest related homolog just upstream of UGT76E12, are not altered when lncNAT-UGT76E12 is overexpressed or knocked out indicating that this regulatory effect is gene-specific.

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296 Structure and degradation of circular RNAs regulate PKR activation in innate immunity

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CircRNAs produced from back-splicing of exons of pre-mRNAs are widely expressed, but current understanding of their functions is limited. These RNAs are stable in general and are thought to have unique structural conformations distinct from their linear RNA cognates. Here we uncover that endogenous circRNAs tend to form 16-26 bp imperfect RNA duplexes and act as inhibitors of double-stranded RNA (dsRNA)-activated protein kinase (PKR) related to innate immunity. Upon poly(I:C) stimulation or viral infection, circRNAs are globally degraded by RNase L, a process required for PKR activation in early cellular innate immune responses. Augmented PKR phosphorylation and circRNA reduction are found in peripheral blood mononuclear cells (PBMCs) derived from patients of autoimmune disease systemic lupus erythematosus (SLE). Importantly, over-expression of the dsRNA-containing circRNA in PBMCs or T cells derived from SLE can alleviate the aberrant PKR activation cascade, thus providing a connection between circRNAs and SLE.

297 Expression and functions of hypoxia regulated long non-coding RNA HABON in hepatocellular carcinoma cells

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The adaptation of tumor cells to hypoxic microenvironment is one of the important characteristics of cancer. Long non-coding RNA (lncRNA) is a class of non-coding RNA that are greater than 200nt in length, which play important roles in tumorigenesis and development of cancers. In our previous work, hypoxia related lncRNAs in hepatocellular carcinoma cells were screened by lncRNA chip-array, and most of them were new RNAs with no report. We validated and analyzed the expression of these lncRNAs, and further screened it with the most important hypoxic-induced factor HIF-1α regulation. Among them, a lncRNA named HABON (Hypoxia Actived BNIP3 Overlapping Non-coding RNA), was not only regulated by HIF-1α but its expression increased significantly under hypoxia in a variety of tumor cells. We knock down or overexpress the amount of HABON in the SMCC-7721 hepatocellular carcinoma cells, and the results show that HABON can prompt the growth and proliferation of these cells and the formation of clones. In the future work, the biologic function of HABON in the adaptive survival and growth under hypoxia will be deeply studied. To decipher the molecular mechanism of HABON's function under hypoxia environment, RNA pull-down as well as mass spectrometry assay have been applied to identify the HABON interaction proteins. Furthermore, the function of these HABON-interaction proteins will be testified in hepatocellular carcinoma cells. This research would provide a new important clue for the adaptive survival and growth mechanism of tumor under hypoxia and would provide new evidence for the diagnosis and treatment of liver cancer.

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298 The susceptibility of sea-island cotton recombinant inbred lines to *Fusarium oxysporum* f. sp. *vasinfectum* infection is characterized by altered expression of long noncoding RNAs

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Disease resistance is one of the most complicated yet important plant traits. The potential functions of long noncoding RNAs (lncRNAs) in response to pathogenic fungi remain unclear. In this study, we sequenced the transcriptomes of four different sea-island cotton (*Gossypium barbadense*) recombinant inbred lines (RILs) with susceptible, highly susceptible, highly resistant, or super highly resistant phenotypes and compared their responses to *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) infection with those of their susceptible and resistant parents. Infection-induced protein coding genes were highly enriched in similar disease resistance-related pathways regardless of fungal susceptibility. In contrast, we found that the expression of a large number of *Fov* infection-induced lncRNAs was positively correlated with plant susceptibility. Bioinformatics analysis of potential target mRNAs of lncRNAs with both trans-acting and cis-acting mechanisms showed that mRNAs co-expressed or co-located with *Fov*-regulated lncRNAs were highly enriched in disease resistance-related pathways, including glutathione metabolism, glycolysis, plant hormone signal transduction, anthocyanin biosynthesis, and butanoate metabolism. Together these results suggest that lncRNAs could play a significant role in the response to pathogenic fungal infection and the establishment of disease resistance. The transcriptional regulation of these infection-susceptible lncRNAs could be coordinated with infection-susceptible mRNAs and integrated into a regulatory network to modulate plant-pathogen interactions and disease resistance. *Fov*-susceptible lncRNAs represent a novel class of molecular markers for breeding of *Fov*-resistant cotton cultivars.

299 Structural characterization of group II introns and long non-coding RNAs

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Non-coding RNAs are implicated in regulating numerous cellular processes such as genome editing, splicing regulation and signaling. While this broad category contains various classes of RNAs, our lab is interested in studying group II introns and long non-coding RNAs. Recent work demonstrates these RNAs fold into discrete modules containing intricate secondary structures that can serve as scaffolds for protein recruitment. We now seek to further our understanding by characterizing these RNAs on a tertiary level. The catalytic activity of group II introns make them an excellent reporter system for refining techniques which we can subsequently apply to other lncRNAs. Using biophysical methods, we have determined conditions that allow us to obtain a homogeneous population of folded RNAs. Currently we are using a combination of chemical probing and single particle cryo-electron microscopy to visualize the native structures of these RNAs. We are optimizing conditions in anticipation obtaining atomic resolution models soon. Overall, these studies will provide valuable insights on how RNA structure can follow function.

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300 Molecular functions of circRNAs in human myoblast proliferation and in rhabdomyosarcoma

Francesca Rossi[1], Ivano Legnini[1], Francesca Megiorni[2], Alessio Colantoni[1], Marco Guarnacci[1], Mariangela Morlando[1], Tiziana Santini[3], Gaia Di Timoteo[1], Dario Dattilo[1], Carlo Dominici[2], Irene Bozzoni[1,3]
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Circular RNAs (circRNAs) are covalently closed RNA molecules, derived from a particular splicing event (back-splicing), ubiquitously expressed among eukaryotes and conserved among different species. Their conservation, tissue-specificity and abundance suggest important biological roles. We previously identified several circRNAs expressed and modulated in myogenesis (Legnini et al, 2017). Among them, we showed that a circRNA (circ-ZNF609) is involved in myoblasts proliferation. Upon its depletion, in fact, the proliferation rate of human primary myoblasts is strongly reduced. To better investigate circ-ZNF609 role in proliferation, we studied it in rhabdomyosarcoma (RMS), a pediatric skeletal muscle tumor. We found that circ-ZNF609 is up-regulated in tissue biopsies from the two major RMS subtypes, embryonal and alveolar, and that its knock-down blocks the proliferation of an RMS-derived cell line at the G1-S checkpoint. Therefore, we investigated circ-ZNF609 effects on two well-known protein factors controlling cell proliferation: Rb (Retinoblastoma) and phospho-Akt. Upon circ-ZNF609 knock-down, we observed an alteration in phospho-Rb/Rb ratio and a decrease in phospho-Akt protein level due to an increased proteasome-mediated degradation. Thanks to bioinformatics analyses performed on RNA-seq data from circ-ZNF609-depleted RMS cells, we identified the affected pathways and the master regulators of cell cycle progression whose activity was altered. Among them we found ZWINT, TCF19 and CENPU (Rossi et al, 2019). We are now performing proteomics experiment in RMS cells upon circ-ZNF609 knock-down, to integrate these results with transcriptomics data, and we are also identifying circ-ZNF609 interactors to deepen our knowledge about its molecular mechanism of action.

Since we are interested in studying other circRNAs involved in RMS onset and progression, we identified a subset of circRNAs differentially expressed among human myoblasts, and alveolar and embryonal RMS cell lines. We experimentally validated them, and we are now starting a phenotypic screening analysis.

301 A novel long noncoding RNA lincSox2-AS regulates the cancer stem cell population in glioblastoma multiforme

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Background: Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults, with a two-year survival rate of less than 25%. The recurrence of GBM has been attributed to the presence of glioma cancer stem cells (CSC), which are thought to play a central role in tumor development and progression.

Methods: Using the Ensembl Genome Browser from the European Bioinformatics Institute, an *in silico*"nearest-neighbor" approach was used to identify 112 lncRNAs that were in close proximity to the transcription factors involved in the maintenance of stemness. The lncRNA candidates were then subjected to a series of *in silico* examinations and *in vitro* experiments. The remaining 20 lncRNA candidates were then validated in human GBMs, followed by 3' and 5' RACE. Finally, a novel lncRNA antisense to the transcription factor Sox2, lincSox2-AS, was identified to be functionally active in glioblastoma.

Results: LincSox2-AS is expressed in normal brain and only in grade IV astrocytoma, i.e., glioblastoma. Additionally, its expression is significantly enriched in CSCs v. normal human fetal neural stem cells (NSCs). Upon differentiation of NSCs into astrocytes, lincSox2-AS decreased in expression. However, lincSox2-AS was significantly enriched after CSCs underwent differentiation, suggesting its role in regulation of the CSC phenotype. The knockdown of lincSox2-AS in CSCs resulted in a decrease in stemness markers, Nestin and Sox2, while showing an increase in neuronal marker, Tuj1, and a corresponding decrease in Cyclin B1 as well as Olig2, suggesting differentiation of CSCs towards a neuronal lineage. In contrast, no such phenotype was observed upon knockdown of lincSox2-AS in NSCs, implicating a cancer-specific role of lincSox2-AS. Bioinformatic analysis of lincSox2-AS revealed a bi-directionally transcribed cell-type specific distal enhancer element for Sox2 in CSCs. Hi-C showed chromatin looping between lincSox2-AS enhancer element and Sox2 promoter.

Conclusion: LincSox2-AS represents an exciting epigenetic target for disrupting the CSC niche. By targeting a cell-specific and cancer-specific lncRNA like lincSox2-AS, we can have a higher chance of success in targeting the CSC niche than targeting a multitude of downstream pathways.

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302 A computational method for precise circular RNA isoform quantification

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Circular RNAs (circRNAs) are circular transcripts originating from non-canonical splicing events by forming a back-splice junctions (BSJ). Predominantly circRNA abundance is estimated by the identification of BSJs from transcriptome sequencing data. The currently existing algorithms for identifying circular isoform abundance, however, are not able to derive all circular splicing isoforms. We here propose a computational pipeline that improves circular RNA isoform identification and quantification and significantly facilitates the discovery of novel circRNAs and their functional roles in the cell.

By comparing control and circle-enriched RNA-seq libraries, we can characterize circRNA isoform profiles through a flow-based algorithm. We then generate a pseudo-linear isoform profile as an index for transcript quantification via expectation-maximization. The transcript quantities estimated by our method are in very good agreement with simulated circRNA read abundances (0.97 correlation).

303 lncRNAs as triggers of RNA editing in breast cancer

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Adenine to inosine deamination is the most common type of RNA editing in mammals. Inosine base pairs with cytosine and is interpreted as guanosine by the cellular machinery, which has a number of functional consequences. The phenomenon affects majority of pre-mRNAs and is catalyzed by ADAR enzymes, which recognize double stranded RNA (dsRNA) structures, especially in Alu repetitive elements located in the 3′-UTR and 5′-UTR of mRNAs and in introns. The medical implications may be extensive as it has been implicated in diseases ranging from cancer and neurological diseases to atherogenesis. In cancer, epitranscriptomic changes by RNA editing contribute to sequence diversity independently of DNA mutations. It is therefore believed that RNA editing studies should complement genome sequence data to understand the full scale of sequence alterations. Keeping this in mind, we performed identification of RNA editing events from RNA-Seq data in several subtypes of breast cancer, including ER+ and triple negative breast cancer (TNBC). Differential editing analysis revealed hundreds of loci that significantly differ between healthy and cancerous samples. Interestingly, these loci almost do not overlap between subtypes of breast cancer.

Another aspect of RNA editing phenomenon is that the substrate dsRNA structure, which is recognized by ADAR enzymes, could be formed either via intra- or intermolecular base pairings. For the latter scenario, we attribute a great regulatory potential to natural antisense lncRNAs (NATs), which are able to come into perfect and often extensive base pairings with their sense mates. Moreover, these lncRNAs often bear Alu elements, are enriched in the nucleus, where RNA editing occurs and, importantly, very often have no biological functions known. Our bioinformatics study confirmed that NATs could indeed be functioning as ubiquitous RNA editing triggers, including RNA editing sites that are changed in breast cancer. To the best of our knowledge, this is the first report of lncRNAs that drive RNA editing events. We are now working towards providing experimental evidence for those findings.

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304 Lnc-ing RNA structure to function

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Long non-coding (lnc)RNAs are key players involved in regulation of plethora of cellular processes, and they perform their functions through structure-mediated interactions with effector molecules. Also, their structure and function can be regulated by epitranscriptomic modifications. We study the association between lncRNA epitranscriptomics and functionality using an established system of Kaposi's sarcoma associated herpesvirus (KSHV) that during lytic reactivation expressed highly abundant and stable polyadenylated nuclear (PAN) lncRNA.

Previously, we have revealed secondary structure of PAN lncRNA inside the nuclear and cytoplasmic compartments of KSHV-infected cells, and within virions[1]. PAN conformation and protein binding profiles varied depending upon biological context, suggesting specific regulatory mechanisms, i.e. epitranscriptomic modifications, dynamic intermolecular contacts, that affect its structure and function.

We have focused our current efforts on revealing PAN RNA-RNA interactome network at latent and lytic stages of KSHV infection. The application of RNA antisense purification (RAP-AMT) identified interacting transcripts of viral origin, which were shown previously to encode proteins involved in immune response modulation,regulation of latency and lytic reactivation. Thehost RNAs interacting with PAN included non-coding (nc)RNAs and mRNAs involved in paraspeckles formation and ncRNAs regulating Cajal bodies functionality. We have also identified interacting miRNAs involved in IFN response and tumorigenesis.

The epitranscriptomic analysis revealed that PAN RNA undergoes dynamic epitranscriptomic modifications that likely dictate its intermolecular contacts. Both, N6-methyladenosine (m^6A) and pseudouridine, have been mapped with single-nucleotide resolution, revealing the first mechanistic insight in the epitranscriptomic deposition process on the full length viral lncRNA.

[1]Sztuba-Solinska J, Rausch JW, Smith R, Miller JT, Whitby D, Le Grice SFJ. 2017 Kaposi's sarcoma-associated herpesvirus polyadenylated nuclear RNA: a structural scaffold for nuclear, cytoplasmic and viral proteins.Nucleic Acids Res. 45(11):6805-6821.

Keywords: lncRNA structure and function, epitranscriptomics, interactome

305 The novel long noncoding RNA lncNB1 promotes tumorigenesis by interacting with ribosomal protein.

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The majority of patients with neuroblastoma due to N-Myc oncogene amplification and N-Myc oncoprotein over-expression die of the disease. Here our analyses of RNA sequencing data identified the novel long noncoding RNA lncNB1 as one of the transcripts most over-expressed in MYCN-amplified, compared with MYCN-non-amplified, human neuroblastoma cell lines and most over-expressed in neuroblastoma, compared with > 10,000 cancer tissues from all other organ origins. lncNB1 bound to ribosomal protein to enhance E2F1 protein synthesis, leading to DEPDC1B gene transcription. The GTPase-activating protein DEPDC1B induced ERK protein phosphorylation and N-Myc protein stabilization. Importantly, lncNB1 knockdown abolishes neuroblastoma cell clonogenic capacity / in vitro/ and leads to neuroblastoma tumor regression in mice, and high levels of lncNB1, ribosomal protein and DEPDC1B in human neuroblastoma tissues predicted poor patient prognosis. This study therefore identifies lncNB1, its binding ribosomal protein and its effect DEPDC1B as key factors in N-Myc-driven oncogenesis and as novel therapeutic targets.

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306 The up-regulated circRNA production blast-resistance rice is associated with alternative splicing complexity

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Circular RNAs (circRNAs) are covalently closed RNA molecules which broadly express in animal and plants. CircRNAs have been documented to play potential roles in plants' response to biotic and abiotic stress. However, the biogenesis and functional roles of plant circRNAs contributing to the pathogen infection response and resistance remain unclear. In this study, we performed circRNA-seq analysis on transcriptomes obtained from young leaves of both Magnaporthe oryzae-resistant and -susceptible rice strains under the blast-infected or uninfected conditions. A total of 2932 high-confident circRNAs were identified. The expressed-circRNAs in blast-resistant rice were significantly more diverse than that in the blast-susceptible one regardless of M. oryzae infection. The higher circRNA diversity in blast-resistant rice was primarily attributed to the increased capability in producing more than one circRNA per host gene, which was further correlated with more frequent alternative 3' and 5'SS back-splicing. We further showed that complex splicing sites presented in three or more splice junctions positively correlated with circRNA generation and their fraction were higher in the blast-resistant rice leaf. The functional link between circRNA production and blast-resistance in rice further supports the biological relevance of circRNAs in plants. We proposed that alternative back-splicing and alternative splicing within circRNAs contribute to the biogenesis and diversity of circRNA in rice and other plants, and the blast-resistance could be linked to the genomic alterations that alter the expression of genes encoding the determinants of circRNA biogenesis in rice.

307 Long noncoding *OIP5-AS1* acts as expression regulator of *OIP5* gene

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Long noncoding RNAs (lncRNAs) constitute an abundant class of transcripts in human, involved in a plethora of cellular processes and also linked to a number of human diseases, including cancers. Functions played by lncRNAs are closely related to their subcellular localization. As an increasing number of studies are focused on nuclear lncRNAs, only a handful of them have been functionally characterized so far. It is however known that nuclear-retained lncRNAs affect gene expression in varied ways, including modulation of alternative splicing and chromatin remodeling. Biologically significant alterations to both mechanisms are frequently observed in tumors. Owing to the fact that thus far only a handful of nuclear lncRNAs have been functionally characterized and a growing body of evidence indicates their deregulated expression in a variety of carcinomas, we set out to investigate the presumed molecular functions of carcinogenesis-linked lncRNA in modulation of alternative splicing and modification of chromatin structure. Based on in silico predictions, we have chosen Cyrano (also known as OIP5 antisense RNA 1 or OIP5-AS1) as the most promising candidate for experimental testing. Cyrano represents a natural antisense transcript (NAT) for a known oncogene, Opa interacting protein 5 (OIP5). Our computational analysis showed that Cyrano is enriched in nucleus. Subcellular fractionation of two human cell lines, HEK293T and K562, followed by quantitative real-time polymerase chain reaction (real-time PCR or qPCR) confirmed our in silico results, thus suggesting OIP5-AS1 might be involved in modulation of nuclear processes. Interestingly, analysis of the transcript per million (TPM) expression data from Gene Expression Profiling Interactive Analysis (GEPIA) revealed that Cyrano expression level is increased in leukemia samples compared with normal ones. As knockdown of Cyrano with antisense LNA gapmers led to inhibited expression of its sense partner, OIP5, implicated in development of various cancer types, we speculate that Cyrano might be an attractive target in cancer therapy. To explore the molecular basis for this effect and test our hypothesis, we are planning a number of experiments. In particular, RNA pull-down assay is expected to provide insight into possible involvement of protein factors in the regulation.

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308 An improved method for circular RNA purification that efficiently removes linear RNAs that have G-quadruplexes or structured 3′ ends

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Thousands of eukaryotic protein-coding genes generate circular RNAs that have covalently linked ends and are resistant to degradation by exonucleases. To prove their circularity as well as biochemically enrich these transcripts, it has become standard in the field to use the 3'-5' exonuclease RNase R. Here, we demonstrate that standard protocols involving RNase R fail to digest >20% of all highly expressed linear RNAs, but that these shortcomings can be easily overcome. RNAs with highly structured 3' ends, including snRNAs and histone mRNAs, are naturally resistant to RNase R, but can be efficiently degraded once a poly(A) tail has been added to their ends. In addition, RNase R stalls in the body of many mRNAs, especially at G-rich sequences that have been previously annotated as G-quadruplex (G4) structures. Upon replacing K^+(which stabilizes G4s) with Li^+in the reaction buffer, we find that RNase R is now able to proceed through these sequences and fully degrade the mRNAs in their entirety. In total, our results provide important improvements to the current methods used to isolate circular RNAs as well as a way to reveal RNA structures that may naturally inhibit degradation by cellular exonucleases.

309 Molecular Recognition of RNA by the Bacterial RNA-Chaperone Protein ProQ

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In bacteria, small (s)RNAs play important roles in gene regulation through base pairing with mRNA targets. The stability and function of sRNAs are often supported by RNA-binding proteins such as Hfq, the paradigmatic bacterial RNA-chaperone protein. A structurally distinct protein, called ProQ, has recently been discovered to act as a global RNA-binding protein in proteobacteria, binding to dozens of sRNA and mRNA sequences to regulate mRNA-expression levels through interactions with both 5' and 3' UTRs. Despite excitement about the regulatory roles ProQ may play in bacteria such as *Salmonella* and *E. coli,* very little is known about the molecular mechanisms of ProQ-RNA interactions. Our goal is to define these mechanisms – mapping both the amino acids on ProQ's surface and nucleotides of RNAs that contribute to binding – using a bacterial three-hybrid (B3H) assay we have recently developed. In the B3H assay, ProQ is fused to RNA polymerase (RNAP) and a hybrid RNA containing either an sRNA or mRNA of interest is tethered to a DNA sequence upstream of a test promoter. Interaction of ProQ with the RNA stabilizes the binding of RNAP to the test promoter and activates transcription of a reporter gene. We have detected B3H interactions of ProQ with several of its RNA partners using an *E. coli*-based genetic reporter. Our data suggest that a conserved N-terminal-domain (NTD) mediates RNA binding and we have identified several point mutations that alter ProQ's interactions with target RNAs. Current efforts are focused on forward genetic screens to fully map the binding interface(s) of ProQ for both sRNAs and mRNAs, and to determine whether these binding sites are distinct or overlapping. We are further using our B3H assay to explore the structures and sequences of RNA that are necessary and/or sufficient for ProQ interaction. Using a previously determined NMR structure for the ProQ NTD and guided by the results of our forward and reverse genetic approaches, we are developing a model of molecular recognition between ProQ and its interacting RNAs.

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310 Cold sensing by a group II intron fine-tunes host gene expression

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Group II introns disseminate across all domains of life by interrupting genes. The *Lactococcus lactis* LtrB (Ll. LtrB) group II intron resides in a conjugative plasmid pRS01 within its host relaxase gene that is responsible for the plasmid's conjugal transmission. Our previous work showed that the Ll.LtrB intron inhibits expression of the host gene and thus the horizontal transfer of pRS01 by mRNA targeting. However, whether and how this regulatory pathway responds to environmental cues have not been determined. Here we show that the splicing of the Ll.LtrB intron in the native *lactococcal* host is inhibited at temperatures that are lower than the physiological one. In-cell structure profiling reveals that key tertiary interactions essential for maintaining the scaffold structure of the intron RNA are disrupted at those lower temperatures. In addition, *in vitro* assays indicate that such splicing inhibition by cold takes place in a protein-independent fashion. However, despite suppression of splicing, both levels of the host gene-encoded mRNA (ligated exons) and the relaxase protein increase, likely due to significant reduction in the mRNA-targeting activity of the intron, leading to increased levels of translatable mRNA. Finally, we show that the pRS01 plasmid conjugation is stimulated as the temperature is reduced. Taken together, this study suggests that the bacterial group II intron can modulate the function of the host gene by sensing and adapting to low temperatures.

311 Analysis of FinO targets in *Salmonella* Typhimurium

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FinO-domain containing proteins mediate post-transcriptional regulation by promoting base pairing between sRNAs and target mRNAs. Interestingly, in *Salmonella enterica* the number of bound sRNAs can range from a single sRNA in the case of the pSLT plasmid encoded FinO to dozens in the case of the chromosome-encoded FinO-domain containing protein ProQ. What defines the ability of a FinO-domain containing protein to bind one or more sRNAs remains elusive. *In vivo* global analysis of the extent of FinO targetome in *Salmonella* remained to be established. *In vivo* suite of FinO bound RNA was determined by RIP-seq at different stages of *Salmonella* growth curve, and binding sites were determined by CLIP-seq. While FinP is the unique sRNA bound, FinO binds in addition dozens of transcripts from both the genome and *Salmonella* plasmids.

We aim to decipher the features that define the specificity of the pSLT encoded FinO to bind exclusively the sRNA FinP in the same cytoplasm where the closely related ProQ binds dozens.Of note, while the FinO domain is conserved, ProQ carries an extended C-terminal domain not present in FinO, however, these differences do not seem to determine the specificity of FinO targetome compared to ProQ. FinO sRNA targetome can be expanded when overexpressed, displaying a similar RNA binding profile when expressed at similar levels than ProQ. Our results point towards protein localization and protein expression level as factors determining the targetome of FinO containing proteins rather than differences at the aminoacidic level.

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312 A census of RNA and protein complexes in a model Gram-positive bacterium reveals exonuclease-mediated stabilization of small RNAs in the competence regulon

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A vast number of RNAs and proteins in a bacterial cell form complexes in order to fulfill their physiological functions. This includes the whole range from small complexes like the signal recognition particle to giant ones like the ribosome. Yet, the *in vivo* interactions of the majority of RNAs and many proteins remain unknown because typical molecular biological strategies that analyze these complexes suffer from selectivity, biases and low throughput. Even more so in Gram-positive species, knowledge of these interactions is lagging behind. With Grad-seq (gradient profiling by sequencing), we recently introduced a high throughput complexomic approach based on gradient partitioning that allows us to analyze the *in vivo* macromolecular state of both RNAs and proteins in a single experiment.

Here, we have applied Grad-seq to the model human pathogen *Streptococcus pneumoniae*, reconstructing the in-gradient sedimentation for >2,200 RNAs and >1,300 proteins. Our data reproduce the limited number of known RNA/protein and protein/protein complexes, verifying the validity of our approach. We then used clustering methods to select for a set of co-migrating small regulatory RNAs (sRNAs) involved in the regulation of competence. Downstream analyses of in-gradient distributions and tag-based capture of these sRNAs led to the identification of a functional interaction with Cbf1 (a.k.a. YhaM). Contrary to expectation, we show that the nucleolytic activity of Cbf1 stabilizes these sRNAs and thereby represses competence. Together, we provide the first Grad-seq map for a Gram-positive organism and unveil Cbf1 as a negative regulator of competence.

313 Mimicking cotranscriptional riboswitch folding via a superhelicase unwinding assay

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Many riboswitches regulate gene expression by coupling small molecule binding to changes in RNA conformation cotranscriptionally. For example, ZTP riboswitches bind the purine biosynthetic intermediate ZMP (and its triphosphorylated form ZTP) to regulate folate biosynthesis genes by modulating transcription termination. Although cotranscriptional folding is critical to riboswitch function, monitoring RNA folding during transcription is challenging. To analyze how the ZTP riboswitch folds and binds ZMP, we have utilized an engineered "superhelicase" to rapidly unwind an RNA/DNA duplex, thereby releasing single stranded RNA in a 5´-to-3´ direction to sequentially fold, mimicking transcription. In this assay, which releases RNA at a rate similar to transcription, RNA folding is observed through FRET and distinguishes conformations corresponding to riboswitch termination and readthrough. In bulk and single-molecule experiments at equilibrium, the riboswitch folds completely into a terminated state incapable of binding ZMP. However, during helicase unwinding, the ZTP riboswitch binds ZMP and folds into a higher FRET readthrough state, consistent with cotranscriptional folding. Consistent with bulk assays, we show that readthrough is promoted by mutating the termination hairpin, folding more slowly, or using a complex mimicking an infinitely paused transcription intermediate. During real time observation of individual unwinding events, RNA folding occurs in seconds and passes through a high FRET intermediate before reaching a terminated state. Moreover, we find that the ZTP riboswitch is kinetically controlled in bulk transcription assays and helicase unwinding experiments. Taken together, these results suggest that the RNA sequence encodes for kinetic control independent of the motile enzyme, whether polymerase or helicase.

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314 The contribution of the Hfq protein to the annealing of Class II sRNAs to their mRNA targets

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The matchmaker protein Hfq facilitates the pairing of small regulatory RNAs (sRNAs) to their target mRNAs in *Escherichia coli* and related bacteria. Hfq utilizes three distinct sites to bind RNA molecules. Canonical, or Class I, sRNAs use 3′-terminal uridine tails to bind the proximal face of Hfq and internal AU-rich sequences to bind the rim of the Hfq ring, while their target mRNAs use A-rich sequences to bind the distal face. Recent *in vivo* studies showed that a subset of sRNAs, called Class II, were dependent on the rim surface of Hfq to regulate target mRNAs. The role of the Hfq rim as the binding site of mRNAs regulated by Class II sRNAs was recently showed *in vitro* for MgrR sRNA.

Here, the effects of mutations in RNA binding sites of Hfq on the kinetics of annealing of ChiX sRNA to *chiP* mRNA were studied to better understand how Hfq contributes to the pairing of Class II sRNAs. It has been previously shown that ChiX was particularly efficient in competition against other sRNAs for binding to Hfq *in vivo*. Similarly as MgrR sRNA, ChiX contains A-rich and U-rich sequences, which bind the distal and proximal faces of Hfq, respectively. As opposed to MgrR, the data showed that the mutation in the proximal face of Hfq did not markedly affect ChiX annealing to *chiP* mRNA, which suggests that ChiX binds more strongly than MgrR to the distal face of Hfq. Similarly as for MgrR, the mutation in the rim of Hfq trapped ChiX on Hfq and prevented its annealing to *chiP* mRNA, which supports the role of the rim in binding mRNAs regulated by Class II sRNAs. Finally, the mutation in the distal face prevented the release of ChiX-*chiP* complexes from Hfq, which supported the role of the distal face in binding of additional sRNA molecule. Overall, the data suggested similar mechanisms of the Hfq contribution to regulation by Class II sRNAs ChiX and MgrR, while highlighting the fine tuning of ChiX interactions with Hfq, which explains its superior efficiency in the competition for access to Hfq *in vivo*.

315 Control of the *tpiA* mRNA abundance by RNase G contributes to glycolysis upon oxygen availability in *Escherichia coli*

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Previous studies have shown that many enzymes such as enolase, alcohol dehydrogenase, and triosephosphate isomerase involved in carbohydrate metabolism are upregulated in *rng* null mutant of *E. coli*. However, molecular mechanisms underlying RNase G-associated regulation of carbohydrate metabolism have not been characterized. Here, we show that *tpiA* mRNA, encoding a glycolytic enzyme, triosephosphate isomerase, is cleaved by RNase G in its 5' untranslated region, leading to destabilization of the mRNA. Nucleotide substitutions within the cleavage site resulted in increased amounts of TpiA protein, implying a direct effect of RNase G on TpiA expression. Additionally, we discovered that increased expression level of *tpiA* in *E. coli* cells under the microaerobic condition is associated with down-regulation of RNase G expression. Our findings show that RNase G contributes to modulation of glycolysis in response to oxygen availability in *E. coli.*

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316 New method for unbiased quantification of riboswitch transcriptional activity

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In bacteria, the expression of significant fraction of genes is controlled by premature transcription termination. An interesting example of such mechanism are riboswitches; regulatory sequences, usually located within the 5' untranslated region (5'UTR) of certain genes, able to bind small cellular compounds, therefore evoking regulatory effect. In vast majority of riboswitches, interaction of ligand-binding domain with a given ligand (metabolite) causes structural rearrangements, resulting in the formation of termination hairpin and consequently, premature transcription termination. Terminated as well as read-through transcripts are detectable by standard experimental procedures (like northern blot or real-time PCR). However, an absolute quantification cannot be easily achieved by currently available methods. This fact was an inspiration for creation of a new method for direct identification and absolute quantification of transcription termination events, as a result of riboswitch activity. The first step in the developed protocol requires a site-directed RNaseH-induced cleavage (covering transcription termination site), resulting in separation of two transcript's populations: 5' part – corresponding to the sum of terminated and read-through transcripts and 3' part, corresponding to full-length transcripts alone. The cleavage efficiency is then determined by primers flanking the cleavage site. An absolute concentration of terminated and read-through transcripts is quantified using the droplet digital PCR (ddPCR) technology. As it is shown, utilization of our protocol allows for precise and unbiased quantitative analysis

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317 Unveiling the Hfq-mediated RNA-RNA interactome of Salmonella Typhimurium

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Post-transcriptional control of mRNAs by base pairing small RNAs in the bacterial model organisms Escherichia coli and Salmonella Typhimurium mainly occurs through the action of the RNA chaperones, Hfq and ProQ. Knowing the targets of sRNAs is critical to understanding the complex post-transcriptional networks in these bacteria. Traditionally, the prediction of sRNA targets was done experimentally by pulse-expression of individual sRNAs or by bioinformatical prediction tools. However, recent studies showed how combining proximity in vitro RNA ligation and high throughput RNA-sequencing technologies (RIL-seq, CLASH and GRIL-seq methods; Ref. 1,2) can yield global maps of RNA-RNA interactions in these bacteria. This study aims to identify the Hfq-mediated RNA-RNA interactome in the human pathogen Salmonella Typhimurium. We will apply RIL-seq in different growth conditions to produce a high-resolution map of the Hfq-RNA-RNA interactome with relevance for infections. One long-term goal is to reveal the post-transcriptional networks of virulence gene regulation in animal infection models.

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318 Comprehensive sequence analysis of group II introns and their phylogenetic profiles in bacteria

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Group II introns are the ribozymes found in genomes of bacteria, archaea as well as eukaryotic organelle. Most group II introns contain Intron Encoded Protein (IEP), and complex of translated IEP and intron RNA promote self-splicing and mobility reactions. These reactions contribute to increasing copy number of the introns in genomes. Several studies have attempted to identify IEP sequences from bacterial genomes by searching for reverse transcriptase domain inside IEP. Gathered IEP sequences are phylogenetically classified into approximately 14 types, based on their sequence similarities. It is known that some species in prokaryotes have particularly high number of group II introns (more than 10 per genome), while only 0-2 group II introns are present in most of prokaryotic genomes. However, phylogenetic distribution of these species with increasing number of introns has not been clarified.

Here, we developed a bioinformatics pipeline to comprehensively detect group II introns containing IEP from genome sequence data. The pipeline is available to calculate the number of the introns inside the genome. We applied this program to representative 1,661 bacterial genomes and obtained a set of 1,760 introns from 372 species. Among these species, 40 bacterial genomes possess 10 or more introns, and we found that Bacterial-C IEP-type introns predominantly proliferate in 25 species of three bacterial phyla. Furthermore, Chloroplast-Like (CL) IEP-type introns were dominant in all 5 Cyanobacteria genomes out of the 40 species. In previous studies, it is reported that target regions during mobility reaction of Bacterial-C IEP-type introns are less specific than that of other introns, including CL IEP-type introns. Therefore, it is implied that at least two different factors act on the remarkable increase of the introns in genomes. Based on these observations, we will discuss the evolution of group II introns in bacteria together with the results of archaeal genome analysis.

319 Exploiting nucleotide signaling molecules for antibiotic development by using riboswitch sensors

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Improved screening methods and new targets are needed to revitalize antibacterial drug discovery. A promising solution involves exploiting natural bacterial riboswitches as sensors for monitoring in vivo metabolic signaling changes in response to small molecules. Riboswitches are noncoding RNAs usually found in 5′ untranslated regions of mRNAs that function to regulate gene expression by selectively binding their cognate ligands. To date, nearly 40 different classes of riboswitches responding to a variety of fundamental metabolites and ions have been discovered and validated (1). Riboswitches have been bioinformatically identified in a wide variety of organisms, including pathogenic bacteria. Signaling pathways, which could be novel and effective antibiotic targets, are naturally monitored by at least five experimentally validated riboswitch classes that respond to RNA-based signaling molecules, including ppGpp and c-di-GMP. An example of effective antibiotics that affect signaling molecule pathways are antifolates. Bacteria use 10-formyl-tetrahydrofolate for a key transformylase reaction with the purine pathway intermediate 5-aminoimidazole-4-carboxamide ribonucleotide (ZMP) to form inosine monophosphate. If folate is deficient, the nucleotide signaling alarmones ZMP and its triphosphorylated form ZTP accumulate. A ZTP riboswitch subsequently triggers expression of folate biosynthesis genes to maintain homeostasis of purine and folate metabolites (2). We have developed a high-throughput screen (HTS) using a ZTP-riboswitch-controlled β-galactosidase reporter gene in Escherichia coli for discovering novel antifolate compounds. We demonstrate that our assay can also be used to establish structure-activity relationships of antifolate derivatives, which can be used to develop compounds with improved activity. Thus, we present a proof-of-concept for exploiting bacterial riboswitch reporters that can be used in HTS endeavors to discover novel small molecules that disrupt pathways affecting critical signaling metabolites.

(1) McCown, P.J., et al. 2017. RNA 23:995-1011.

(2) Kim P.B., et al. 2015. Mol Cell 57:317-28.

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320 A draft RNA landscape of the colorectal cancer-associated germ *Fusobacterium nucleatum*

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The anaerobic gram-negative bacterium *Fusobacterium nucleatum ss. nucleatum* (*FNN*) is increasingly gaining attention due to its role in multiple diseases outside of its natural habitat. Recent studies found a significant association of *FNN* with colorectal cancer (CRC) or adenoma tissue for which its increased presence correlates with worsened patient prognosis and resistance to the common chemotherapeutic. Despite this, only few genes such as the virulence factor FadA have been characterized and even less is known about the transcriptional regulation in *FNN* and its possible connection to CRC.

Hence, to aid in a better understanding of this enigmatic bacterium, we are generating a functional RNA map of FNN by utilizing differential RNA-seq (dRNA-seq) during different growth stages. The method has been widely used for the analysis of transcriptomes of various bacteria allowing the precise identification of transcriptional start sites (TSS), 5 ‘UTRs as well as the discovery of small RNAs (sRNAs). In order to additional dissect regulatory processes in *FNN*, we analyzed the global transcriptomic changes under different, potential infection relevant environmental conditions such as iron stress.

Our global RNA-seq approach identified shared but also growth phase-dependent TSS. This enabled the identification of promoters important for the transcriptional regulation during growth but also of specific genes and regulons induced by different stress stimuli. Our data further allowed the improvement of the current annotation and led to the discovery of the first regulatory sRNAs in *FNN* differentially in growth or environmental stress conditions. Combined, this enabled us to begin study the role of these novel sRNAs in the context of different stress conditions, which may shed light on the physiology of this cancer-associated pathogen.

321 A Cas12a-mediated CRISPRi screen for small noncoding RNAs in the human commensal *Bacteroides thetaiotaomicron*

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Gram-negative, obligate anaerobic *Bacteroides thetaiotaomicron* is one of the dominant members of the intestinal bacterial consortium in healthy human beings. Transposon mutant libraries of *B. thetaiotaomicron* were generated and screened to identify genetic determinants required for colonization of germ-free (Goodman et al, CH&M, 2009) and gnotobiotic (Wu et al, Science, 2015) mice. This approach, whose underlying principle is that the stochastic insertion of a transposon disrupts the function of the targeted gene, has however been largely restricted the screening of protein-coding genes and operons.

Small noncoding RNAs (sRNAs) are widely used within the bacterial kingdom to adapt gene expression in response to myriads of external and intrinsic cues. We recently identified hundreds of sRNA candidates in the *B. thetaiotaomicron* genome, whose functions are currently not known. However, due to their short length (typically 50-500 nt), sRNAs are less likely to be hit by random mutagenesis and, consequently, they are poorly reflected in existing Tn-seq (transposon sequencing) datasets.

For this reason, in this work we seek to employ CRISPR interference (CRISPRi) to identify and characterize *B. thetaiotaomicron* sRNAs essential for growth under defined conditions. We *in-silico* screened several protospacer adjacent motifs (PAMs) to identify the one with the highest occurrence among the sRNA pool. The analysis resulted in the AT-rich 5'-TTV PAM recognized by Cas12a (Cpf1), in line with the low GC content of the *B. thetaiotaomicron* genome. We also employed a cell-free transcription and translation system (TXTL) to screen potential nucleases to express in our library. Finally, by using the Nanoluc luciferase as a reporter, we assessed how the position and strandness of the targeted region inside the reporter gene affect the extent of transcriptional repression by Cas12a.

The CRISPRi approach described in this study will allow us to pinpoint functionally important sRNAs in *B. thetaiotaomicron* grown under defined conditions, including the ones that mimic the bacterium's *in-vivo* niche.

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322 The transcriptomic landscape of the dominant human gut commensal, *Bacteroides thetaiotaomicron*

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The major human gut symbiont *Bacteroides thetaiotaomicron* plays a vital role in maintaining healthy host metabolism and colonization resistance against pathogenic bacteria. To do so, *B. thetaiotaomicron* must adapt its global gene expression to the harsh environment of the large intestine that includes ever-changing nutrient availabilities, high concentrations of bile salts, host immune surveillance, high bacteriophage levels, and intense resource competition. However, investigations of the underlying regulatory networks have so far been mainly restricted to protein-mediated, transcriptional control mechanisms, largely neglecting the possible involvement of complementing RNA-mediated gene expression control.

Key to the systematic identification of RNA-based expression control mechanisms is a high-resolution transcriptome map of the respective organism. To address this, we performed differential RNA sequencing (dRNA-seq, Sharma et al.), which involves the selective enrichment of primary transcripts and consequently enables the identification of native transcription start sites at high resolution. Specifically, dRNA-seq was applied to *B. thetaiotaomicron* grown in rich medium to three defined growth stages in vitro - early-log, mid-log and stationary phase. We identified a total of 3,936 transcription start sites (TSSs), categorized into primary (1,792), secondary (381), internal (942), antisense (1086) and orphan TSSs (248) based on their proximity to, or location within, annotated coding sequences. The analysis confirmed previous findings on promoter architecture, and informed about transcript organization and operon structure in this bacterium. Additionally, our approach revealed hundreds of noncoding RNA candidates from any of the major classes including cis- and trans-encoded antisense RNAs, intergenic as well as 5´ and 3´UTR-derived small RNAs (sRNAs), and putative riboswitches and RNA thermometers. High-confidence sRNA candidates are currently being investigated at a mechanistic level. The resulting data will improve our understanding of post-transcriptional control in *B. thetaiotaomicron* and - more generally - anaerobic commensals.

References: Sharma, Cynthia M., et al. "The Primary Transcriptome of the Major Human Pathogen Helicobacter Pylori." Nature, vol. 464, no. 7286, 2010, pp. 250-55, doi:10.1038/nature08756.

323 The recognition of small RNAs and regulated mRNAs by *E. coli* ProQ protein

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Small regulatory RNAs (sRNAs) are involved in numerous processes in bacterial cells. sRNAs act by base pairing with target mRNAs, in this way affecting their translation or stability. Those sRNAs, which are *trans*-encoded pair with partly complementary mRNA sequences, and are mainly involved in the bacterial adaptation to environmental stress and maintenance of cellular homeostasis. On the other hand, *cis*-encoded sRNAs are fully complementary to regulated mRNAs, and several of them are involved in the control of plasmid replication or in the toxin-antitoxin systems. The main matchmaker protein involved in the regulation by *trans*-encoded sRNAs is the Hfq protein. It has been recently shown that another protein, ProQ, binds many *cis*-encoded sRNAs in *E. coli* and *S. enterica*. ProQ consists of the N-terminal ProQ/FinO domain and the C-terminal Tudor-like domain, which are connected by an extended linker.

To better understand how the ProQ protein recognizes RNA molecules the equilibrium binding of *E. coli* ProQ and Hfq proteins to several sRNAs known to interact *in vivo* with either ProQ (3´-UTR-*cspE*, SibA, RaiZ) or Hfq (MicA, ChiX) was analyzed using a gelshift assay. Additionally, to explain the role of ProQ in sRNA-mRNA interactions the unassisted annealing of several sRNAs to complementary mRNAs was compared to the corresponding reactions in the presence of ProQ. The results of these studies should help to better understand the determinants of RNA recognition by ProQ and the contribution of ProQ to sRNA pairing with their mRNA targets.

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324 RNA-seq-based approaches for the characterization of small proteins involved in *Salmonella* Typhimurium virulence

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Due to their nature, small proteins (<50 aa in length) are difficult to identify and investigate with standard biochemical approaches. Information regarding their presence has been slowly building up in recent years, and functional characterization of a few candidates demonstrated their involvement in diverse cellular processes.

In order to identify virulence-related small proteins in the bacterial pathogen *Salmonella* Typhimurium, we re-analyzed existing RNA-seq data, as *Salmonella* infects epithelial host cells (Westermann *et al.*, 2016). This highlighted the mRNA of the putative protein YjiS (54 aa long) as one of the most strongly induced transcripts upon infection. Its translation has been verified by genomic tagging as well as ribosome profiling performed in infection-relevant conditions, confirming the coding sequence boundaries. Infection experiments with *yjiS*-deficient and -proficient strains revealed that YjiS serves as an anti-virulence factor, with its absence promoting *Salmonella* escape from the host and re-infection of neighboring cells. Comparative host-pathogen transcriptomics - on host cells infected with either wild-type *Salmonella* or a Δ*yjiS* mutant - indicated that YjiS hardly affects the bacterial transcriptome, but instead exerts a subtle effect on the host response at 5 h post infection.

MgrB is a 48 aa-long protein, conserved within *Escherichia coli* and *Salmonella*, and a known regulator of the PhoPQ two-component system (TCS) (Salazar *et al.*, 2016). As this TCS is involved in *Salmonella*‘s adaptation to the intracellular environment, we studied the role of MgrB during host cell infection. Comparing the transcriptomes of wild-type and Δ*mgrB* strains grown in infection-relevant conditions indicated its positive effect on several motility- and chemotaxis-related genes as well as the sRNA CsrB.

Together, comparative host-pathogen RNA-seq represents a useful approach for pinpointing the molecular footprints of virulence-associated small proteins in the bacterial transcriptome during infection and the corresponding host response. This knowledge may serve as the starting ground for tailored functional studies to decipher the molecular mechanisms of infection-relevant small proteins in *Salmonella* and other bacterial pathogens.

325 Investigation of noncoding RNAs in the protozoan parasite *Leishmania braziliensis*

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Leishmania is a genus of trypanosomatid protozoan parasites and the causative agent of leishmaniases. *Leishmania braziliensis* species is the etiological agent of the mucocutaneous leishmaniasis, a morbid form of the tegumentary leishmaniasis in Central and South America. Gene expression in these parasites is regulated via post-transcriptional mechanisms comprising the action of cis and trans-regulatory elements. In this context, noncoding RNAs have been poorly explored as factors involved in the regulation of gene expression in *Leishmania* and should be examined. In this study, the whole transcriptome, coding, and ncRNAs, of *L. braziliensis* was investigated. To uncover ncRNAs a computational pipeline was designed and 11,372 putative ncRNAs were identified in *L. braziliensis*, allowing classification into different ncRNAs classes such as lncRNA, antisense ncRNAs, and UTR derived RNAs (uaRNAs). Two hundred and thirty-nine ncRNAs were differentially expressed across the life cycle of *L. braziliensis*. A small group of ncRNAs was selected and subjected to Northern blotting to confirm size and differential expression. A group of sense long ncRNAs (lncRNAs) and a couple of antisense ncRNAs are under investigation: knockout lines (KO) were generated, and the phenotypic analysis for three of the KO transfectants on in vitro macrophage infections suggested that attenuation of virulence occurred in the absence of these ncRNAs. Protein interacting with these ncRNAs were investigated with pull-down essays, a group of functionally related proteins has been detected, and RNA targets for the antisense ncRNAs have been identified and are under investigation. This work represents an outline of *L. braziliensis* transcriptome contributing to improving the understanding of the composition and functional role of ncRNA in the parasite.

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326 Study of the existence and relevance of human microRNA-encoded peptides (miPEPs) in hematological malignancies.

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MicroRNAs (miRNAs) play key roles in normal and malignant hematopoiesis. While the mechanisms by which miRNAs affect tumorigenesis start to be better understood, how miRNA expression is regulated has not yet been fully elucidated. A new mechanism of regulation has been recently identified in plants, showing that peptides encoded by short open reading frames (sORFs) within the primary miRNA transcripts (pri-miR) can specifically activate the transcription of their associated miRNA. Whether pri-miRs conserve this bivalent non-coding/coding function in mammalian cells and whether the accumulation of their associated mature miRNA is the prevalent mechanism of action of miRNA-encoded peptides (miPEPs) in human cells is currently unknown. In the canonical miRNA biogenesis pathway the pri-miRs are efficiently processed in the nucleus by the Drosha-DCGR8 microprocessor complex to give rise to the 70-nucleotide long pre-miR stem-loop structure. However, in contrast with the canonical biogenetic mechanism, our preliminary data indicate that some pri-miR species, containing several evolutionary conserved sORFs, can be found in the cellular cytoplasmic compartment and might have coding potential. Therefore, based on our preliminary observations, we hypothesize that the mechanism of miRNA regulation by miPEPs is conserved in human cells. We now seek to identify naturally expressed miPEPs in human cells using a combination of ribosome profiling and mass spectrometry approaches, and we then will evaluate the function and therapeutic potential of these miPEPs in hematological malignancies.

327 Regulatory functions of nested retrocopies derived from protein-coding genes

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Retrocopies of protein coding genes are duplicates created in the process of retrotransposition in which mature mRNA is reverse transcribed into cDNA and subsequently inserted into the genome. A great number of reports show that many of retrocopies are transcriptionally active and thus they can be important players involved in various molecular processes.

In our research we are focused on roles of nested retrocopies in gene expression regulation on DNA and RNA level. We performed the bioinformatic analyses to identify retrocopies overlapping protein-coding and non-coding genes in human genome. Retrocopies may overlap within exonic and/or intronic regions, thus we decided to analyzed their possible role on two different levels of regulation. Due to the fact that retrocopies are duplicates of parental genes, the presence of their antisense sequence in exons of other genes can be considered as a potential source of trans-NATs. We found 169 retrocopies incorporated into exons in antisense orientation to the parental genes. Based on the transcript support level and length of retrocopy-derived sequence, we selected 15 strong candidates for further experimental analysis.

Besides exonic retrocopies, we analyzed those located in introns in the context of transcriptional interference. For this purpose, we select retrocopies localized in intron but also downstream of at least one shorter splice variant. We found 51 retrocopies meeting these requirements from which we select 3 pairs for further analysis. We experimentally verified their expression by RT-PCR as well as, based on various online sources, we predicted their promoter regions. To examine the influence of retrocopy expression on host gene transcription, we decided to delete the retrocopy and/or part of promoter region from the genome in CRISPR-Cas9 experiments. Interestingly, in the case of retrocopy *retro_hsap_4044* located in intron of *ERLIN2* gene, we observed changes in splicing.

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328 Nucleic Acid Profiling in Differentiating Mesenchymal Stem Cells and their Extracellular Vesicles

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Mesenchymal stem cells (MCSs) are adult stem cells present in various tissue types. They have the potential to self-renew, as well as to differentiate to various cell linages (chondrocytes, osteocytes, adipocytes, myocytes and neurons). MSCs are extensively studied and tested with respect to their therapeutic potential. Transplanted MSCs can engraft in damaged tissue and promote regeneration. Interestingly, it is proposed that their regenerative potential is at least partially mediated by abundantly produced extracellular vesicles (EVs). Extracellular vesicles are a heterogeneous population of cell derived membraneous particles that are generated either by the endosomal system (exosomes) or shed from the plasma membrane (microvesicles). They contain proteins, lipids, DNA and various RNA species (micro RNAs, messenger RNAs, long non-coding RNAs and circular RNAs). EVs are produced by MCSs, as well as other cell types and serve cell-to-cell communication purposes. They were also proposed to function as a means of removal of unneeded compounds from cells. Released EVs can interact with target cells in close vicinity or at distant sites through ligand-receptor binding, fusion with plasma membrane or endocytosis into the cells. Just like parental mesenchymal stem cells, MCS-derived extracellular vesicles (MSC-EVs) have the potential to enhance regenerative processes of damaged tissues in living organisms. The attractiveness of EVs in therapeutic applications are their physiochememical stability, biocompatibility and lack of reported side effects. In order to understand how the MSC-EVs exert their function, it is important to know their cargo and how it is changing during the process of differentiation to cell types of interest. In the current project, we conduct differentiation of umbilical cord- and adipose tissue-derived MCSs to chondrocytes and osteoblasts and test the influence of graphene-based scaffolds on the differentiation and regenerative potential of the cells and their EVs. This task will be accompanied by global analysis of mRNAs, lncRNAs and miRNAs expression profiles in undifferentiated and differentiating cells and their EVs. The aim of this study is to correlate the intracellular and EV nucleic acid content with their differentiation efficiency and therapeutic potential.

329 Exosome-mediated MIR211 modulates tumor microenvironment via the DUSP6-ERK5 axis and contributes to BRAFV600E inhibitor resistance in melanoma.

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The microRNA MIR211 is an important regulator of melanoma tumor cell behavior. Previous studies suggested that in certain tumors, MIR211 acted as a tumor suppressor while in others it behaved as an oncogenic regulator. When MIR211 is expressed in BRAFV600E-mutant A375 melanoma cells in mouse xenografts, it promotes aggressive tumor growth accompanied by increased cellular proliferation and angiogenesis. We demonstrate that MIR211 is transferred to adjacent cells in the tumor micro-environment via exosomes. Cross-species genome-wide transcriptomic analysis showed that human tumor-derived MIR211 interacts with the mouse transcriptome in the tumor microenvironment, and activates ERK5 signaling in human tumor cells via the modulation of a feedback loop. Human miR211 directly inhibits human DUSP6 protein phosphatase at the post-transcriptional level. We provide support for the hypothesis that DUSP6 inhibition conferred resistance of the human tumor cells to the BRAF inhibitor vemurafenib and to the MEK inhibitor cobimetinib, with associated increases in ERK5 phosphorylation. These findings are consistent with a model in which MIR211 regulates melanoma tumor proliferation and BRAF inhibitor resistance by inducing ERK5 signaling within the complex tumor microenvironment. We propose that the MIR211-ERK5 axis represents an important and sensitive regulatory arm in melanoma with potential theranostic applications.

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330 FUS controls the processing of snoRNAs into smaller RNA fragments that can regulate gene expression

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FUS is a multifunctional protein involved in many steps of RNA metabolism, including transcription, splicing, miRNA processing and replication-dependent histone gene expression. Here we show for the first time that FUS binds and negatively regulates the levels of a subset of snoRNAs in cells. Scanning of available human small RNA databases revealed the existence of smaller RNA fragments that can be processed from FUS-dependent snoRNAs. Therefore, we suggest that FUS mediates the biogenesis of snoRNA-derived small RNAs, called sdRNAs. Further in silico approaches enabled us to predict putative targets of selected FUS-dependent sdRNAs. Our results indicate that sdRNAs may bind to different regions of target mRNAs as well as to noncoding transcripts and influence the posttranscriptional level or translation of these targets.

331 The orphan C/D box snoRNA SNORD116 missing in Prader-Willi syndrome regulates microexon usage

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Prader-Willi syndrome (PWS) is a common genetic form of syndromic obesity, associated with intellectual disability. Central to the etiology of PWS is a cluster of 29 orphan SNORD116 C/D box snoRNAs falling into five related classes. Knock-down experiments using gapmers showed that SNORD116 represses more than 50 microexons (exons smaller than 50 nt) but has no detectable effect on longer alternative exons. Most of these microexons are surrounded by Alu-elements.

We concentrated on a 10 nt long exon in the Synaptophysin like 1 (SYPL1 pre-mRNA) regulated by SNORD116. SYPL1 contains four transmembrane domains and the protein works in vesicle fusion. Inclusion of the microexon deletes two transmembrane domains and the corresponding protein variant is no longer associated with membranes.

Deletion of the flanking Alu-elements activates the microexon which is repressed in most cell types. Expression of two of the five classes of SNORD116 also activates the exon through binding to a 12 nt long motif, showing for the first-time differences between SNORD116 copies. The binding sites are within a predicted secondary RNA structure and suggesting that SNORD116 binding changes this structure to de-repress the microexon.

Biochemical fractionation experiments showed that about one-third of SNORD116 is present in a nuclear fraction devoid of fibrillarin, and pull-down experiments show that SNORD116 is associated with hnRNPs. This suggests that most of SNORD116 forms protein complexes different from canonical, methylating C/D box snoRNAs where the RNA is stabilized by hnRNPs. Thus, SNORD116 acts more like an oligonucleotide stabilized by proteins.

To validate this model, we tested oligonucleotides representing a consensus binding sequence of all SNORD116 copies. These oligos activate exon usage, similar to SNORD116, suggesting that its loss can be substituted in PWS.

In summary, SNORD116 regulates a protein acting in vesicular transport, likely by generating a dominant negative isoform through inclusion of a 10 nt long microexon. This is similar to SNORD115, another C/D box snoRNA missing in PWS, which regulates the localization of the serotonin receptor 2C through a dominant negative isoform and points to aberrant localization of membrane proteins as a contributor to PWS, which can be treated with oligonucleotides.

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332 Spb1p: a yeast methyltransferase with a pre-60S remodeling function

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Remodeling events during ribosome biogenesis often involve a reorientation of rRNA helices in a precisely orchestrated manner through the participation of numerous *trans*-acting factors. Yet, our understanding of the peculiar role of these *trans*-acting factors is far from complete. Here, through the analysis of available cryo-EM structures of yeast pre-60S particles, we report an undocumented remodeling event that involves the 841 amino acid long multidomain yeast Spb1p methyltransferase (MTase) and the H34/H35/H35a subdomain II helices. This event is associated with a key U-turn to Z-turn transition of the conserved H35a GAAA(G) cap This transition is essential for the docking of this Z-turn motif to a newly uncovered subdomain IV Z-turn receptor, a vital step in maturation of the large subunits of all organisms (Z-turns are isosteric to UNCG tetraloop folds). More specifically, we show that the U-turn state of the GAAA H35a cap is encapsulated by the Spb1p C-terminal domain that blocks the remodeling step up to the completion of the PTC Gm_{2922} methylation, an essential eukaryotic modification. Upon release of Spb1p, the GAAA U-turn adopts a Z-turn through a tetra-to-pentaloop transition and docks to its receptor. This is the first documented instance of a remodeling and a MTase activity associated with a large multidomain *trans*-acting factor essential for rRNA biogenesis. In archaea, bacteria and mitochondria, no equivalent Sbp1p C-terminal domain was found, suggesting that this and the associated DUF3381 domain formed during eukaryogenesis and that the U-turn protection mechanism is not present in these organisms/organelles.

333 The human RNA helicase DHX37 is required for release of the U3 snoRNP from pre-ribosomal particles

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Ribosome synthesis is an essential cellular process, and perturbation of human ribosome production is linked to cancer and genetic diseases termed ribosomopathies. During their assembly, pre-ribosomal particles undergo numerous structural rearrangements, which establish the architecture present in mature complexes and serve as key checkpoints, ensuring the fidelity of ribosome biogenesis. RNA helicases are essential mediators of such remodelling events and here, we demonstrate that the DEAH-box RNA helicase DHX37 is required for maturation of the small ribosomal subunit in human cells. Our data reveal that the presence of DHX37 in early pre-ribosomal particles is monitored by a quality control pathway and that failure to recruit DHX37 leads to pre-rRNA degradation. Using an *in vivo* crosslinking approach, we show that DHX37 binds directly to the U3 small nucleolar RNA (snoRNA) and demonstrate that the catalytic activity of the helicase is required for dissociation of the U3 snoRNA from pre-ribosomal complexes. This is an important event during ribosome assembly as it enables formation of the central pseudoknot structure of the small ribosomal subunit. We identify UTP14A as a direct interaction partner of DHX37 and our data suggest that UTP14A can act as a cofactor that stimulates the activity of the helicase in the context of U3 snoRNA release.

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334 Genome-wide RNAi screen identifies new players in 60S subunit biogenesis in human cells

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The assembly of eukaryotic ribosomes is a complex, highly compartmentalized process which involves more than 200 non-ribosomal proteins, known as trans-acting factors. Current knowledge on ribosome biogenesis, in particular for the large subunit, mostly derives from data in yeast, however less is known about this process in mammalian cells.

To unravel the cellular machinery supporting 60S assembly in human cells, we performed a genome-wide RNAi screen in HeLa cells using a microscopy-based assay relying on the localization of RPL29-GFP as a readout. Among the identified hits are several expected factors, including the ribosomal proteins, known ribosome biogenesis factors and members of the splicing and translation machinery. In addition, we identified several proteins which have so far not been linked to ribosome biogenesis, including members of signaling pathways, metabolic enzymes and uncharacterized proteins. We are currently confirming and characterizing the function of selected hits.

335 Molecular mechanism of stress response ribosome-binding trGTPase, BipA.

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BPI-inducible protein A (BipA) is a highly conserved ribosome-binding translational GTPase (trGTPase) across bacteria species and even found in plant. BipA is dispensable when the culture condition is optimum, but environmental stressors such as cold shock and acid stress are found to cause growth defect in bipA deletion mutant. Furthermore, loss of bipA apparently affects the ability of the host to swim in 0.3% LB agar, whereas plasmid overexpressing bipA overcompensates the swimming ability. As BipA binds 70S ribosome, polysome profiling is carried out and reveals the accumulation of 30S and reduction in 70S, a phenomenon that is seen in other literatures as well. While the accumulation of 50S is not as strong as seen in 30S, a peak representing immature 50S or pre-50S can be seen when bipA deletion mutant is cultured under suboptimal temperature, implying the role of BipA in ribosome assembly. Surprisingly, loss of rluC, a gene expressing 23 rRNA pseudouridine$^{955/2504/2580}$ synthetase, can alleviate the growth defect, the weakened swimming, and the abnormal ribosomal distribution phenotypes. Our preliminary results also include total RNA and ribosome profiling analyses on mild-acidic stressed *Escherichia coli* (*E.coli*), and tandem mass tag mass spectrometry analysis on cold-shocked *E.coli*. In the nutshell, BipA is theorized to be a ribosome assembly factor which is assisting in ribosome biogenesis in terms of rRNA scaffolding and r-protein recruitment.

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336 Structural modular evolution of the ribosome through extensive local duplications

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Understanding the evolution of the ribosome before the emergence of the last Universal Common Ancestor (LUCA) remains a great challenge in biology. Through an extensive structural analysis of the ribosomal RNAs, surveyed across an array of organismal contexts, we have garnered a great amount of information on the way RNA self-assemble and fold into complex three-dimensional architectures. Organized three-dimensional networks of interactions often take advantage of recurrent structural modules (or motifs) that specify localized arrangements of conserved and semi-conserved nucleotides. Within the ribosomal RNAs, structural modules are found in a variety of combinations to form distinctive and specialized local architectures (or native folds) able to perform specific operations including intermolecular recognition, catalytic, or mechanical functions. Using these structural modules as molecular signatures, we have identified multiple instances of duplication and recombination of RNA structural modules within the small and large ribosomal subunits. While few of these modules are late additions to the pre-LUCA ribosome, more than two third of them are involved in rRNA regions that are among the most conserved and ancient parts of the ribosome. Our structural analysis of the ribosomal RNAs sheds new lights on the way the translational apparatus emerged and evolved before the rise of LUCA. A new scenario for the modular structural evolution of the ribosome is proposed.

337 Pseudouridylation enzyme RsuA influences 30S ribosome assembly

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The ribosome is responsible for protein biosynthesis in all living organisms. Bacterial ribosome biogenesis is a complex process that requires synchronization of various cellular events including ribosomal RNA (rRNA) transcription, ribosome assembly, RNA processing and post-transcriptional modification of rRNA. Ribosomal RNA nucleotide modifications and their respective modification enzymes can modulate rRNA folding and ribosome assembly. The only pseudouridine modification found in *E. coli* 16S ribosomal RNA is located at position 516 of 16S helix 18 which forms the central pseudoknot of the 30S ribosomal subunit. Our circular dichroism spectroscopic data suggest that the helix 18 model RNA undergoes Mg^{2+}-dependent structural changes only in the presence of the pseudouridine modification at position 516. A FRET-based RsuA binding assay developed in our lab shows thermodynamic anti-cooperativity between ribosomal protein S4 and RsuA that catalyzes pseudouridylation of U516. Furthermore, as observed with a reverse transcriptase-based activity assay, RsuA is catalytically cooperative with ribosomal protein S17. Our data suggest that the RsuA enzyme binds preferably to non-pseudoknoted (extended) helix 18.

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338 Structural basis of alternative ribosome rescue by ArfA and RF2

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Translating ribosome often stalls on an mRNA in a variety of situations. To avoid accumulation of stalled ribosomes in the cell, rescue systems are available in Escherichia coli. ArfA is a protein molecule to carry out this task with the help of RF2, whereas it is not clear how ArfA and RF2 recognize the target ribosome to hydrolyze peptidyl-tRNA.

Here, using cryo-electron microscopy, we characterize the structure of the Escherichia coli 70S ribosome bound with ArfA, RF2, a short non-stop mRNA and a cognate P-site tRNA. The C-terminal loop of ArfA occupies the mRNA entry channel on the 30S subunit, whereas its N terminus is sandwiched between the decoding centre and the switch loop of RF2, leading to marked conformational changes in both the decoding centre and RF2. Despite the distinct conformation of RF2, its conserved catalytic GGQ motif is precisely positioned next to the CCA-end of the P-site tRNA. These data illustrate a stop-codon surrogate mechanism for ArfA in facilitating the termination of non-stop ribosomal complexes by RF2.

339 Strategies for structure determination of a poorly characterized and extremely complex mitochondrial ribosome from trypanosomes

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Translation of mRNA into protein is a universally conserved cellular process catalyzed by ribosomes, two-subunit ribonucleoprotein assemblies in which the ribosomal RNA (rRNA) plays a dominant functional and structural role. While cytoplasmic ribosomes display a high degree of structural similarity, ribosomes from the mitochondrial organelle (mitoribosomes) considerably diverged during evolution in both their rRNA and protein content, leaving only the catalytic core of the particle unaltered. Here, we present the atomic cryo-EM structure of the most complex ribosomal assembly described so far, the 4.5 MDa mitoribosome from the unicellular parasite *Trypanosoma brucei* [1]. It harbors the smallest known rRNAs embedded in an huge outer shell formed by 127 ribosomal proteins, many of which were previously uncharacterized and display novel folds. To identify these additional protein components, we predicted protein sequences directly from the EM maps and used them to search protein databases and our mass spectrometry libraries for homologs. Our structure reveals that the differences between cytoplasmic ribosomes and the trypanosomal mitoribosome are most pronounced in the "small" subunit, which even exceeds the size of the "large" subunit. The architecture of the particle results in an exceptional rRNA:protein ratio of 1:6 and allows us to define the minimal catalytic core needed for ribosomal function. We further show how ribosomal proteins take over the scaffolding function from the rRNA, which in large portions is stabilized by the proteins in a single stranded form. Our current research focuses on understanding the assembly of mitochondrial ribosomes using the trypanosomal mitoribosome as an example.

[1] Ramrath *et al*., Science 362, p. 422/eaau7735 (2018)

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340 Cryo-EM visualizes translation elongation with authentic substrates and reveals continuous mRNA decoding

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Understanding how ribosomes accurately decode mRNA requires visualization of GTP-catalyzed elongation, which has remained a challenge. Here, time-resolved cryo-EM revealed 33 states, from aminoacyl-tRNA delivery by EF-Tu•GTP through peptidyl transfer, visualizing the elongation mechanism. Instead of locking cognate tRNA upon initial recognition, the ribosome continuously monitors codon-anticodon interactions before and after GTP hydrolysis by EF-Tu. Upon GTP hydrolysis, EF-Tu extends away and gradually releases from tRNA. The 30S subunit locks cognate tRNA in the decoding center and rotates allowing the tRNA to bypass 23S rRNA protrusions during accommodation into the peptidyl transferase center. By contrast, ribosomes fail to stabilize interactions with near-cognate tRNA, hindering GTP hydrolysis, accommodation and elongation with an incorrect amino acid. Thus, the ribosome ensures accurate translation by continuously verifying incoming tRNA until peptidyl transfer. Our work demonstrates the power of cryo-EM to monitor dynamic multi-enzyme processes at near-atomic resolution.

341 Modulation of rRNA processing pathways conserves energetic reserves and maintains nucleolar structure during stress

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Production of ribosomes is a major energetic task of a growing cell. The energetic costs are compounded by the fact that the rRNA is transcribed as a ~13,000 nt pre-rRNA, termed the 47S rRNA, that must be reiteratively processed to release the mature 18S, 5.8S and 28S rRNAs. Building ribosomes requires precise regulation of rRNA synthesis along with the delivery of newly synthesized ribosomal proteins to the nucleolus.

In response to stress, energetic reserves are redirected from pro-growth activities, such as ribosome production, to pro-survival tasks. We have shown that various acute stresses causes global translation inhibition following eIF2α phosphorylation, which results in formation of stress granules (SGs), non-membranous liquid-liquid phase separations of untranslated mRNPs. As translation of mRNAs encoding ribosomal proteins is preferentially inhibited and targeted to SGs upon eIF2α phosphorylation, the cessation of ribosomal protein synthesis necessitates coordinative regulation of the rRNA synthesis. Here, we hypothesized that failure in such coordination results in a misallocation of energetic resources leading to nucleolar dysfunction.

We show that certain stresses that induce eIF2α phosphorylation and SG formation also cause inhibition of rRNA synthesis. Transcription inhibition via TIF-1A phosphorylation is not involved, as has been reported for other stresses. Instead, there is a rapid (<30 min) suppression of the first step in rRNA processing. As the rate of rRNA transcription is intrinsically tied to the efficiency of rRNA processing, this has the ultimate effect of repressing the rate of rDNA transcription. Moreover, this mechanism allows for the maintenance of nucleolar structure during stress. As RNA is a major driver of liquid-liquid phase separations in RNA granules (such as SGs or nucleolus), by maintaining unprocessed 47S pre-rRNA, the nucleolus remains stable. This is in contrast to stresses or drugs (e.g. Act D) that directly inhibit Pol I, where nucleolar integrity is compromised. Despite being coordinated with eIF2α phosphorylation, stress responsive modulation of rRNA processing is regulated by an independent, yet parallel, signaling pathway. Significantly, upon analysis of a panel of chemotherapeutic drugs we show that many of these drugs similarly induce both eIF2α phosphorylation and stress dependent rRNA processing modulation.

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342 Unexpected requirements for nucleolar structure and function revealed by genome-wide RNAi screen for increased nucleolar number in human cells

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Ribosome biogenesis initiates in the large, phase-separated body called the nucleolus. In the human MCF10A cell line, we observe 2-3 nucleoli per nucleus; however, intriguingly we have shown that siRNA depletion of factors required for ribosome biogenesis cause a decrease in this number from 2-3 to 1. This observation led to a genome-wide siRNA screening campaign to identify novel proteins required for making ribosomes (Farley-Barnes et al., 2018). While the published work has focused on proteins revealed by the one nucleolus phenotype, this screen also uncovered hits that cause an increase in nucleolar number to 5 or more. Focusing on this 5+ phenotype (unpublished), we screened 18,017 genes and identified 186 distinct hits, that do not overlap with the hits that yield the one nucleolus phenotype. Hits were then filtered by expression and viability leaving 103 proteins that, interestingly, are largely not known to function in ribosome biogenesis as determined by analysis using the Ingenuity Pathway Analysis software (Qiagen). Instead, these hits are associated with cancer and development, and cellular processes including mitosis and the cell cycle, cellular assembly and organization, and DNA replication and repair. Biochemical follow-up on these hits reveals proteins required for protein synthesis, and to a lesser extent, rDNA transcription and pre-rRNA processing. Taken together, these data suggest that we identified a unique subset of proteins required for nucleolar function. The significance of this study thus lies in the elucidation of novel mechanisms required for maintaining nucleolar function, and leads us to new insights into the requirements for nucleolar assembly post-mitosis, broadening our understanding of the pathogenesis of diseases associated with nucleolar dysfunction including congenital developmental disorders (e.g. the ribosomopathies) and cancer.

343 Structural basis for the auto-inhibition of the DEAH-box helicase Dhr1

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Eukaryotic ribosome biogenesis requires the specific activity of the atypical DEAH-helicase Dhr1. Here we present the 2.3-Å crystal structure of the Dhr1 functional core. The structure reveals the molecular basis of the Dhr1-specific auto-inhibitory loop, which keeps the enzyme in an open conformation and prevents enzymatic catalysis by occupying the same path as substrate RNA.

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344 Ribosome maturation factor Nop53 controls association of the RNA exosome with pre-60S particles

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Eukaryotic ribosomal biogenesis is a highly energy demanding and complex process that requires hundreds of trans-acting factors for processing of pre-rRNAs and assembly of the subunits 40S and 60S. Each ribonucleoprotein complex is composed of specific rRNAs and ribosomal proteins which are structured in functional domains. In order to properly fold the ribosomal RNAs in coordination with the recruitment of specific ribosomal proteins, the pre-rRNA processing pathway must be orchestrated following a hierarchy of maturation factors. The RNA exosome complex plays a crucial role as one of the pre-60S processing factors, as the RNase responsible for the initial processing of the pre-rRNA 7S, which will give rise to the mature 5.8S rRNA. The yeast pre-60S assembly factor Nop53, has previously been shown to interact with Nop7 in the middle stages of nucleoplasmic pre-60S and with the exosome catalytic subunit Rrp6, thus considered as the adaptor responsible for recruiting the exosome for 7S processing. Here we highlight new interactions through which Nop53 can modulate the exosome activity in the context of 60S maturation, and show evidence that in addition to recruiting, Nop53 may also be important for the release of the exosome from the pre-60S.

345 The kinase Rio1 regulates the entry of nascent pre-40S ribosomes into the translating pool

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Ribosome synthesis requires a series of quality control mechanisms to prevent misassembly of ribosomes and dysregulation of ribosome concentrations, which are associated with diseases including an increased risk of cancer development in humans. To reduce mistakes in translation due to misassembled ribosomes reaching the translating pool, a concerted effort of over 200 transiently-binding assembly factors coordinate rRNA processing with ribosomal protein binding and verify proper ribosomal structure and function prior to translation initiation. During the final stages of maturation of the small, 40S ribosomal subunit, the endonuclease Nob1 cleaves the 3'-end of 20S pre-rRNA to produce the mature 18S rRNA. Our data show that Nob1 inhibits mRNA recruitment and must be released in order for ribosomes to enter the translating pool. rRNA cleavage is required before Nob1 release and regulates the binding of the essential kinase Rio1 to pre-40S. Rio1 binds Nob1 and the assembly factor Pno1 directly in an ATP-dependent manner. Upon ATP-hydrolysis, Rio1 releases both Pno1 and Nob1 from pre-40S. Thus, Rio1 serves to coordinate the final assembly steps in 40S ribosome maturation and, by releasing Nob1, regulates translation initiation of nascent 40S subunits.

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346 Mapping rRNA 2'-*O*-Methylation by RiboMethSeq and characterization of snoRNAs in *Arabidopsis*

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Transcription and processing of ribosomal RNA precursors (pre-rRNA) is a keystone of ribosome biogenesis. The 45S pre-rRNA is transcribed by RNA polymerase I and contains the 18S, 5.8S and 25S structural rRNAs separated by internal (ITS1 and ITS2) and external (5'ETS and 3'ETS) transcribed spacers. Processing of 45S pre-rRNA into mature 18S, 5.8S and 25S rRNA include removal of ITS and ETS sequences and the modifications of rRNA positions. The most abundant rRNA modifications are methylation at the ribose moiety (2'-*O* methylation) and uridine isomerization (pseudouridylation), driven by small nucleolar RNA (snoRNA) of C/D-box or H/ACA-box type, respectively. In yeast and mammalian cells, 2'-*O* methylation and/or pseudourylidation impact translational fidelity and initiation or the affinity for specific (tRNA/IRES) ligands. The impact of rRNA modifications for ribosome structure/function is not yet known in plants.

In *Arabidopsis thaliana* plants, around 200 C/D-box snoRNAs have been identified. Most of the C/D-box snoRNAs targets are predicted 2'-*O*-methylation rRNA sites. Others, the so-called orphan snoRNAs, may target mRNA or act as miRNA or siRNA precursors. We used the recently developed Illumina-based RiboMethSeq approach [1] for mapping all 2'-*O*-methylation rRNA sites in *A. thaliana* plants. This analysis detected most of the rRNA 2'-*O*-methylations predicted as box C/D-box snoRNA targets and/or experimentally detected in previous reports. Remarkably, the RiboMethSeq approach also identified new 2'-*O*-methylation sites without obvious C/D snoRNA guides. It is expectable that rRNA may be 2'-*O*-methylated by non-predicated or canonical C/D-box snoRNAs guides. We performed immunoprecipitation to identify novel (small or large non-coding) RNAs interacting with fibrillarin and that might be required for 2'-*O*-methylation. Our results provide a comprehensive cartography of 2'-*O*-methylated sites of rRNA and enlarge the repertoire of C/D-box snoRNAs. Furthermore, we investigated 2'-*O*-methylation in *Arabidopsis* NUC1 knock-out plants. NUC1 is a functional conserved nucleolar protein involved in transcription and processing of pre-rRNA in all eukaryotes organisms and in *Arabidopsis*, gene disruption provokes growth and developmental defects [2]. This analysis revealed a subset of NUC1-sensitive modified positions and provide evidences for differential 2'-*O*-methylation in plants.

1. Marchand et al.,. NAR, 2016. **44**(16); 2. Pontvianne et al., PLoS Genet, 2010. **6**(11).

347 Pol5 is required for assembly of the large ribosomal subunit and the recycling of small subunit biogenesis factors

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The synthesis of ribosomes is an essential process in all growing cells that requires the correct assembly of the small and the large ribosomal subunits (SSU and LSU respectively). Imbalance in the production of the two ribosomal subunits alters translation, but only little is known about how cells coordinate SSU and LSU synthesis. In eukaryotic cells, RNA polymerase I produces a single transcript containing three out of the four ribosomal RNAs. Thus, the initial maturation steps of both ribosomal subunits take place in the same ribonucleoprotein particle.

As published by several groups, defects in LSU biogenesis establish a negative feedback loop on the biogenesis of SSU. We have characterized the nucleolar protein Pol5 as a novel assembly factor of the LSU. Pol5 associates transiently with pre-60S particles and is required for the correct formation of the peptide tunnel. Interestingly, several assembly factors required for SSU formation are not released from the pre-rRNA in the absence of Pol5. Therefore, Pol5 is required both for the correct assembly of the LSU and for the recycling of factors involved in SSU biogenesis.

We will discuss a possible role of Pol5 as a link coupling the correct assembly of pre-LSU particles with the recycling of SSU assembly factors. Our model provides a rationale for the regulatory feedback loop that ensures the stoichiometric production of ribosomal subunits.

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348 Iron-dependent cleavage of ribosomal RNA during oxidative stress in the yeast *Saccharomyces cerevisiae*.

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Living cells encounter various stressful conditions in the environment during their lifespan. Oxidative stress, caused by exposure to elevated levels of reactive oxygen species (ROS), can damage biomolecules and influence essential cellular processes. Translation is affected by elevated levels of ROS on many levels, including oxidation and inactivation of translation factors, base modification and strand breaks in mRNA, tRNA and ribosomal RNA (rRNA). Stress-induced strand breaks in rRNA have been observed in a wide range of organisms, but the mechanisms of their origin are not well understood. In this study, we used a yeast model of oxidative stress and found that site-specific rRNA cleavages can be initiated in cells through a chemical rather than enzymatic mechanism. Analysis of oxidant effects in different yeast strains revealed an increased level of 25S rRNA cleavages occurring in cells with abnormal iron homeostasis. In cells lacking the mitochondrial monothiol glutaredoxin Grx5, cleavages in rRNAs appeared almost immediately upon oxidant exposure and eventually led to extensive rRNA fragmentation correlating with a decline in cell viability. Reducing the labile iron pool in *grx5D* cells decreased the extent of cellular rRNA degradation and improved cell viability. Importantly, an *in vitro* iron/ascorbate reaction with purified ribosomes or synthetically-generated 25S rRNA fragment precisely recapitulated the 25S rRNA cleavage pattern observed in cells, indicating that redox activity of iron bound to the ribosome in sufficient to induce the cleavage. Our current model is that excessive intracellular iron ions can replace other divalent metals within specific ribosomal structures, priming rRNA for cleavage. Iron-mediated, site-specific breaks in rRNA may provide a mechanism for redox-sensitive tuning of the ribosome function, contributing to cell-fate decisions in stressed cells.

349 YBEY is a ribosome assembly factor required for the mitochondrial small subunit biogenesis

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Ribosome biogenesis relies on a number of specific factors. Some of them are remarkably conserved, suggesting that they play essential roles even in distant evolutionary contexts. This is namely the case for the UPF0054 protein YBEY found in all Bacteria, but also in many Eukarya. Proposed to act as an endoribonuclease processing the 3' end of 16S rRNA, YBEY is critically required for translation in model bacteria and plant chloroplasts. However, ribosomal RNA processing pathways are poorly conserved between distant phyla, suggesting that YBEY may have another important function in ribosome biogenesis.

We studied the human YBEY homologue and found that it localises in mitochondria. The human mitochondrial rRNAs are flanked by tRNA genes and thereby processed by mitochondrial RNase P and RNase Z, making other ribonucleases superfluous. Yet, CRISPR-mediated knockout of the YBEY gene resulted in a decrease of the mitochondrial small ribosomal subunits (SSU), abolished translation in the organelles and, as a result, led to the inability of the knockout cells to respire. Mapping the ends of the mitochondrial rRNAs revealed no processing defects. Similarly, although human YBEY did show robust RNase activity in vitro and in vivo, mutations in key catalytic residues did not abolish its ability to complement the knockout phenotypes. High-resolution glycerol gradient analyses revealed a slight shift in the sedimentation of both subunits. In particular, SSU of the knockout cells showed a ~26S peak instead of the canonical 28S, suggesting that it was lacking proteins and stuck in a late stage of assembly. A mass spectrometry analysis of the mitoribosomes identified a distinct set of SSU proteins, mostly located in the head and the platform, to be significantly depleted in the absence of YBEY, including uS11m, required for translation initiation. Importantly, uS11m was the only SSU protein found to directly interact with YBEY in vitro, in vivo and in situ. The sum of our data supports the model where YBEY functions as an essential ribosome biogenesis factor by recruiting uS11 in order to complete the assembly of translationally active SSUs.

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350 Dynamics of the early steps in ribosome biogenesis.

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During the early steps of ribosome biogenesis more than 70 assembly factors (AFs) associate with the nascent transcript to form the first pre-ribosomal precursor. This pre-ribosomal particle is required for the biogenesis of the small subunit and it has been called SSU-processome. Several assembly factors including the U3 snoRNP act as primary binders of the nascent pre-rRNA and participates in the hierarchical recruitment of other components of the SSU-processome (including AFs and ribosomal proteins). The association of the U3 snoRNA with the pre-rRNA has been largely described, but its role during assembly of the SSU-processome remains unsolved. Recent advances in electron microscopy allowed the observation of the SSU-processome at atomic resolution. However, the observed structures represent, most likely, steady-state complexes where flexible and heterogeneous regions remain invisible. To study the dynamics of ribosome assembly, new approaches need to be developed. In this work, we describe the use of mutants in the U3 snoRNA to "freeze" assembly states of the SSU-processome that are characterized in terms of protein composition and maturation stage of the pre-rRNA. In addition, structure probing methods based in tethered enzymes have been developed to investigate the folding state of the pre-rRNA during SSU-processome formation. We will discuss possible roles in ribosome assembly for the different domains of the U3 snoRNA.

351 Comparative genomics analysis of CPR bacteria with their complete genomes: Minimized profiles of ribosomal proteins and ribosomal RNAs

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Candidate phyla radiation (CPR) is a large bacterial supergroup recently described. It consists of uncultured bacterial lineages and forms a monophyletic radiation separated from the other well-known and ordinary bacteria, such as *Escherichia coli* and *Bacillus subtilis* (Hug *et al*. *Nature Microbiology* 2016. 1: 1-6). CPR bacteria have been reported to have small genomes lacking many genes involved in some essential metabolic pathways. In this study, we conducted comparative genomic analysis of CPR bacteria with focus on their gene length, to characterize their evolutionary positions.

We analyzed the length of proteins predicted from coding genes, and rRNA genes using 18 complete genomes of CPR bacteria collected from NCBI GenBank. As a result, although the mean protein length in each CPR bacteria was 272 to 338 amino acids (aa) and was not much different from that of *E. coli* (306 aa), the distributions of protein length, represented by the density curve, were very different between CPR and the ordinary bacteria. In CPR bacteria, the distribution of protein length represented two peaks at around 150 and 350 to 400 aa, and the former peak was remarkably larger than the latter. The similar distribution profiles were observed in the parasitic bacteria such as *Carsonella ruddii* and *Wolbachia pipientis*. Interestingly, we found that approximately 20 percent of proteins corresponding to the former peak were ribosomal proteins. Furthermore, we confirmed that there were long 16S and 23S rRNA genes with intron-like sequences in some CPR bacteria (Brown *et al*. *Nature* 2015. 523: 208-211). Meanwhile, we found that WWE3 bacteria in CPR had notably short 16S and 23S rRNA genes. Based on the alignment analysis, it was revealed that WWE3 lacks several regions of 16S rRNA gene and these regions correspond to each individual stem-loop structure such as helixes 17 and 39. In contrast, deletion sites of the 23S rRNA gene were concentrated in its 3' end region and the regions correspond to the domains V and VI. We speculated that analysis of these small genes found in CPR bacteria might contribute to find essential regions of these molecules.

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352 Characterising gene-gene and gene-environment interactions within the fitness landscape of a snoRNA

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Empirical fitness landscapes allow a previously hypothetical concept describing the genotype-phenotype relationship to be visualised and dissected. The fitness landscapes of entire molecules have only recently begun to be elucidated, with gene-gene and gene-environment interactions being largely unexplored in this context. U3 small nucleolar RNA (snoRNA) represents a suitable model in which to examine these interactions, with the strategy of deep mutational scanning enabling mutational effects to be studied at a single nucleotide resolution. In yeast, U3 is involved in three essential cleavage events of the pre-ribosomal RNA (pre-rRNA), facilitated by complementarity between the 5' region of U3 snoRNA and the 35S pre-rRNA transcript. These cleavage events along with folding of the pre-rRNA are facilitated by a series of proteins, some of which bind with U3 snoRNA to form the U3 snoRNP.

Since U3 snoRNA interacts with several proteins, it is an ideal candidate to study the molecular mechanisms behind certain gene-gene interactions. The auxin inducible degron system was used to downregulate eight proteins that interact with U3 snoRNA, with the fitness landscape then measured. The disruption of these essential genes demonstrated intermolecular epistasis, altering the fitness landscape in a gene specific manner. Conditionally deleterious mutations were enriched in functionally important regions of U3 snoRNA, particularly those whose role is related to that of the hypomorphic protein.

To simulate the changing environments that an organism may face, and observe the resulting impact, the fitness landscape of U3 snoRNA was measured in three different temperature environments. This highlighted numerous gene-environment interactions, related to structural and protein binding regions of U3 snoRNA. Various genotypes tolerated at physiological temperature become deleterious at higher and lower temperatures.

353 These can go up to eleven: shedding light on the molecular mechanism of RNA counting in rotaviruses

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Genome segmentation offers certain evolutionary benefits to a number of rapidly evolving RNA viruses, including rotaviruses and influenza viruses. As the number of genomic RNAs per virus particle increases, such viruses must solve the task of maintaining the integrity of their genomes by always selecting a single copy of each type of the RNA segment, all of which are equally represented in the viral population. A hallmark of rotavirus assembly in cells is the stoichiometric association of eleven distinct RNAs prior to their packaging into a virus particle. Elucidating the mechanism of accurate self-assembly of eleven distinct RNAs in rotaviruses will advance our knowledge of similar mechanisms employed by other segmented RNA viruses.

We have employed multi-colour cross-correlation spectroscopy (FCCS) to detect stable, RNA-RNA interactions between rotavirus RNAs. In order to identify the genomic sequences involved in the formation of such contacts, we have employed an RNA-RNA SELEX methodology. Using both approaches, we have shown that the formation of inter-segment contacts requires binding of the rotavirus-encoded RNA-binding protein NSP2, which is essential in virus replication and is involved in assembly of RNA-containing membraneless organelles in cells. Single-molecule fluorescence studies suggest that NSP2 possesses an RNA chaperone activity *in vitro*, consistent with the RNA probing and structure modeling that confirms that NSP2 binding results in the RNA remodeling required for the assembly of multiple RNAs *in vitro*. Finally, we are developing new tools for simultaneous imaging of eleven distinct RNA transcripts to explore their dynamics and assembly in rotavirus-infected cells. We have used this approach to visualize the assembly of multiple RNA transcripts and use a number of small-molecule compounds to interrogate the RNA assembly process during rotavirus replication.

References

1. Borodavka A, Dykeman EC, Schrimpf W, Lamb DC: **Protein-mediated RNA folding governs sequence-specific interactions between rotavirus genome segments**. *Elife* 2017, **6**.
2. Bravo JPK, Borodavka A, Barth A, Calabrese AN, Mojzes P, Cockburn JJB, Lamb DC, Tuma R: **Stability of local secondary structure determines selectivity of viral RNA chaperones**. *Nucleic Acids Res* 2018, **46**:7924–7937.

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354 A specific mRNA scaffold promotes *cis*-translational interaction of proteins important for P-body assembly.

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P-bodies (PBs) are cytoplasmic mRNA-protein (mRNP) granules conserved throughout eukaryotes which are implicated in the repression, storage and degradation of mRNAs. PB assembly is driven in part by proteins with self-interacting and prion-like protein domains, such as Edc3 and Lsm4 (decapping activators) in yeast. Non-translating mRNA is also required for PB assembly, however no studies to date have explored whether particular mRNA species are more critical than others in facilitating PB assembly. A previous genome-wide microscopy screen revealed that *rps28bΔ* (Ribosomal protein subunit-28B) mutants do not form PBs under normal growth conditions. In this work, we demonstrate that the *RPS28B* 3'UTR is important for PB assembly, consistent with the fact that this is a known Edc3 binding site. However, expression of the *RPS28B* 3'UTR in isolation is insufficient to drive normal P-body assembly. Intriguingly, chimeric mRNA studies revealed that Rps28 protein, translated *in cis* from an mRNA bearing the *RPS28B* 3'UTR facilitates Rps28 interaction with Edc3. This Edc3-Rps28 interaction correlates with PB assembly. We are currently investigating how this interaction facilitates PB assembly and how the mRNA itself contributes to PB assembly *in vitro*. In summary, our work indicates that PB assembly may be preferentially nucleated by specific mRNA "scaffolds", which may be a common theme in RNP granule assembly. Furthermore, this is the first description to our knowledge of a *cis*-translated protein interacting with another protein in the 3'UTR of the mRNA which encoded it, that in turn drives assembly of a macromolecular complex. Our finding is complementary with work by the Mayr lab which indicates that 3'UTRs can drive protein-protein interactions with functional consequences and this phenomenon may occur broadly in eukaryotes.

355 A novel tudor-domain protein promotes germline differentiation through post-transcriptional gene regulation in cytoplasmic RNA granules

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Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development including post-transcriptional gene regulation. Recent work in our lab identified the previously uncharacterized tudor protein, Tudor5-prime (Tdrd5p), which promotes male germline identity in germline stem cells (GSCs) in the testis, but is repressed by the RNA binding protein Sex lethal (SXL) in female GSCs. Interesting, Tdrd5p is also expressed in the differentiating germline in both sexes, indicating that it may also act to control germline differentiation in both sexes.

Previously we reported that Tdrd5p localizes to the periphery of a previously uncharacterized germline RNA granule in both sexes. In the males, numerous smaller Decapping protein 1 (Dcp1) granules co-localize with the periphery of the Tdrd5p granule. This suggests that Tdrd5p granules are docking with processing bodies, and could play a role in post-transcriptional gene regulation. Additionally a subset of Tdrd5p granules of smaller size co-localize with Survival Motor Neuron (SMN), suggesting that Tdrd5p might also function in RNA processing. To understand what RNA regulatory pathway Tdrd5p functions in, we conducted RNAi against the deadenylase *twin* in mutant gonads, which revealed a genetic interaction between *tdrd5p* and the CCR4-NOT deadenylation complex. In both males and females, knockdown of *twin* in *tdrd5p* mutants results in sterility. Additionally, we found similar genetic interactions between *tdrd5p* and both *dcp1* and *gawky(gw)* further suggesting a role for *tdrd5p* in post-transcriptional gene regulation. Recent investigation into the role Tdrd5p plays in the female germline suggests that Tdrd5p could function in maternal RNA deposition. Interestingly, Tdrd5p localizes to a single large granule at the anterior of the oocyte directly adjacent to the ring canals. To further investigate this possibility we stained for Gurken(Grk) in *tdrd5p* mutant ovaries and wild type ovaries. In wild type flies we see normal Grk translation in the anterior dorsal corner of the oocyte, while in *tdrd5p* mutant ovaries we see translation of Grk in the nurse cells. Taken together, our data suggests TDRD5P functions to ensure the proper development of the germline, however the mechanism by which it regulates differentiation is different between males and females.

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356 Regulating the dynamicity of stress induced cytoplasmic ribonucleoprotein granules via liquid-liquid phase separation.

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Regulation of gene expression is essential for cells to respond to different environmental cues and developmental changes. Key regulatory events occur post-transcriptionally and, for example, in response to certain stress conditions, cytoplasmic mRNAs localize to membraneless organelles such as processing bodies (PBs) and stress granules (SGs). PBs and SGs are dynamic, phase-separated cellular compartments involved in degradation and/or storage of mRNAs and assemble via a variety of multivalent but weak RNA-protein, protein-protein and RNA-RNA interactions; a phenomenon called liquid-liquid phase separation (LLPS). Yet, how cells regulate assembly and disassembly of these membraneless organelles remains poorly characterized.

Our lab recently identified the DEAD-box ATPase Dhh1 as a critical regulator of PB dynamics. Mutations in Dhh1 that prevent ATP hydrolysis, or that affect the interaction between Dhh1 and Not1, the central scaffold of the CCR4-NOT complex and an activator of the Dhh1 ATPase, prevent PB disassembly in vivo. Interestingly, this process can be recapitulated in vitro, since recombinant Dhh1 and RNA, in the presence of ATP, phase-separate into liquid droplets that rapidly dissolve upon addition of Not1 (Mugler et al., 2016).

Here, we demonstrate that Pat1 a scaffolding protein involved in mRNA decay and translational repression antagonizes Not1 and promotes PB assembly via Dhh1. Pat1 binding to Dhh1 induces oligomerization and promotes PB assembly in vivo. Intriguingly, this can be recapitulated in vitro, since recombinant Dhh1 and RNA, in the presence of ATP, phase-separate into liquid droplets, a process, which is enhanced by the addition of recombinant Pat1 (Sachdev et al., 2019). Our results provide novel insight into the mechanisms of how cells control PB assembly and disassembly in different growth conditions.

Intriguingly, mRNP granules can also undergo maturation and solidification, and the aberrant formation of irreversible mRNP granules has been implicated in disease development. We will present results that shed light on this poorly understood process and its functional consequences in adaptation to stress and impact on cellular health and aging.

357 Identification of the first small-molecule regulator of mRNA decapping and processing-body turnover.

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An emerging area of research into metabolic stress in eukaryotic cells studies stabilization of mRNAs and their reversible, liquid phase separation into non-membranous, cytoplasmic processing bodies (PBs). Herein, we present a novel observation that mRNA stability and PB formation are coordinated by a small-molecule signaling pathway. This work follows prior identification of mRNA decay through 5'-decapping by NUDT3. The latter hydrolyzes the triphosphate linker between the 7-methylguanosine cap and the most 5'-nucleotide, in a transcript subset serving expression of pro-inflammatory and pro-migratory proteins; we chose four mRNAs as proxies for NUDT3 activity: ITGB6, Fibronectin, LCN2, S100A8. Decapping in vitro was inhibited by a small-molecule signal: the "inositol pyrophosphate", IP7. To examine biological significance, we genetically elevated IP7 levels 3-fold in intact HCT116 and HEK cells, through CRISPR knockout of the enzymes (PPIP5Ks) that metabolize IP7. Quantitative PCR analysis of PPIP5K-/- vs wild-type cells exposed inhibition of NUDT3-mediated decapping, with our target mRNAs decaying slower after Actinomycin D addition: ITGB6, by 80% in HCT116 cells, 160% in HEK cells; Fibronectin, 30% and 105%; LCN2, 40% and 55%; S100A8, 30% and 330%. This was associated with >2 fold higher steady-state levels of each of these transcripts in PPIP5K-/- cells. Importantly, treatment of PPIP5K-/- cells with "TNP", an inhibitor of IP7 synthesis, reverted transcript levels to those of wild-type cells, validating that the inhibition of NUDT3 decapping is IP7-mediated. Interestingly, despite the higher target mRNA levels in the PPIP5K-/- cells, levels of the corresponding proteins were similar to those of wild-type cells. These data indicate translational repression in PPIP5K-/- cells, raising a potential connection of decapping inhibition with PB assembly. To assess this possibility, we immunostained a PB marker, DCP1a. For each experimental condition, data from >400 randomly-selected cells were acquired by confocal microscopy. Total PBs/cell was 2.4-fold higher in PPIP5K-/- vs wild-type cells. Finally, we incubated wild-type HCT116 cells with liposomes containing exogenous IP7 for 6hr; this novel method for intracellular delivery of IP7 elevated PBs/cell 3.4-fold compared to uptake of empty liposomes. Supervision of mRNA stability and PB assembly by a small-molecule signal is a new paradigm for controlling gene expression.

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358 TIAR specifies stress-induced nuclear compartments during G2/M checkpoint activation

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The G2/M checkpoint ensures that DNA is fully replicated before cells enter mitosis and partition their chromosomes. The checkpoint relies on the ATR kinase pathway that controls the activity of the central mitotic kinase CDK1. We recently discovered that the RNA-binding protein TIAR is essential for activation of the G2/M checkpoint in the presence of unreplicated DNA. Depletion of human TIAR sensitizes cells to replication stress, elevates CDK1 activity and accelerates entry into mitosis. As a consequence, loss of TIAR leads to pronounced mitotic defects, chromosomal aberrations and DNA damage, all of which can be reversed by inhibition of CDK1. During late G2 and prophase, replication stress causes TIAR together with RNA polymerase II, core splicing factors as well as replication stress response proteins to assemble in nuclear foci that we termed G2/M transition granules (GMGs). Importantly, TIAR retains CDK1 in GMGs and strongly attenuates its activity. We propose that GMGs assemble on stalled replication forks and serve as a stress-induced compartment that delays mitotic entry and thereby controls timing of the G2/M checkpoint (Lafarga et al., EMBO Rep 2019).

We now extended our work to the analysis of G2/M checkpoint activation upon translation suppression through ribotoxins. This pathway depends on p38-MAPK signaling, and also requires TIAR for full activation of the checkpoint. Interestingly, ribotoxic stress causes TIAR to redistribute into distinct nuclear foci that are related to speckles, and were hence termed checkpoint speckles (ChSs). ChSs form specifically during S-phase in a p38-MAPK kinase-dependent manner. Mutation of either the RNA recognition motifs or the glutamine-rich domain of TIAR abolished both its recruitment to ChSs as well as its function in G2/M checkpoint activation. Besides TIAR, RNA polymerase II, U1 snRNP and U2AF showed enhanced accumulation in ChSs, suggesting a function in regulating co-transcriptional splicing. In line with this notion, transcriptome-wide splicing analysis revealed a global decrease in the intron/exon ratio during checkpoint activation, compatible with enhanced splicing activity. Taken together, our study identifies a novel role for stress-induced nuclear compartments in G2/M checkpoint activation.

359 Molecular mechanisms of the paraspeckle formation as distinct nuclear bodies

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NEAT1 long noncoding RNA (lncRNA) serves as an essential architectural component of phase separated membraneless organelles called paraspeckle nuclear bodies (NBs). Paraspeckles were initially defined as the foci found in close proximity to nuclear speckles, which are other phase-separated NBs, and sequestrate specific RNA-binding proteins and RNAs to control gene expression. In the paraspeckles, multiple proteins containing intrinsically disordered regions or prion-like domains are highly enriched, and phase separation of these proteins leads to the paraspeckle formation. In the cell nucleus, various NBs are separately present in spite of having no membrane structure, however, it remains unknown how these NBs co-exist without fusion.

During CRISPR/Cas9-mediated dissection of functional domains of NEAT1 in human haploid cells, we unexpectedly observed that, unlike in wild type cells, paraspeckles were incorporated into nuclear speckles in the specific NEAT1 mutant cell lines. This result raised an intriguing possibility that specific NEAT1 region(s) are required for formation and maintenance of paraspeckles as distinct nuclear bodies. Therefore, we used this mutant cell line to investigate the molecular mechanism how NBs segregate each other. We hypothesized that proteins associating with the specific NEAT1 regions were involved in the segregation process of paraspeckles from nuclear speckles. We then found that several proteins were less recruited to paraspeckles in the mutant cell line. Thus, we considered them as candidate proteins of the NB segregation. To examine whether these proteins play a role in this process, we used MS2 artificial tethering assay. The tethering of specific candidate proteins onto the NEAT1 mutant could rescue the segregation defect, leading to separation of paraspeckles from nuclear speckles. We are now investigating how domains and amino acids of identified proteins contribute to this segregation process.

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360 De novo missense changes in the helicase DDX6 cause intellectual disability and dysmorphic features by disrupting RNA regulation and P-body assembly.

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mRNA fate in the cytoplasm comprising translation, degradation, repression or storage, is depending on the associated RNA binding proteins (RBPs), constituting mRNPs which are found concentrated in cytoplasmic granules like P-bodies, stress granules, neuronal granules... We previously showed that P-bodies provide coordinated storage of mRNAs encoding proteins with regulatory functions (1) and that the RNA helicase DDX6 and the RBPs LSM14A and 4E-T are 3 key factors whose interactions are crucial for P-body assembly (2,3). However, the in vivo significance of P-bodies is still questioned.

Recently, genetic analyses showed that 5 young patients suffering of intellectual disability (and mild microcephaly at least in 2 cases) and various dysmorphic features each carry a de novo missense mutation impacting the same region of DDX6. We asked if these variants are associated with functional defects of DDX6. Our transcriptomic analysis of patient-derived cells revealed defects in RNA metabolism. We also observed that patient cells contain much less P-bodies than control cells. Using RNA interference strategy and complementation assays as a functional test in human cell lines, we demonstrated that these mutations result in a strong defect in P-body assembly. Co-immunoprecipitation analyses of DDX6 variants indicated that they are also defective for interaction with some of their partners, notably 4E-T and LSM14A. Altogether, our results provide the first evidence of a role of DDX6 and/or P-bodies in human; further experiments will be required to characterize their function in brain development.

[1] Hubstenberger, A., et al. (2017). P-Body Purification Reveals the Condensation of Repressed mRNA Regulons. Mol. Cell 68, 144-157.e5.

[2] Ayache, J., et al.. (2015). P-body assembly requires DDX6 repression complexes rather than decay or Ataxin2/2L complexes. Mol. Biol. Cell 26, 2579-2595.

[3] Kamenska, A., et al.. (2016). The DDX6-4E-T interaction mediates translational repression and P-body assembly. Nucleic Acids Res. 44, 6318-6334.

361 RNA is a critical element for the sizing and the composition of phase-separated RNA-protein condensates

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Liquid-liquid phase separation is thought to be a key organizing principle in eukaryotic cells to generate highly concentrated dynamic assemblies, such as the RNP granules. Numerous in vitro approaches have validated this model, yet a missing aspect is to take into consideration the complex molecular mixture and promiscuous interactions found in vivo.

Here we report the design of a versatile scaffold, called ArtiG (ArtiGranule), to generate concentration-dependent RNA-protein condensates within living cells, as a bottom-up approach to study the impact of co-segregated endogenous components on phase separation. We demonstrate that intracellular RNA seeds the nucleation of the condensates. Moreover, it provides molecular cues to locally coordinate the formation of endogenous high order RNP assemblies at the surface of ArtiGs, which is reminiscent of stress granules forming on pre-existing P-bodies in stressed cells.

Interestingly, the co-segregation of intracellular RNPs by ArtiGs ultimately impacts the size of the phase-separated condensates. Thus, RNA arises as an architectural element that can influence not only the composition but also the morphological outcome of the condensate phases in an intracellular context.

Navarro MGV, Kashida S, Chouaib R, Souquere S, Pierron G, Weil D, Gueroui Z. 2018. RNA is a critical element for the sizing and the composition of phase-separated RNA-protein condensates. ***BioRxiv, https://doi.org/10.1101/457986***

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362 Phase separation behaviour of the *Arabidopsis* SERRATE protein

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The phenomenon of phase separation is particularly important for self-organisation in living cells. It can lead to the spontaneous emergence of special membrane-less structures performing some dedicated functions in the cell. In the case of liquid-liquid phase separation (LLPS), a liquid mixture spontaneously separates into two immiscible phases that maintain different chemical composition.

We observed spontaneous droplet formation in samples of purified N-terminal fragment of the *Arabidopsis* SERRATE protein under molecular crowding conditions. This behaviour is characteristic for LLPS. SERRATE is a multifunctional nuclear protein acting e.g. in miRNA biogenesis, mRNA splicing and communication between splicing, miRNA biogenesis and RNA Polymerase II. SERRATE has a structured core and two disordered N- and C-terminal tails. The fragment of SERRATE covering amino acid positions 1–142 (SE_{1-142}), rich in arginine, proline and serine residues, was verified to be disordered by the circular dichroism spectroscopy. The attachment of Atto488 to the N-terminus of SE_{1-142} confirmed that the protein was present only in droplets. Further investigations confirmed the droplet formation behaviour of the full-length SERRATE protein, but not the protein mutant lacking the N-terminal disordered tail (amino acids 1-193).

We observed also an interesting correlation between the *in vitro* experiments performed and subcellular localization of SERRATE. When overexpressed in protoplasts with the green fluorescent protein fused to its N-terminus, the full-length SERRATE exhibits a speckled distribution in the nucleus. Gradual shortening of the protein starting from the N-terminus results in stepwise loss of speckles, showing the homogenous distribution upon removing the first 150 amino acids. These speckles contain also other proteins acting in miRNA biogenesis and splicing. Moreover, removal of the SERRATE N-terminal tail results in more homogenous distribution of U1 snRNP axillary proteins: atPRP39b and atPRP40a. Based on the results we propose a role of phase-separation properties of the SERRATE protein in formation of membrane-less nuclear bodies acting in *Arabidopsis* RNA metabolism.

363 Exploiting pathogen-associated molecular patterns present in RNA for improved diagnostics of infectious diseases

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Virus-derived transcripts can often differ from the cytosolic RNA of the host by several features, such as the presence of long stretches of exposed double-stranded RNA or the lack of some post-transcriptional modifications. These key differences are exploited by the vertebrate innate immune system: specialized receptors recognize the unusual RNA features as pathogen-associated molecular patterns, which leads to initiation of antiviral signaling cascades and mounting of the interferon response. We propose to employ components of these mechanisms to improve diagnostic procedures in detection of infectious diseases. This research is funded by the National Centre for Research and Development, Poland, under the grant agreement LIDER/039/L-6/14/NCBR/2015.

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364 Transcriptional and translational control of the host antiviral response by ILF3

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During viral infections, the presence of viral-derived dsRNA triggers an extensive transcriptional and translational response in the host. The antiviral response activates the expression of type I interferons and around 500 interferon stimulated genes (ISGs), but, at the same time, inhibits cap-dependent translation initiation affecting both host and viral-derived transcripts. Intriguingly, transcriptionally induced antiviral genes escape this translational shut-off, suggesting a regulatory control mechanism that selectively allows the expression of essential self-defence genes.

We have identified Interleukin enhancer binding factor 3 (ILF3) as an essential host factor required for efficient translation of interferon B1 (IFNB1) and a large subset of ISGs which are critical for establishing an effective antiviral state in the cell. By combining polysome profiling and high-throughput sequencing, we also show that ILF3 is required to enhance the antiviral-driven changes in transcription, alternative splicing and translation. Our data suggests that ILF3-mediated regulation of RNA metabolism is necessary to establish a robust cellular antiviral response.

365 CD44 intragenic DNA methylation is an indicator of its alternative splicing during tumor progression

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During tumour progression, the role of DNA methylation at promoters has been well characterized; but DNA methylation is also a suspected modulator of alternative splicing, while splicing defects in turn are involved in tumour formations nearly as frequently as DNA mutations. Yet, the possibility that DNA methylation impacts on tumorigenesis via its effect on splicing has not been thoroughly explored. Here, we have examined the effect of the inactivation of DNMT1 and DNMT3b in HCT116 colon carcinoma cells, which virtually eliminates DNA methylation. This results in an epithelial to mesenchymal transition (EMT) and a subsequent modified splicing of the CD44 cell surface adhesion receptor which loses extracellular regions encoded by alternative exons. This modified splicing is in part explained by altered splicing or expression of genes encoding splicing and chromatin factors. Yet, locally at the CD44 gene, changes in levels of intragenic DNA methylation had a sufficiently strong direct impact on the outcome of CD44 splicing to have a predictive value. This was further tested in a model for breast cancer progression and in patients with acute lymphoblastic leukemia (B ALL) that both showed changes of intragenic DNA methylation correlating with perturbations of CD44 alternative exon inclusion. Altogether, our observations suggest that, although DNA methylation may have multiple avenues to alternative splicing, the direct effect of intragenic CD44 DNA methylation allows to estimate the outcome of splicing of this key tumour progression marker.

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366 A non-amyloid prion particle that activates a heritable gene expression program

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Spatiotemporal gene regulation is often driven by RNA-binding proteins that harbor long intrinsically disordered regions in addition to folded RNA-binding domains. We report that the disordered region of the evolutionarily ancient developmental regulator Smaug drives self-assembly into gel-like condensates. These proteinaceous particles are not composed of amyloid. Yet they are infectious, allowing them to act as a protein-based epigenetic element: a prion. In contrast to many amyloid prions, condensation of Smaug enhances its function in mRNA decay, and its self-assembly properties are conserved over large evolutionary distances. Yeast cells harboring the [*SMAUG*$^{+}$] prion downregulate a coherent network of mRNAs and exhibit improved growth under nutrient limitation. Smaug condensates formed from purified protein can transform naïve cells to acquire the prion. Our data establish that non-amyloid self-assembly of RNA-binding proteins can drive a form of epigenetics beyond the chromosome, instilling adaptive gene expression programs that are heritable over long biological timescales.

367 Codon optimality regulates the epigenetic silencing of transposons in plants

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Transposons are stretches of DNA that can move around in the genomes and present in almost all eukaryotic organisms. Due to the nature of mobility, they are one of the most detrimental threats to genome integrity. Particularly in plants, transposons make up the largest part of chromosomal DNA, for example, over 85% of the genomes of major crops such as barley, wheat and maize are composed of transposons. Crop genomes are therefore exposed to continuous danger of genetic mutations caused by transposons. Those unwanted jumping of transposons is frequently observed during in vitro culture of plant tissues, which is broadly used for regenerating genome-edited crops. We have witnessed that recent advances of genome editing technologies such as CRISPR/Cas system have revolutionized the crop breeding field. However, the potential threat of uncontrolled transposition causing deleterious genomic instability still exists significantly hampering the development of stable and safe genome-edited crops. Despite the inherent threat of transposon jumping during the establishment of genome-engineered crops, we are still far from understanding the precise mechanisms for the transposon inhibition by the host genomes. Research in our group specifically aims to understand how transposons are initially recognized as genome invaders and thereafter repressed at the post-transcriptional level by switching on the epigenetic silencing machineries of the host. In this talk, I will discuss that transposon RNAs in plants are rich in suboptimal codons and thereby exhibit significantly reduced translational rate. In addition, we suggest that the translation-coupled RNA decay pathway is required for the 21- or 22-nt siRNA production, probably through forming phase-separated membrane-less granules in cytoplasm. Our work provides novel insights into how the host genomes differentiate self and non-self RNA and trigger RNA silencing pathways.

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368 Deep into cellular dark matter – role of rDNA array in stress response

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In many eukaryotic genomes genes encoding the ribosomal RNA (rDNA) are organized in highly repetitive locus which is known to exhibit greater instability relative to the rest of the genome. *Saccharomyces cerevisiae* wild-type cells preferentially maintain a stable number of rDNA copies, which suggests underlying genetic control of the size of this locus. However even in genomes of well known organisms rDNA is still poorly characterized loci.

The main function of rDNA is to produce RNA components of ribosomes but it also plays a pivotal role in nuclear organization by assembling the nucleolus. The rDNA array is believed to be involved in gene regulation and stress response. To determine the effects of the loss of the rDNA array on the viability and phenotype of yeast cells, I have utilized the rDNA array-deficient yeast strain with the only copy of rRNA genes located on the plasmid.

I have shown that this strain is more sensitive to different stress conditions, including temperature, antibiotic and osmotic stress but more resistant to oxidative stress. In addition, I have observed that rDNA array-deficient yeasts show slow growth phenotype. Bioinformatic analysis revealed connections between organization of rDNA and stress response. Importantly, observed differences are not due to the decreased expression of rRNA species, as rRNA levels are comparable in cells containing plasmid-born single rDNA gene or entire rDNA array. These results suggest that rDNA array is a part of regulatory mechanism involved in stress response, possibly via organization of the nucleolus.

369 Exploring the role of chromatin decondensation at a gene locus in stochastic gene expression

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Individual cells within isogenic populations show great variability or "noise" in gene expression. This variability is exploited by unicellular organisms for adaptation. Cancer relapse is suggested to be a consequence of variable gene expression, because it allows small fractions of cells to escape cancer therapy. A previous model suggests that noise is due to the random opening and closing of chromatin at the promoters, creating "on" and "off" states of gene expression. We aim to characterize the molecular mechanisms involved in the noisy gene expression by perturbing the chromatin specifically at a single gene locus.

We constitutively tethered the catalytic histone acetyltransferase domain of p300, a transcriptional co-activator, to the regulatory regions of the cyclooxygenase-2 (Cox-2) and collagenase I (Col I) genes. This constitutive tethering was accomplished by fusing the histone acetyltransferase domain of p300 to an endonuclease-deficient CRISPR-associated protein 9 (dCas9). Stable cell lines were created that express the dCas9 system along with small guide RNAs designed to guide the fusion protein to the target genes. We show that tethering a histone acetyltransferaseto regulatory regions of the target genes cause a significant increase in chromatin accessibility rendering the locus constitutively open.

We then measured the cell-to-cell variability in the expression of the Cox-2 and Col I genes in cell lines expressing the dCas9 system compared to the variability of the same genes in unmodified cells. To measure the variation in gene expression, we imaged and quantified each target mRNA in single cells using single-molecule FISH and then statically characterized the cell populations. Our results show that cells with the Cox-2 or Col I promoter region that are constitutively open due to tethering of histone acetyltransferase at their promoters, exhibit reduced variability in the number of mRNA transcripts. These results support the hypothesis that promoter accessibility plays an important role in heterogeneous gene expression.

Through an understanding of the fundamental molecular mechanisms of gene expression, future studies can be performed to control or lessen the magnitude of gene expression variability within cell populations, enabling an exploration of the phenotypic consequences.

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370 DOT1L suppresses nuclear RNAi originating from enhancer elements in *Caenorhabditis elegans*

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Methylation of histone H3 on lysine 79 (H3K79) by DOT1L is associated with actively transcribed genes. Earlier, we described that DOT-1.1, the *Caenorhabditis elegans* DOT1L homologue, cooperates with the chromatin-binding protein ZFP-1 (AF10 homologue) to negatively modulate transcription of highly and widely expressed target genes. Also, reduction in ZFP-1 levels has long been associated with lower efficiency of RNA interference (RNAi) triggered by exogenous double-stranded RNA (dsRNA), but the reason for this is not clear. Here, we demonstrate that DOT1L suppresses bidirectional transcription, including that producing enhancer RNAs, thereby preventing dsRNA formation and ectopic RNAi. This ectopic elevation of endogenous dsRNA may engage the Dicer complex and, therefore, limit efficiency of exogenous RNAi. Our insight provides a novel perspective on the underlying mechanisms of DOT1L function in development, neural activity, and cancer.

371 Differential epigenetic silencing of nuclear tRNA gene repeats in *Arabidopsis thaliana*

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The *Arabidopsis thaliana* nuclear genome contains around 600 tRNA genes (tDNAs). In general, each isoacceptor tRNA family owns a number of tDNA copies proportional to the corresponding codon usage frequency. However, Tyr, Ser and Pro tDNAs exhibit an excess number of genes[1] organized in clusters of repetitive elements. These copies contain all required *cis*-elements for their transcription by RNA polymerase III. Aside from this, we recently revealed, using northern blots and tRNA-derived fragments libraries analysis, that tRNAs from these clusters are undetectable in normal growth conditions. Based on these observations, we decided to further explore the molecular reasons of their silencing.

Using publicly available ChIP-seq and whole-genome bisulfite sequencing data, as well as other molecular approaches, we unveiled the epigenetic profiles of these clustered tDNAs. Correlated with their silencing, Tyr and Ser tDNA tandem repeats display repressive heterochromatic profiles similar to those of transposable elements loci or 5S ribosomal RNA gene tandem repeats. Indeed, at these tDNAs, CG, CHG and CHH appear methylated, mainly through the maintenance methyltransferases DDM1, MET1, CMT2 and, CMT3. Concomitantly to DNA methylation, KYP-dependent histone H3 lysine 9 dimethylation (H3K9me2) and H3K27me1/me2 are also found on the chromatin of Tyr and Ser tDNA repeats. Conversely, Pro tDNA repeats are only methylated in a MET1-dependent CG context and their chromatin is enriched in euchromatic histone marks typical of RNA polymerase II actively transcribed genes (i.e. H2B monoubiquitination and H3K4 and H3K36 methylation). This represents an intriguing epigenetic profile.

Altogether, we highlighted new features of particular plant tDNA gene clusters as to their arrangement into two diametrically opposed types of DNA methylation and chromatin contexts. These two types likely implies distinct biological functions related to the structural organization of chromosomes in the nucleus and/or the response/adaptation to environmental stress.

[1] Michaud, M., et al., Plant J. 66, 80-93 (2011).

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372 An attempt to reveal differences in RNA methylation of transcriptome of colon cancer model cell line (LoVo) as a result of azacitidine action.

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Among different types of modification of nucleotides in RNA, the most prevalent one is the methylation of the adenosine base at the nitrogen-6 position (m6A). m6A can be found in polyadenylated mRNA and long non-coding RNA and plays an important role in gene expression and splicing patterns. Moreover, it has been proven that there is a link between m6A modification and human diseases, especially numerous types of cancer. Despite RNA methylation is a phenomenon known since the 1970s, there was lack of suitable methods to analyze it until high-throughput techniques have been developed. CLIP is a group of methods that combines UV cross-linking with immunoprecipitation in order to analyze protein interactions with RNA. In the miCLIP variant, cross-link is made between N6-methyladenosine and anti-m6A antibody, that allows generating a pool of RNA fragments for RNA-Seq, resulting in revealing exact RNA methylation pattern. Here we present the application of the miCLIP method to study changes in RNA methylation in the LoVo colon cancer cell line. The study design involved a 72-hour incubation of LoVo cells with azacitidine at a concentration of 10 μM. Azacitidine, a drug used in the treatment of certain hematological cancers, is a chemical compound that leads to DNA demethylation. The aim of the study was to investigate whether the use of a substance that causes DNA demethylation will translate into changes in RNA methylation. The miCLIP technique was used to compare the extent of RNA methylation derived from treated and untreated cells with azacitidine. Surprisingly, we found that methylation of transcripts of only very few genes has been changed. The biggest differences have been observed in transcripts of CASC19, PIGT and CLN3 where immense (FC ~10) m6A demethylation occurred after incubation with azacitidine. Further experiments are required as obtained data suggests that there is no direct link between DNA and RNA demethylation processes.

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373 Links between chromatin status and 3′-end mRNA processing

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The eukaryotic genome is organized into complex structures of DNA and protein called chromatin. The basic unit of chromatin, or nucleosome, consists of 147 base pairs of DNA wrapped around a histone octamer comprised of two copies each of histone H2A, H2B, H3 and H4. Post-translational modifications (PTMs) on the tails of these core histone proteins establish a code that modulates how genes are expressed. Proper gene expression also relies on the processing of nascent RNA transcripts to become mature mRNA. Conventionally, PTMs on histones alter chromatin accessibility, but recent work in *Drosophila melanogaster* using a "histone replacement" system identified a link between modification of the K36 residue on histone H3 and the quality of the mRNA transcripts generated. This study revealed that H3K36R mutants produce transcripts with decreased poly(A) tail length and fewer mature, polyadenylated RNA transcripts of a select group of highly expressed genes. This initial evidence suggests that PTMs not only influence chromatin state, but also directly impact the 3'-end of the transcript and, thus, the stability of the RNA produced. The relationship between histone modifications and mRNA transcript stability extend the possible functional implications of histone modifications to the regulation of post-transcriptional processes. Thus, we hypothesize that specific PTMs on H3K36 are required for proper recruitment of protein machinery that adds and processes the poly(A) tail on the 3'-end of the elongating transcript. We have generated *Saccharomyces cerevisiae* expressing a histone H3 variant altered at the K36 residue using genome editing. Complementary genetic and biochemical approaches will be employed to explore interactions between changes in H3K36 and the evolutionarily conserved 3'-end formation machinery. We will characterize these mutants with regards to growth and drug sensitivity and determine how they affect 3'-end formation. Additionally, we will use discovery-based approaches to identify specific factors and pathways that are impacted by mutations at the H3K36 residue. These results will be complemented by experiments performed in *Drosophila*. Information learned from this study will determine how histone modifications influence gene expression through a novel mechanism: by impacting the stability of the RNA transcript via generation and protection of the 3'-end.

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374 Splicing influences Set2 recruitment and H3K36me3 at intron-containing genes in *Saccharomyces cerevisiae*

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Disruption of splicing leads to altered chromatin modifications. In particular, there is growing evidence for tri-methylation of lysine residue 36 in histone H3 (H3K36me3) being a strategic link between splicing and chromatin regulation. In metazoans, nucleosomes with H3K36me3 mark the boundary between introns and exons [1]. In human cells, inhibition of splicing reduced the recruitment of the H3K36 methyltransferase, SETD2, at intron-containing genes [2], and reduced the level of H3K36me3 and/or caused a shift of its distribution toward the 3' ends of genes [3].

However, more mechanistic questions remain unanswered, for example: Which stage of splicing and which factors are responsible for H3K36me3 loss? How is H3K36me3 demethylated?

To gain more insights into how splicing affects H3K36 methylation in budding yeast we combined two approaches: 1) trans-acting splicing factors were conditionally and rapidly depleted by auxin-induced degradation (AID), and 2) the effect of a cis-acting splice site mutation was analysed using a reporter gene. We monitored both Set2 recruitment and H3K36 methylation by ChIP-qPCR in these two systems.

AID depletion of individual splicing factors indicates that specific steps in the splicing cycle are necessary to stimulate H3K36 tri-methylation at specific gene locations during transcription. In particular, the depletion of a U1 or U2 snRNP protein leads to reduction of both Set2 recruitment and H3K36me3 at several endogenous intron-containing genes. Disruption of later-acting factors does not reduce Set2 recruitment, however, further progress through the spliceosome assembly pathway is required for normal H3K36me3 levels.

Our high-resolution kinetic studies reveal a fluctuation of Set2 and H3K36me3 levels at an inducible reporter gene following induction of transcription. However, a cis-acting mutation reduces both Set2 recruitment and H3K36me3. Interestingly, deletion of the histone demethylase Rph1 partially suppresses this tri-methylation defect. We propose a model in which splicing factors and Rph1 may have competing roles in the recruitment of Set2 and H3K36me3 deposition at intron-containing genes.

[1] P. Kolasinska-Zwierz et al. (2009) Nat Genet 41: 376-381.

[2] Kim et al. (2011) PNAS 33: 13564-13569

[3] de Almeida et al. 2011 Nature Structural & Molecular Biology 18: 977-983

375 Genome surveillance by non-coding transcription

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RNA Polymerases IV and V are plant-specific DNA-dependent RNA polymerases specialized to work in RNA-directed DNA methylation (RdDM). This conserved process silences transposons and controls expression of several genes. Pol IV produces precursors for siRNA biogenesis, however small RNAs cannot base-pair with DNA and instead recognize their target loci in chromatin by base-pairing with nascent transcripts. In plants these long nascent noncoding RNAs are produced by Pol V. The existing model of RdDM predicts that both Pol IV and Pol V transcribe in highly locus-specific manners and initiation of their transcription targets specific loci for silencing. We show data suggesting that this model may be incorrect. Pol V transcription is much more widespread than the presence of canonical silencing targets. This indicates that Pol V may transcribe a significant fraction of the genome to make it competent for silencing by siRNA. Our findings have significant implications for the understanding of how newly integrated or activated transposons are first recognized. Given the parallels between transcriptional silencing in plants and other eukaryotes, it is possible that pervasive transcription may have a conserved role in genome surveillance and transposon silencing.

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376 Polycistronic miRNA processing regulates the good and the bad of self-renewal

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Almost a half of microRNAs (miRNAs) in mammalian cells are generated from polycistronic primary transcripts encoding more than one miRNA. Polycistronic clusters produce mature miRNAs that frequently regulate complementary sets of target mRNAs. Why miRNAs are organized in clusters and how the processing of individual miRNAs within the cluster is controlled in vivo is not well understood. By taking a 'functional RNAomics' approach, we have previously uncovered how the RNA binding protein Serine-arginine rich splicing factor 3 (SRSF3) regulates the processing of a coordinated network of RNAs encoding factors with critical functions in pluripotency. The determination of SRSF3 regulated noncoding RNAs in pluripotent cells identified miRNA clusters as key SRSF3 targets, with SRSF3 binding to the previously identified CNNC motif downstream of the miRNA stem loop. Individual miRNAs within the SRSF3 target clusters were differently regulated and gave rise to >70% of all miRNAs in pluripotent cells. SRSF3 binding position within the miRNA cluster determined the pri-miRNA processing efficiency and relative expression of individual miRNAs from the cluster.Target analysis of the SRSF3 regulated miRNAs demonstrated that SRSF3 confers some of its self-renewal promoting properties through the regulation of the polycistronic miRNAs both in normal pluripotent stem cells and cancer stem cells. Furthermore, analysis of a cohort of colorectal cancer patients demonstrated that SRSF3-regulated polycitronic miRNA pathway is characteristic of poorly differentiated stem cell like tumours, suggesting a key role in controlling both the good and bad of self-renewal.

377 The roles of RNA helicases during large ribosomal subunit biogenesis

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RNA helicases are ubiquitously expressed enzymes that play important roles in many key cellular pathways through their energy-dependent functions in structurally remodeling RNA molecules and ribonucleoprotein (RNP) complexes. One key pathway that requires numerous RNA helicases is the synthesis of ribosomes, a complex and dynamic process that involves the processing, modification and folding of precursor ribosomal RNAs as well as the correct assembly of approximately 80 ribosomal proteins. In *S. cerevisiae*, 21 RNA helicases are involved in ribosome biogenesis. While several RNA helicases have been shown to be required for the release of specific snoRNAs from pre-ribosomes, others are implicated in structural rearrangements that promote recruitment or release of subsets of ribosomal proteins and/or biogenesis factors. However, the precise functions of several RNA helicases still remain unknown. To address this, we analyze pre-rRNA processing upon depletion of specific RNA helicases involved in biogenesis of the large ribosomal subunit (60S) and determine the composition of the pre-ribosomal complexes they associate with using mass spectrometry. Together, these approaches reveal insights into the timing of their recruitment to pre-60S particles. We employ crosslinking and analysis of cDNAs (CRAC) to identify specific RNA helicase binding sites on pre-ribosomes, thereby facilitating further functional analysis. For example, dimethyl sulfate (DMS) structure probing or mass spectrometry performed on pre-ribosomal particles isolated in the absence or presence of a given RNA helicase can be used to analyze RNA helicase-dependent rRNA rearrangements or changes in protein composition. Alternatively, quantitative PCR of snoRNA levels on pre-ribosomes and RiboMeth-Seq analysis of rRNA 2'-*O*-methylation can provide insights into potential roles in regulating snoRNP dynamics and rRNA modification. Altogether, our data suggest diverse role for RNA helicases during the course of pre-60S maturation.

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378 Role of the RNA-binding protein Csde1 in oncogene-induced senescence

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Cellular senescence is a state of permanent cell cycle arrest characterized by a complete remodelling of gene expression and cell morphology triggered by a variety of insults. Despite the diversity of trigger-dependent senescence features, senescent cells share a unique common trait, the secretion of pro-inflammatory molecules known as the senescence-associated secretory phenotype (SASP). We have previously found that Csde1 behaves as an oncogene in melanoma (1). We now find that, in normal primary mouse keratinocytes (PMK), Csde1 behaves as a tumor suppressor, at least in part by promoting SASP expression.

PMK over-expressing H-Ras undergo senescence efficiently. However, depletion of Csde1 leads to senescence bypass and to establishment of an immortalized phenotype. Accordingly, BrdU incorporation and colony formation assays show increased proliferation of Csde1-depleted cells. In addition, conditioned-media analysis shows impairment of the SASP response. Interestingly, identification of Csde1 targets by irCLIP revealed senescence-related transcripts that are differentially bound by Csde1 upon senescence induction. Taken together, these results suggest that Csde1 plays a crucial role in oncogene-induced senescence by dynamically binding and regulating senescence-associated transcripts. The dual, context-specific roles of Csde1 in tumor promotion or suppression will be discussed.

379 Single Molecule Investigations of Small Molecule, G-Quadruplex, and Protein Interactions

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Guanine-rich segments of the genome are prone to folding into G-quadruplex structures (GQs), which are more prominent at telomeric overhangs and promoters of DNA and untranslated regions of RNA. Telomeric GQs have emerged as potential targets for GQ stabilizing small molecules that inhibit telomerase activity in cancer cells, while GQs in promoters and untranslated regions of RNA regulate gene expression at transcription or translation level, respectively. Due to their high thermal stability, GQs require protein activity to be unfolded. We present single molecule fluorescence measurements probing the interactions of GQs, proteins (Bloom helicase-BLM, and Replication Protein A-RPA), and small molecules. Using a fluorescently labeled small molecule, we measured the dwell time of this GQ stabilizing molecule on GQ at the single molecule level. We also quantified the impact of several prominent small molecules on the dynamics and steady state BLM-mediated GQ unfolding activities using two different approaches. We report 2-3 fold reduction in BLM activity due to these small molecules. Finally, we present RPA, GQ, and small molecule interactions while the GQ is maintained under tension using a force transducer based on a short looped DNA construct. We demonstrate an order of magnitude reduction in RPA-mediated GQ unfolding in the presence of a GQ-stabilizing small molecule.

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380 Mapping and Quantifying Stress-Responsive RNA Binding by the Conserved Ssd1 Protein in *Saccharomyces cerevisiae*

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Ssd1 is an RNA binding protein conserved across fungi that is required for stress tolerance in *Saccharomyces cerevisiae* as well as virulence in the fungal pathogen *Candida albicans*. In unstressed cells, Ssd1 is known to associate with, and repress translation of, mRNAs encoding proteins with roles in cell wall biosynthesis and remodelling. We have mapped stress-responsive RNA binding by Ssd1 using CRAC (crosslinking and cDNA analysis) to identify precise sites of RNA interaction. In unstressed cells, Ssd1 binds predominantly on the 5' UTRs of the previously identified mRNAs. An RNA oligonucleotide derived from a 5'-UTR cross-linking site associated with a consensus motif binds recombinant Ssd1 protein *in vitro*. Upon short exposure to heat stress at 42°C, Ssd1 binding was reduced for many of these mRNAs, whereas binding increased for heat shock protein mRNAs. Moreover, these interactions are more generally dispersed across the mRNAs, including their 3'-UTRs. Consistent with this, mRNAs bound to Ssd1 under non-stressed conditions are recruited to heat shock granules upon heat stress, however Ssd1 is not. Amongst mRNAs showing the greatest increase in Ssd1 binding after heat shock are HSP104 and STI1, which encode protein-disaggregase chaperones that are required for induced thermotolerance. It was reported previously that Ssd1 is required for induced thermotolerance, and that Ssd1 influences the ability of Hsp104 protein to hexamerise, interact with its chaperone Sti1 and bind heat-induced protein aggregates. Thus, coordinated control of translation and post-translational interactions by Ssd1 may regulate Hsp104-mediated protein disaggregation following heat stress.

381 Discovering RNA-binding proteins involved in T-cell acute lymphoblastic leukemia

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T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive haematological cancer which is characterized by excessive proliferation of malignant T-lymphocyte progenitors. To date, research on T-ALL has mainly provided insights on the transcriptional level of the disease. We, however, are interested in the post transcriptional level – particularly in RNA-binding proteins (RBPs). RBPs are implicated in a number of physiological processes including RNA splicing, translation and decay. During the development and progression of many cancers, RBPs become abnormally regulated and can contribute to cancerous processes such as uncontrolled proliferation, evading immune surveillance and inducing metastasis. Various mechanisms can lead to RBP dysregulation, including aberrant post-translational modifications (PTMs). PTMs are presumed to fine-tune RBP activity and recent studies revealed that the bindings sites of RBPs are hotspots for PTMs such as acetylation. Histone deacetylases (HDACs) remove such acetylation marks from histone and non-histone proteins. In T-ALL, elevated expression levels and enhanced activity of HDACs were reported. Moreover, HDAC inhibitors showed anti-leukemic effects and have recently been approved for the treatment of T-cell lymphoma (e.g. Vorinostat).

We hypothesize that a number of RBPs are involved in T-ALL which have so far remained undiscovered and that RBPs are new potential targets for the treatment of T-ALL with HDAC inhibitors. Consequently, this study aims at uncovering the complete repertoire of RBPs in T-ALL cells, including alterations in the RBP composition upon HDAC inhibition. We use the recently established enhanced RNA interactome capture (eRIC) technique in which RBPs are UV cross-linked to polyadenylated RNAs. After cell lysis, the covalently bound RBPs are captured with a locked nuclei acids (LNA)-based probe and subjected to stringent washes. Quantitative mass spectrometry identification of the purified proteins is used to determine the mRNA interactome. As a model for the disease and the response to HDAC inhibition, we use non-treated and Vorinostat-treated T-ALL cell lines. This study will provide information on an additional level of regulation in T-ALL. Ultimately, it will help to further our understanding of the pathogenesis and the response to therapy and potential new treatment options involving HDAC inhibition, either alone or in combination with other drugs.

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382 Epicardial SRSF3 function in myocardial repair

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The mammalian heart is unable to effectively repair itself following myocardial infarction (MI). During development, the outer heart layer, the epicardium, contributes vascular smooth muscle cells and a subpopulation of endothelial cells to the coronary vasculature but also fibroblasts and up to 10% of the heart's cardiomyocytes. Epicardial cells (EPDC) are quiescent in the adult, but become stimulated after MI and facilitate neovascularisation of the ischaemic heart by undergoing epithelial to mesenchymal transition (EMT), migration and differentiation.

Alternative splicing generates mRNA diversity and allows remodelling of the transcriptome to orchestrate complex physiological transitions, e.g. cardiac morphogenesis, via coordinated splicing transitions. The importance of splicing for the heart is evident from knockout (KO) mouse lines, such as members of the SRSF family of splicing factors (Srsf1, Srsf2 and Srsf10), which display late embryonic cardiac phenotypes, preceded by mis-regulation of alternative splicing.

A number of key epicardial genes are subject to alternative splicing, but the functional significance of alternative splicing in an epicardial context has not been explored, but has a clear potential to govern the multipotency and activity of EPDCs. A putative interaction with a regulator of epicardial fate determination led us to hypothesise that a member of the SRSF family, SRSF3, can regulate epicardial gene splicing during critical periods of epicardial activity i.e. embryonic development and post-MI.

Staining of mouse heart sections before and after MI, demonstrated re-expression of SRSF3 in re-activated epicardium together with other epicardial markers. To investigate the RNA binding role of SRSF3 in epicardium, we optimised irCLIP of endogenous SRSF3 in a mouse epicardial cell line. We compared SRSF3 RNA-binding and splicing profiles in the epithelial and mesenchymal state of epicardial cells to identify SRSF3-governed transcriptome changes that could determine cell fate decisions. We further investigated the role of SRSF3 in vivo, using an epicardial-specific knock-out mouse model and observed altered EPDC differentiation, confirming a key role for SRSF3 in cell fate determination in the heart and making its reactome an interesting target for treatments to improve heart repair after MI.

383 RNA-binding protein MSI-1 affects *let-7* microRNA-regulated stem cell-like division of the epithelial seam cells in *Caenorhabditis elegans*

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The RNA-binding proteins (RBPs) Musashi1 and Musashi2 are two closely related RNA-recognition motif (RRM) containing proteins that regulate neural stem cell proliferation in mammals via controlling the fate of various target mRNAs. Recently, the Musashi proteins were found to be overexpressed in a variety of cancers, including colorectal, lung, and pancreatic cancers; glioblastoma; and several leukemias, associated with poor prognosis. The Musashi proteins play a carcinogenic role by regulating mRNA stability and protein translation of multiple genes involved in oncogenic signaling pathways or stemness maintenance. In *C. elegans*, expression of the sole Musashi-1 (MSI-1) has been detected in embryogenesis intensely and also in multiple neuron cells and the gut at later developmental stages. Mutation of the *msi-1* gene has been implicated in defective male mating behavior and memory loss. So far, whether the *C. elegans* MSI-1 could play roles in post-embryonic stem cell-like division outside the nervous system, such as those in vulval precursor cells or epithelial seam cells, remains unclear.

In an attempt to identify RBPs involved in the *let-7* microRNA-regulated gene pathways in *C. elegans* by feeding RNAi screen, we found that *msi-1(RNAi)* significantly suppressed the lethal *let-7(n2853)* vulval bursting phenotype. The *let-7(n2853)* mutation changes the fifth G to an A in the mature *let-7* miRNA, leading to destabilization of target interactions and up-regulation of *let-7* target mRNAs, including *lin-41*. The well-characterized conserved *let-7*/LIN-41 regulation determines cell differentiation in human and cancer stem cells. In *C. elegans*, the *let-7*/LIN-41 pathway regulates downstream *lin-29*, encoding a transcription factor homologous to human EGR (Early Growth Response) family proteins, which promotes terminal differentiation of stem cell-like epithelial seam cells. In *let-7(n2853)* mutant worms, impaired *let-7* function results in reiterated division of seam cells that fail to terminally differentiate due to the lowered LIN-29 activity at the young adult stage. Interestingly, we found that *msi-1(RNAi)* also suppressed this retarded seam phenotype by microscopic examination of worms carrying a seam cell marker, pSCM::GFP. By contrast, *msi-1(RNAi)* failed to suppress the same retarded seam phenotype in *lin-29(n333)* loss-of-function worms. Our results indicate that MSI-1 may affect *let-7*-regulated epithelial seam cell differentiation in *C. elegans*.

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384 Coregulation of primary microRNA processing by the SR proteins SRSF1 and SRSF3

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The serine and arginine-rich (SR) proteins are a conserved family of RNA binding proteins with multiple roles in post-transcriptional gene regulation. Recently, the SR proteins SRSF1 and SRSF3 were linked to regulation of microRNA biogenesis, however the mechanisms remain enigmatic[1-4]. To determine how SRSF1 influences microRNA processing we used individual nucleotide resolution crosslinking immunoprecipitation (iCLIP) to characterize interactions with microRNA-containing transcripts in HEK293 cells on a global scale. This experiment revealed SRSF1 binding sites located 30-50 nt upstream of the basal stem in ~300 miRNAs. Using both cell based and in vitro assays, we demonstrate that binding sites in the 5' leader sequence of primary microRNA 10b are required for SRSF1-dependent processing. Chemical probing of wild type primary miRNAs or those lacking SRSF1 binding sites demonstrates that defects in miRNA processing are associated with different RNA structures. SRSF3 was recently shown to interact with sequences on the 3'end of the basal stem and promote microprocessor complex recruitment. Since SR proteins coordinately regulate exon definition during pre-mRNA splicing, we hypothesized that SRSF1 and SRSF3 may function together to promote microRNA biogenesis. Using both in vivo and in vitro assays we found that SRSF1 and SRSF3 enhance miRNA and processing. Taken together, our results expand the constellation of RNA regulatory elements involved in microRNA biogenesis and clarify the roles of SR proteins in noncoding RNA processing.

1. Fernandez, N. et al. Genetic variation and RNA structure regulate microRNA biogenesis. Nat. Commun. 8, 15114 (2017).

2. Auyeung, V. C., Ulitsky, I., McGeary, S. E. & Bartel, D. P. Beyond secondary structure: primary-sequence determinants license pri-miRNA hairpins for processing. Cell 152, 844-858 (2013).

3. Wu, H. et al. A splicing-independent function of SF2/ASF in microRNA processing. Mol. Cell 38, 67-77 (2010).

4. Kim, K., Nguyen, T. D., Li, S. & Nguyen, T. A. SRSF3 recruits DROSHA to the basal junction of primary microRNAs. RNA 24, 892-898 (2018).

385 Structural Determination of Dicer-2 Complexes

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RNA interference (RNAi) is essential for gene silencing and fighting viral infection in *Drosophila melanogaster* (*D.mel.*). A key protein in this pathway is Dicer-2 (*dm*Dcr-2) which processes double-stranded RNA (dsRNA) to make siRNAs. We recently solved a 6.8 Å resolution structure of *dm*Dcr-2's helicase domain in complex with blunt dsRNA which revealed a second module for termini recognition (Sinha et al., Science, 2018). Blunt dsRNA, a mimic of viral dsRNA, is threaded through the helicase domain and processively cleaved by the RNase III domains into products of various lengths. This is in contrast to dsRNAs that harbor two nucleotide 3' overhangs, often found on cellular dsRNAs, which interact with the PAZ/Platform domain and are cleaved into canonical 22 nucleotide products by the RNase III domains. We now seek to extend our structural work by solving a cleavage-competent structure of *dm*Dcr-2 in complex with blunt dsRNA. Our strategy entails using a biotin-monovalent streptavidin-blocking mechanism to trap the blunt dsRNA in the helicase domain. We demonstrate that blunt dsRNA with an internal biotin block incubated with monovalent streptavidin leads to partial dsRNA threading through the helicase domain. By monitoring cleavage products over time by denaturing gel electrophoresis, we established that the addition of monovalent streptavidin to biotinylated blunt dsRNA results in the loss of cleavage products greater than 20 base pairs in size, suggesting the blocking mechanism is effective. We also use gel-mobility-shift assays to demonstrate that the biotin-monovalent streptavidin-blocked blunt dsRNA incubated with *dm*Dcr-2 forms a stable complex. By negative staining TEM, particles appear structurally homogeneous and mono-disperse. We are now in the process of optimizing cryo-EM grid conditions for eventual data collection on our new Titan Krios for 3D structure determination. Achieving higher-resolution structures of *dm*Dcr-2 in complex with dsRNA will provide new mechanistic insights into helicase-mediated processing of its substrates, including how processive threading and unwinding occur. Our system also provides a platform that enables structural studies of *dm*Dcr-2 in complex with its protein binding partners, such as R2D2 and Loqs-PD.

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386 Characterizing the stimulation of spliceosomal DEAH-box helicases by G-patch proteins

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In eukaryotes the vast majority of non-coding intron sequences present in precursor mRNAs are excised via the spliceosome, a multimegadalton molecular machine composed of numerous protein and RNA components. Two key-players during pre-mRNA splicing are the DEAH-box helicases Prp2 and Prp43. While Prp2 has only ATPase- but no helicase activity and is necessary for the catalytic activation of the spliceosome, Prp43 is a genuine helicase and catalyzes the disassembly of the intron-lariat spliceosome. Both helicases are known to be stimulated by G-patch proteins, named after their characteristic glycine-rich motif. The G-patch protein Spp2 stimulates the RNA-dependent ATPase activity of Prp2. Besides the function in the spliceosome, Prp43 is involved in ribosome biogenesis and apoptosis. Its ATPase and helicase activity is stimulated by four different G-patch proteins, depending on the pathway (Pfa1, Ntr1, Gno1 and Cmg1). In addition to stimulation, these proteins have also been shown to recruit Prp43 to certain cellular locations, thereby determining its function.

To shed light onto the interaction between spliceosomal ATPases and their G-patch cofactors, we solved different crystal structures of a complex formed of Prp2-ADP and the G-patch motif of Spp2. The N-terminal region of the G-patch forms an α-helix that binds through mainly hydrophobic interactions to the winged-helix domain of Prp2. The C-terminal part of the G-patch motif interacts with the RecA2 domain and is able to adopt two alternative conformations. Additionally, we could show that the G-patch motif of Pfa1 increases the affinity of Prp43 towards single stranded RNA, which could lead to a reinforcement of processivity. To further investigate the role of the G-patch in stimulating the ATPase cycle of Prp43, we characterized its influence on nucleotide binding using steady-state and pre-steady state techniques.

387 Loss of DHX36 helicase activity leads to accumulation of translationally inactive target mRNAs with G-rich structures in untranslated regions

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Translation efficiency can be affected by mRNA stability and secondary structures, including G-quadruplex structures (G4s). The highly conserved DEAH-box helicase DHX36/RHAU resolves G4s on DNA and RNA *in vitro*, however a systems-wide analysis of DHX36 targets and function is lacking. We globally mapped DHX36 binding to RNA in human cell lines and found it preferentially interacting with G-rich and G4-forming sequences on more than 4,500 mRNAs. While DHX36 knockout (KO) resulted in a significant increase in target mRNA abundance, ribosome occupancy and protein output from these targets decreased, suggesting that they were rendered translationally incompetent. Considering that DHX36 targets, harboring G4s, preferentially localize in stress granules, and that DHX36 KO resulted in increased SG formation and protein kinase R (PKR/EIF2AK2) phosphorylation, we speculate that DHX36 is involved in resolution of rG4 induced cellular stress.

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388 RNA translocation mechanism of spliceosomal DEAH-box ATPases

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The spliceosome-mediated excision of non-coding intron sequences in precursor mRNAs requires the action of eight DExD/H-box ATPases. These proteins use the energy from ATP hydrolysis to ensure compositional and conformational rearrangements during the assembly, activation, catalysis and disassembly steps of a splicing cycle. DEAH-box ATPases are implicated in the three latter steps and were long thought to fulfill their tasks by direct unwinding of RNA duplexes. Recent studies, however, suggest that they rather function as ssRNA translocases and remodel the spliceosome from a distance by a mechanism called winching. Crystallographic studies on DEAH-box ATPases over the last decade have tried to shed light onto the motor function of this protein family and the analyses of different ligand-bound states have been key to unravel the molecular dynamics. While early ADP-bound structures of Prp43 revealed the global architecture, later ATP- and RNA-bound structures unmasked insights into the RNA-loading and -binding mechanism and first domain movements could be described. More recently, adenosine nucleotide-free structures of Prp22 showed that DEAH-box ATPases are able to adopt more open conformations of the helicase core in the absence of ADP/ATP. By toggling between these closed and open conformations of the helicase core they are able to translocate along an ssRNA in 3'-5' direction with a step-size of one RNA nucleotide per hydrolyzed ATP. These dynamics additionally pose a particular challenge for other interaction partners, such as G-patch proteins that modulate the function of Prp43 and Prp2. The G-patch motif of the intrinsically disordered protein Spp2 is mostly unfolded in solution but its N-terminal part stably binds Prp2 *via* an amphipathic α-helix, while the C-terminal part of this motif adopts two alternative conformations in different crystal structures. The increased conformational freedom of this region likely allows the G-patch to adapt to the different adenosine nucleotide-dependent conformations of the helicase core.

389 Global analysis of molecular interactions underlying functions of NANOS1 and NANOS3 proteins in human germ cell model

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Nanos RNA-binding proteins are critical factors of germline development throughout the animal kingdom and their dysfunction causes infertility. They act as posttranscriptional regulators by binding to target mRNAs followed by recruitment of CCR4-NOT deadenylation complex for repression. Mammalian Nanos paralogues play divergent roles in germ cell development i.e. NANOS1 promote germ cell proliferation while NANOS3 is critical for maintenance of germ stem cell characteristics. However, the molecular basis of their functional differences have not been defined yet. We hypothesized that different roles of NANOS1 and NANOS3 paralogues stem from regulating distinct pools of mRNAs by binding to different protein co-factors. To test this, first we have performed a global analysis of mRNAs being under NANOS1 and NANOS3 control by overexpression followed by RNA-Sequencing (RNA-Seq) in a model of human male germ cell line, TCam-2. RNA-Seq revealed largely distinct groups of mRNAs regulated by NANOS1 and NANOS3. In order to predict functional consequences of the regulation of proteins they encode we built high confidence protein-protein interaction networks based on STRING database and identified functional complexes by MCODE. The above analysis suggests that NANOS1 and NANOS3 regulate some diverse biological processes in TCam-2 cells. Second, we have performed a global search for protein interacting with NANOS1 and NANOS3, by Co-IP followed by mass spectrometry (Co-IP/MS). This revealed that NANOS1 and NANOS3 mostly interact with different sets of proteins, several of them containing RNA-binding domains. To further check whether this may explain divergent pools of target mRNAs interacting with each NANOS protein we are currently searching for motifs recognized by RNA-binding domains of NANOS1 or NANOS3 interacting proteins in those mRNA targets.
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390 Identification of novel RNA-binding proteins in human mitochondria with focus on poly(A)-binding proteins

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Binding of proteins to RNA is crucial for every step of gene expression and RNA metabolism. For instance, interaction of poly(A)-binding proteins with polyadenylated nucleus-encoded transcripts influences their stability and translation. It is known that mitochondrial mRNAs also possess stable poly(A) tails, however mitochondrial poly(A)-binding proteins remain unknown. The project's aim is to identify novel human mitochondrial RNA-binding proteins with particular focus on poly(A)-binding proteins. To accomplish this goal, mitochondrial protein extracts were subjected to affinity chromatography using poly(A) conjugated beads and purified proteins have been identified by mass spectrometry. Initial experiments showed relatively high background. A series of optimization experiments has been conducted in order to discard false positives from among identified proteins. In the improved approach the affinity chromatography has been extended to poly(U) and poly(C) conjugated beads. A group of putative mitochondrial RNA-binding proteins has been identified. This method allowed to capture known mitochondrial RNA-processing proteins and identify novel putative RNA-binding proteins. Selected identified proteins are being subjected to functional studies.

391 Development a FRET-based RNA biosensor.

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The ability to detect the presence of RNA with sequence specificity is an essential research tool for many fields. In our work we are currently developing a fluorescent protein-coupled RNA detection system that is both very sensitive and also sequence-specific.

A family of RNA binding proteins known as the pentatricopeptide repeat (PPR) proteins are a potential target for designing sequence-specific RNA binding proteins. These proteins are defined by a repetitive 35 amino acid sequence, with multiple repeats forming an alpha-solenoid structure. Each repeat binds to a single RNA base with specificity through the interactions of two amino acid residues, binding the nucleotide according to binding 'code'.

From the natively occurring proteins, we have created designer proteins (dPPR) that are able to be targeted to novel RNA sequences not found natively with high affinity and specificity. While studying these designer proteins we discovered unique conformational changes that occur upon RNA binding, that can be exploited in a FRET system as a reporter for RNA binding.

This FRET-dPPR system can detect picomolar levels of RNA in in-vitro assays and provides binding affinities and kinetics for the system. This technique can be applied on the single-molecule level, gaining insight relating to the binding kinetics of individual proteins and conformational changes in the proteins. This same RNA detection technique can allow for the in vivo monitoring of specific RNA in living cells and holds promise for the use in biosensors and biotechnology for in the detection of RNA.

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392 Nuclear and Cytoplasmic Cofactors of the RNA Exosome Play Unique Roles in Regulation of RNA Transcripts in Conjunction with RNA-Binding Protein Nab2

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RNA binding proteins play important roles in the processing and precise regulation of both coding RNAs and non-coding RNAs. Highlighting the biological importance of RNA binding proteins is the increasing number of human diseases that result from mutations in genes that encode these proteins. We recently discovered that mutations in the *ZC3H14* gene, which encodes an evolutionarily conserved poly(A) tail RNA-binding protein, cause intellectual disability. The majority of the studies that have provided insight into the function of ZC3H14 have exploited the budding yeast model to study the ZC3H14 orthologue, Nuclear Poly(A) Binding protein 2 (Nab2). The *NAB2* gene is essential in S. cerevisiae and conditional *nab2* mutants cause defects in a number of steps in RNA processing. To explore the critical functions of the Nab2/ZC3H14 protein family, we have performed a high-copy suppressor screen on *nab2* mutant yeast cells. This screen identified genes encoding two core subunits of the RNA exosome Rrp41 and Rrp42 as well as Nrd1 and Ski7, nuclear and cytoplasmic cofactors of the RNA exosome, respectively. Nrd1 is an RNA binding protein that is part of the Nrd1-Nab3-Sen1 (NNS) complex, which plays an important role in transcription termination of non-coding RNAs. Ski7 is a GTP-binding protein that mediates interaction between the RNA exosome and the Ski complex, which targets RNA transcripts to the exosome for processing and degradation in the cytoplasm. We have used structure function analysis to determine that the RNA binding function of Nrd1 is required for the suppression of growth defects in *nab2* mutant cells, while the Nab3-interacting and CTD-interacting functions are not required. In the case of the cytoplasmic cofactor, the RNA exosome-interacting domain is required for Ski7-mediated high copy suppression of *nab2* growth defects. To explore the functional interactions between the RNA exosome and Nab2, we are using RNA-seq analysis to identify the coding and noncoding transcripts most impacted by overexpression of these exosome cofactors in *nab2* mutant cells. This study uncovers functional interactions between the RNA exosome and Nab2 in both the nucleus and the cytoplasm.

393 The MTR4 helicase recruits nuclear adaptors of the human RNA exosome using distinct arch-interacting motifs

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The nuclear exosome and its essential co-factor, the RNA helicase MTR4, play crucial roles in several RNA degradation pathways. Besides unwinding RNA substrates for exosome-mediated degradation, MTR4 associates with RNA-binding proteins that function as adaptors in different RNA processing and decay pathways. Here, we identify and characterize the interactions of human MTR4 with distinct and unrelated adaptors. We show that the unstructured regions of these adaptors contain short linear motifs that resemble arch-interacting motif (AIM) of yeast rRNA processing factors and that they bind the MTR4 arch domain in a mutually exclusive manner. Our results suggest that nuclear exosome adaptors have evolved non-canonical AIM sequences to target human MTR4 and demonstrate the versatility and specificity with which the MTR4 arch domain can recruit a repertoire of different RNA-binding proteins.

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394 Differential analysis of RNA binding to hnRNPA2/B1 between exosomes and cells in human hepatocellular carcinoma model (HepG2)

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RNA-binding proteins (RBPs) regulate RNA expression by influencing RNA stability, processing and transport. hnRNPA2/B1 is an RBP associated with several cellular processes and preferential binding motifs depending on the cellular compartment. hnRNPA2/B1 is also known to be involved in sorting of small RNAs into extracellular microvesicles (EVs).

Here, we aimed to elucidate the specificity of RNA binding to hnRNPA2/B1 in cells and EVs by analysing the exact binding motifs and RNA types in both compartments via the irCLIP technique. irCLIP was performed on HepG2 cells and EVs extracted from culture supernatants. The libraries were sequenced on Illumina MiSeq. Alignment and annotation was performed using the iCount and the kallisto software. Statistical analyses were performed in R.

We found that hnRNPA2/B1 binds more often to non-coding RNAs (FC ~ 2) and 3' UTRs (FC ~ 3), and less often to introns (FC ~ 0.25) in EVs as compared to cells. Previously known GC-rich binding motifs seem to be chatacteristic for cells, whereas A-enriched motifs were seen in EVs. In the whole transcriptome analysis we detected: that specific non-coding RNAs (eg. VTRNA1-1, VTRNA1-2, RNVU1-7) bind more often to hnRNPA2/B1 in EVs; while both mRNAs and lnc-RNAs (eg. ZEB1, HNRNPH1, PCBP1-AS1) are bound preferentially in cells and are mostly associated with transcription (FOXP1), translation (EIF4A2) and histone acetylation (HDAC8). Analysis of binding to small (<200nt) non-coding RNAs showed that hnRNPA2/B1 preferentially bind splicesomal RNAs in cells and small-nucleolar RNAs and short-non-coding RNAs (piRNAs, U-RNAs) in EVs.

In conclusion, we have shown that the irCLIP protocol may be applied not only to different cellular compartments, but also to EVs. Our analysis proves that hnRNPA2/B1 binding peaks are enriched for non-coding RNAs in EVs, while in cells these peaks overlap with regulatory regions of genes associatred with the transcription and translation processes.

395 A non-canonical RNA binding domain in splicing factor SF3A1

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During splicing of pre-mRNA, 5' and 3' splice sites are brought within proximity by cumulative interactions between the U1 and U2 snRNPs followed by recruitment of the tri-snRNP and assembly of the mature spliceosome. These cross-intron interactions must occur with a high degree of specificity to produce functional mRNAs. Previously, we identified an interaction between the stem-loop 4 (SL4) of U1 snRNA and the U2 snRNP specific protein SF3A1 that is critical for splicing (Sharma et al. Genes and Dev. (2014) 28:2518-31). Although harboring many annotated domains, SF3A1 lacks a canonical RNA binding domain. To identify the U1-SL4 binding region in SF3A1, we expressed N- and C-terminal deletion constructs using an in vitro system that is based on HeLa cell-free extract (CFE). Extracts expressing SF3A1 proteins were then used in binding reactions containing uniformly 32P-labeled U1-SL4. UV crosslinking of the RNA-protein complexes has identified the C-terminal ubiquitin-like (UBL) domain of SF3A1 as the U1-SL4 interacting region. This domain is present in higher eukaryotes, but not in yeast, and contains conserved, positively charged and cyclic residues which may contribute to U1-SL4 binding. To quantify the interaction by electrophoretic mobility shift (EMSA) and surface plasmon resonance (SPR), GST-UBL fusion proteins were expressed and purified from Escherichia coli. The wildtype SF3A1-UBL domain was found to bind U1-SL4 with a Kd of ~97 nM. Mutations of two conserved tyrosine residues (Y772 and Y773) caused a reduction in the binding affinity of SF3A1 to U1-SL4. Competition EMSA with various RNA ligands also indicated that the double-stranded G-C rich stem is an important feature for UBL RNA binding. These data demonstrate that the UBL domain of SF3A1 (aa 703-793) can function as an RNA binding domain and that mutations in this region may interfere with U1-SL4 binding; possibly contributing to the underlying molecular aberrations and splicing dysregulation in diseases like myelodysplastic syndrome (MDS) where a Y772C mutation has been detected.

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396 Dissecting the role of ARS2 in nonsense mediated decay

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Nonsense mediated decay is a mRNA quality control system that prevents the production of aberrant proteins and modifies the expression of mRNAs to respond to varying physiological conditions, such as stress, cell cycle progression, and differentiation. The precise mechanism(s) of how mRNAs are recognized and targeted has not been fully elucidated. The nuclear cap-binding complex, consisting of CBP20 and CBP80, is necessary for the targeting of mRNA by NMD, and promotes the interaction of UPF1 and the exon junction complex (EJC). Recently, ARS2 has been shown to be an essential component of the nuclear cap-binding complex, and facilitates correct processing of several classes of RNA Polymerase II transcripts such as snRNA, mRNA and miRNA. However, whether ARS2 participates in NMD has not been addressed.

We first tested whether ARS2 may be involved in NMD through a tethering assay. Direct tethering of ARS2 to the 3' UTR of a firefly luciferase reporter induced the degradation of the reporter in a manner that is dependent on expression of NMD component UPF1. We next employed a dual-color fluorescent reporter for quantification of NMD activity by flow cytometry. These data demonstrated that overexpression of ARS2 blocks degradation of the NMD reporter, while downregulation of ARS2 promotes degradation of the NMD reporter. We further investigated the effects of ARS2 on endogenous NMD cellular targets. In our assay, depletion of ARS2 promotes the specific degradation of endogenous NMD substrates while NMD insensitive transcripts remain unaffected. Furthermore, this effect on endogenous targets can be rescued by restoring ARS2 expression. Taken together, our data demonstrate that ARS2 acts as an inhibitor of the NMD pathway. Based in previous interactome data, we hypothesize that ARS2 competes with NMD components for the interaction with CBP20/CBP80 and that must be displaced from the cap complex for NMD to proceed. We are currently performing BioID experiments utilizing NMD targets to better elucidate ARS2 interactions with the NMD pathway and how this may be involved in the role of ARS2 in stress responses such as arsenic sensitivity.

397 Modulation of G-quadruplex structural integrity by hnRNP HF controls cellular stress response linked to tumor progression in glioblastoma

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Glioblastoma (GBM) is one of the most lethal primary brain tumors. Treatment options have remained limited in part because of the still incomplete understanding of the basic biology of GBM. Although it is well known that deregulation of protein synthesis contributes to GBM progression and response to current therapeutic treatments, the molecular mechanisms and therapeutic targets remain to be fully elucidated. Accumulating evidences from others and us (Cammas Oncotarget 2016), demonstrated that RNA-binding proteins are abnormally expressed in cancer cells and play a critical role in the link between protein synthesis and cancer by impacting both global and mRNA specific translation. In this study we focused on the RNA-binding protein hnRNP HF, a post-transcriptional regulator that is highly expressed in GBM. We uncovered a novel role for hnRNP HF in translational regulation. hnRNP HF silencing impacts GBM processes known to underpin the spread/relapse of GBM, and regulates tumor growth and response to GBM treatments (temozolomide and irradiation). The underlying mechanism of regulation involves the binding of hnRNP HF to RNA G-quadruplex (RG4) forming sequences and the modulation of RG4 structural integrity in synergy with RNA helicases. Together, our results suggest that hnRNP HF is an essential regulatory hub in GBM networks that drives translational control of genes contributing to GBM outcome.

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398 A Viral RNA-Binding Protein with a Divergent RNA Sequence Preference

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The tristetraprolin (TTP) protein family is highly conserved in eukaryotes, but has not been found in bacteria, archaea or viruses. TTP-like proteins regulate diverse physiological processes in mammals such as the inflammatory response and early embryonic development. TTP-like proteins in higher eukaryotes possess two domains: a pair of CCCH zinc fingers for binding RNA, and a CNOT1 binding domain that recruits the CCR4-NOT (CNOT) complex. TTP-like proteins bind AU-rich RNA elements in the 3' untranslated regions of mRNA, and recruit the CNOT complex to the mRNA, resulting in the mRNA's decay. However, a viral TTP-like protein has not been characterized. Exploring this knowledge gap would explain how the properties of TTP-like proteins could be used by viruses to usurp host gene expression machinery or evade innate immune responses.

A protein BLAST using the TZF domain of human TTP to query viral protein sequences yielded a viral protein with significant sequence similarity encoded by the *scale drop disease virus* (SDDV), called ORF_096L. SDDV infects farmed sea bass with high mortality. Surprisingly, an assay performed with random pools of RNA sequences and the recombinant ORF_096L yielded the binding of an RNA sequence that differed from the one bound by eukaryotic TTP-like proteins. However, co-immunoprecipitation experiments suggest that an interaction between ORF_096L and host CNOT1 protein is possible. Furthermore, the ORF_096L may interact with CNOT1 protein through amino acids present in its c-terminal domain.

In conclusion, *scale drop disease virus'* ORF_096L encodes a RNA-binding protein that binds to RNA with sequence specificity. The RNA sequence bound by ORF_096L is distinct from the TTP-like proteins expressed by eukaryotes. However, similar to eukaryotic TTP-like proteins ORF_096L has the potential to recruit the CNOT complex to RNA by an interaction with the CNOT1 subunit of the complex. Future studies will test the hypothesis that RNA bound by ORF_096L have reduced half-lives as a result, and identify RNAs that are regulated by ORF_096L upon infection.

399 Structural RNA components affect ZFP36L2-RNA interaction

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Protein-RNA interaction can modulate RNA translation, localization and stability. We are interested in mRNA regulation mediated by the adenine uridine rich element binding proteins, ARE-BPs. Specifically, the Tris-Tetra-Prolin (TPP) family of proteins share a similar tandem zinc finger domain responsible for RNA binding. When one of the TTP-family members, ZFP36L2 (or L2), is expressed at low levels in a mouse model, we observed complete female infertility accompanied by decreased ovulation rate. The role of L2 in ovulation is mediated by its effect on at least one transcript, luteinizing hormone receptor (LHR) mRNA. Using different biological assays we had confirmed the modulatory effect of L2 on LHR mRNA at the post-transcriptional level. LHR mRNA contains three potential ARE sites, however L2 binding is specific to a single ARE located in the 3'-UTR, which we term the 'functional ARE'. Other TTP family members do not bind to the LHR mRNA, suggesting binding specificity of L2 to this particular transcript. We found that RNA structure mediates and a significant component of the specificity in this interaction. We modeled the secondary structure of the LHR mRNA using *in vitro* SHAPE-MaP. Our structural data support a model in which maximum binding occurs only in a specific structural context, where the functional ARE is presented in a loop. Also, the proximal stem in this loop is a key modulator of the binding affinity to L2. When the nonamer (UUAUUUAUU) known to support high affinity for the conserved tandem zinc finger domain of the TTP family was located in a loop with a rigid proximal stem minimal binding was observed. To explore this RNA-protein interaction *in vivo* we used multiple chemical probing methods in cells. Our preliminary results suggest that L2 is bound uniquely to one ARE of LHR mRNA *in vivo*. Understanding of L2-LHR mRNA interactions may shed light into novel RNA therapeutics and thus a provocative new way to modulate ovulation independent of hormone therapy. The development of small molecule(s) able to modulate the RNA secondary structure of this RNA-protein interaction has the potential to regulate binding affinity to L2 and consequently LHR mRNA half-life.

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400 Identification of Pentatricopeptide repeat proteins that bind organellar transcripts in plants to coordinate nuclear and organellar gene expression

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Pentatricopeptide repeat (PPR) proteins are RNA-binding proteins found essentially in all eukaryotic genomes. In land plants this family of RNA-binding proteins is largely expanded. The several hundred PPR proteins are predicted to primarily localize in mitochondria and chloroplasts. Encoded in the nuclear genome and targeted to bind organellar transcripts, PPR proteins are believed to represent important players to coordinate nuclear and organellar gene expression. Coordinated gene expression of the three genomes in plants is essential to build the photosynthetic complexes in chloroplasts and the electron transport chain for oxidative phosphorylation in mitochondria. All of these complexes consist of both organellar and nuclear encoded subunits. In the two DNA-containing organelles, PPR proteins are heavily involved in posttranscriptional processes including group I and group II intron splicing, RNA editing, translation and RNA stabilization. PPR proteins that are important for RNA stabilization often have the ability to block exoribonucleases i.a. PNPase, RNase II and RNase J. We show that the ability to act as "road blocks" results in the generation of *in vivo* footprints of PPR proteins. These footprints accumulate as abundant small RNAs, usually between 20 and 30nt in length and can be readily identified by small RNA sequencing. We applied small RNA sequencing in several mutants of PPR proteins and successfully identified their RNA ligands *in vivo*. Using evolutionary information and known RNA base preferences of amino acids at critical positions in the PPR proteins, the so called PPR code, we predicted PPR proteins for evolutionary conserved footprints. Using this approach we identify an atypical PPR protein with a kinase domain which is important for the correct expression of psbC, a central subunit of photosystem II.

401 Grad-Seq in mycobacteria

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The pathogen *mycobacterium tuberculosis* (Mtb) infects and resides in the human macrophage, where it is exposed to numerous hostile environmental conditions e.g. oxidative stress, low pH and nutrient limitation. Bacteria adapt to these kinds of stresses through a variety of mechanisms, including the regulatory function of small non-coding RNAs (sRNAs). Although in recent years several studies addressed the identification and functional characterization of sRNAs in mycobacteria, the exact mechanism of mycobacterial sRNA action still remains elusive due to the lack of homologs of the RNA chaperon proteins Hfq and ProQ that facilitate sRNA-target mRNA interaction.

In order to identify novel RNA-binding proteins involved in mycobacterial sRNA function the technique of gradient profiling by sequencing (Grad-seq; Smirnov et al., 2016) was applied in *mycobacterium smegmatis* (Msmeg). By combining the size- and shape-dependent separation of ribonucleoprotein (RNP) complexes within a linear glycerol gradient with subsequent analysis of each gradient fraction by RNA-sequencing and mass spectrometry, Grad-seq allows the global detection of RNA-protein-complexes and thereby the identification of candidate sRNA-binding proteins. Here, we present our first analysis of Grad-seq data in Msmeg.

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402 Long LARP1 isoform is dominantly expressed in a wide range of human cancer cell lines

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Over the past decade, a family of hitherto unknown RNA binding proteins (RBPs) has been identified, called the La-related proteins (LARPs). Each of this seven gene family (LARP 1, 1B, 3, 4A, 4B, 6 and 7) have distinct, but incompletely characterised, roles in transcription and/or mRNA translation. LARP1 is oncogenic and required for ribosome biogenesis and cancer cell survival. With emerging functional roles in cancer behaviour, there is interest in the protein as a potential therapeutic target. However, there is controversy around which one of two distinct LARP1 protein isoforms is the canonical form for use in in vitro studies. The "long" (NP_291029.2) and "short" (NP_056130.2) LARP1 isoforms encode proteins of 1096 and 1019 amino acids respectively and differ in their first exon. Here, we conducted a series of in silico, biochemical and cellular assays to elucidate the expression profile of LARP1 in cancer and normal cells and conclude that the long isoform is dominantly-expressed whilst the short isoform is undetectable. For future studies using ectopic expression of LARP1 in cellular systems we would therefore recommend that the long LARP1 isoform is used as this is the canonical form.

403 RNA-binding protein NANOS1 is necessary for regulation of mRNAs encoding apoptotic pathways in human germ cells

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Nanos is a morphogen of germ cells which is characterized by the highly conserved nanos-type $(CCHC)_2$ zinc finger RNA-binding domain. This domain enables Nanos to bind to target mRNAs and act as a posttranscriptional regulator of gene expression. While Nanos-mediated maintenance of germ cells in *Drosophila* has been related to downregulation of apoptosis, the relevance of these findings to human physiology is uncertain. Here, by using MTS test and Anexin V including FACS analysis, we show that overexpression of wild-type NANOS1 causes upregulation of proliferation and downregulation of apoptosis of the human male germ cell line TCam-2. Interestingly, overexpression of a mutated NANOS1 instead, which has previously been associated with a lack of germ cells in the testes of infertile patients, switches NANOS1 function to anti-proliferative and pro-apoptotic. To further investigate the role of NANOS1 in apoptosis, we performed RNA-seq analysis upon NANOS1 overexpression and identified 17 mRNAs encoding pro-apoptotic factors being under NANOS1 repression. Following this, in order to understand how the mutation effects the role of NANOS1 in apoptosis, we are investigating NANOS1-mediated regulation of selected pro-apoptotic genes by studying changes of their expression profile at RNA and protein levels, as well as NANOS1 binding upon overexpression of wild-type or mutant NANOS1. This report underscores the conservation of Nanos from flies to humans as a regulator of apoptosis-related mRNAs in germ cells, and provides a mechanistic basis for understanding NANOS functions in human health. This study was supported by a grant from the National Science Centre Poland, no 2014/15/B/NZ1/03384.

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404 Hypoxia-induced response of RNA binding proteins in leukemia cells

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Activation of pro-survival pathways and loss of genomic stability seem to be major mechanisms that lead to the development and progression of therapy resistance of leukemia cells. We have previously shown that phosphorylation of eIF2alpha upon stress response induces resistance of leukemia cells to imatinib treatment [Kusio-Kobialka, 2012]. We have discovered that expression of *BCR-ABL* oncogene in chronic myeloid leukemia cells induces formation of protein complexes by T-cell intracellular antigen-1 (Tia) proteins and leads to TIAR-dependent down-regulation of BRCA1 protein synthesis [Podszywalow-Bartnicka, 2014; Wolczyk, 2017]. Given the fact that leukemia cells migrate along diverse environments, from hypoxic in the bone marrow to oxygen-reach in the blood circulation (so called normoxia), activation of stress response pathways might modulate sensitivity of leukemia cells to therapy. Herein, we would like to present our results concerning adaptation of leukemia cells to the low oxygen level. The study was performed on various leukemia cell lines (K562, Lama-84, BV173, HL-60 and Nalm-6) cultured in the normoxia or in the hypoxia workstation (1.5% O2, 5% CO2) utilized to secure stable conditions for long-term studies. We monitored apoptosis and cell cycle progression using flow cytometry. We have observed that exposition of leukemia cells to hypoxia activates distinct signaling pathways during acute phase (first hours) than in the chronic phase (over 72h). Western blotting and immunostaining showed an enhanced shuttling of RNA binding proteins (RBPs) to the cytoplasm in the acute phase. Upon adaptation, the RBPs accumulate in the nucleus, while total RBPs protein level remains unchanged. Mass spectrometry analysis of protein composition of cytoplasmic RBPs complexes of normoxia versus hypoxia-adopted cells revealed that oxygen conditions determine the activity of RBPs in the cytoplasm. Several ribosomal proteins that under normoxia interact with FMRP, in hypoxia were found in complex with TIAR, indicating oxygen-dependent regulation of the proteins activity. Our results point to translational control as important component of leukemia cells adaptation to hypoxia (work supported by research grants from the National Science Centre: UMO-2014/15/D/NZ3/05187 to P.P.-B and UMO-2016/23/N/NZ3/02232 to M.W.; P.P.-B. is a recipient of the Hollis Brownstein research grant from the Leukemia Research Foundation for 2018/2019).

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405 CRKL regulates alternative splicing of cancer-related genes in cervical cancer samples and HeLa cell

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Background: Aberrant spliced isoforms are specifically associated with cancer progression and metastasis. The cytoplasmic adaptor CRKL (v-crk avian sarcoma virus CT10 oncogene homolog-like) is a CDPK-related kinase like proto-oncogene, which encodes a SH2 and SH3 (src homology) domain-containing adaptor protein. CRKL is tightly linked to leukemia via its binding partners BCR-ABL and TEL-ABL, upregulated in multiple types of human cancers, and induce cancer cell proliferation and invasion. However, it remains unclear whether signaling adaptors such as CRKL could regulate alternative splicing.

Methods: We analyzed the expression level of CRKL in 305 cervical cancer tissue samples available in TCGA database, and then selected two groups of cancer samples with CRKL differentially expressed to analyzed potential CRKL-regulated alternative splicing events (ASEs). CRKL was knocked down by shRNA to further study CRKL-regulated alternative splicing and the activity of SR protein kinases in HeLa cells using RNA-Seq and Western blot techniques. We validated 43 CRKL-regulated ASEs detected by RNA-seq in HeLa cells, using RT-qPCR analysis of HeLa cell samples and using RNA-seq data of the two group of clinical cervical samples.

Results: The expression of CRKL was mostly up-regulated in stage I cervical cancer samples. Knock-down of CRKL proliferation led to a reduced cell proliferation. CRKL-regulated alternative splicing of a large number of genes were enriched in cancer-related functional pathways, among which DNA repair and G2/M mitotic cell cycle, GnRH signaling were shared among the top 10 enriched GO terms and KEGG pathways by results from clinical samples and HeLa cell model. We showed that CRKL-regulated ASEs revealed by computational analysis using ABLas software in HeLa cell were highly validated by RT-qPCR, and also validated by cervical clinical samples.

Conclusions: This is the first report of CRKL-regulation of the alternative splicing of a number of genes critical in tumorigenesis and cancer progression, which is consistent with CRKL reported role as a signaling adaptor and a kinase. Our results underline that the signaling adaptor CRKL might integrate the external and intrinsic cellular signals and coordinate the dynamic activation of cellular signaling pathways including alternative splicing regulation.

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406 Multi-omics characterization of the *Salmonella* RNA-binding protein ProQ during infection in the mouse gut

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RNA-binding proteins are increasingly known to serve as global regulators in bacterial pathogens; however, in most cases their specific roles in infection remain unclear. One such example is the recently discovered RNA-binding protein ProQ, which has been shown to affect the expression of hundreds of transcripts and induce a virulence defect in animal models of infection. Here, we take a multi-omics approach to understand the role of ProQ in *Salmonella enterica* serovar *Typhimurium* infection. Our initial experiments revealed an unexpected dependence of the ProQ virulence defect on the host microbiota in the well-studied murine typhoid model: a strong virulence defect was observed for the *Salmonella* ProQ deletion in conventional mice, but was not observed in germ-free mice. We have applied RNA-seq, metagenomics, and metatranscriptomics to both wild-type and ProQ deletion strains of *Salmonella*, using both germ-free and conventional mice. We present initial findings from our multi-omics investigations of these infection models, illustrating the insights to be gained from this approach, and speculate on the regulatory mechanisms of ProQ that may impact *Salmonella* infection in the murine gut.

407 RNApdbee 2.0: a web platform for comprehensive RNA structure annotation

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Reliable RNA secondary structure, including non-canonical base pairs, is of crucial importance in the RNA 3D structure prediction. To assess topological agreement of RNA 3D models within the context of the reference RNA 3D structure or experimental data, the extended secondary structure extraction from RNA 3D structure is required.

RNApdbee, a freely-accessible web server, allows users for extraction and visualization of RNA secondary structure encoded in extended dot-bracket notation from 3D RNA structures retrieved from PDB or uploaded by the users. First, canonical as well as non-canonical base pairs are identified using the selected tool among incorporated tools (i.e., RNAView, MC-Annotate or 3DNA/DSSR). Next, an iterative decomposition is performed in order to accurately encode pseudoknots' orders, especially in case of complex RNA structures.

Here, we present RNApdbee 2.0, a multifunctional platform for comprehensive analysis of relationships between the RNA 3D and secondary structure. This is one of the few among currently available tools processing efficiently large 3D RNA structures stored in PDBx/mmCIF format only. The system integrates new tool for base pairs identification named FR3D. The user can decide whether the non-canonical and isolated base pairs should be considered. Moreover, several new algorithms for RNA structure topology encoding are provided. Finally, RNA secondary structures diversity can be observed, especially for complex, pseudoknotted RNAs, as a result of concurrent execution of all provided algorithms and base pairs identification tools in a single computationally efficient process. Current version of RNApdbee allows also for identification of structural elements that can be easily applied into refined 3D RNA structure modeling using the RNAComposer.

Visualization of non-canonical base pairs combined with support of multi-stranded RNA structures allows users for analysis and visualization of RNA structures including specific motifs, e.g., G-quadruplexes.

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408 Profiling the alternative splicing landscape of senescent cells

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Cellular senescence is an irreversible cell cycle arrest in response to potentially oncogenic stimuli, characterised by a pro-inflammatory secretome that is protective when limiting the replication of preneoplastic cells or deleterious when the accumulation of senescent cells with ageing impairs tissue homeostasis. Despite the recent characterisation of senescent cells' gene expression heterogeneity and the suggested role of alternative splicing in the regulation of senescent phenotypes, namely through the recently found PTBP1-mediated alternative splicing program controlling the tumour-promoting secretome in oncogene-induced senescent cells, alternative splicing profiles associated with specific senescence inducers remain elusive. As accumulating evidence suggests some drugs are able to selectively kill specific forms of senescence, thereby improving tissue function, the comprehensive characterisation of senescent transcriptomes is crucial.

Next-generation sequencing of RNA (RNA-seq) allows alternative splicing quantification with unprecedented precision. Alternative sequence inclusion can be quantified from RNA-seq reads mapping to exon-exon and exon-intron junctions through the ratio between reads supporting inclusion and total reads supporting both inclusion and exclusion. However, percent spliced-in (PSI) values do not convey information on the number of reads used in the quantification (coverage), although read numbers directly reflect the evidence for measured transcript abundance.

Alternatively, by modelling alternative sequence inclusion using the beta distribution (the conjugate prior distribution of the binomial), the precision of its estimates is proportional to the associated coverage and reflected on the significance of differences in alternative splicing between samples. We employed our beta distribution-based differential alternative splicing pipeline to public and own RNA-seq datasets and were thus able to rank senescence-associated differentially spliced events according to a compromise between the magnitude of splicing changes and the amount of associated supporting evidence.

Our differential splicing analyses based on beta distribution modelling reproducibly identified, at the transcriptome-wide level, the alternative splicing changes specifically related with different types of induced senescence in multiple types of cells, providing molecular leads for therapeutically targeting senescent cells.

409 Analysis of CLIP data using iMaps

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The investigation of protein-RNA interactions has begun to illuminate the essential roles of post-transcriptional modification, protein expression regulation, and other mechanisms across a spectrum of biological processes and diseases. CLIP (cross-linking and immunoprecipitation) is an established method to study protein-RNA interactions. Multiple variations of the technique have been developed, and while the wet-lab procedures differ, the resulting (high-throughput sequencing) data is consistently the same. Likewise, the analysis procedures require only minor variations on a general theme: mapping of reads to a reference genome, identification of protein-RNA crosslink sites, and grouping these positions into wider areas (peaks) that define protein binding sites. Until now, the tools for data analysis and quality assessment have been neither standardized nor commoditized to enable adoption of CLIP among the broader research community. Moreover, data from disparate sources has been analysed with a range of public and custom tools, yielding incomparable apples-to-oranges results.

We have developed iMaps (https://imaps.genialis.com) as a tool for the community to make CLIP data analysis easy, robust and consistent. iMaps is a freely-available web application that allows users to explore a growing collection of published CLIP data. The data is organised into collections based on the original scientific papers, and is made findable and accessible through rich metadata (controlled vocabulary). All public data is processed with a single pipeline allowing direct comparisons and meta-analyses.

Users may also upload and analyse their own data. The recommended and thoroughly validated analysis pipeline can be triggered through a graphical user interface with just a few clicks. Computational and storage resources are provided free of charge for an introductory period. Results include quantified crosslink events, protein binding peaks, oligonucleotide motif enrichment, and visual depictions of protein binding patterns around specific landmarks (e.g. intron-exon sites). Several QA/QC metrics are also built in.

We intend for iMaps to empower the wider RNA research community to advance our understanding of the mechanisms by which epitranscriptomic modifications and RNA-binding proteins regulate gene expression.

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410 SimRNA: a coarse-grained method for RNA folding simulations and 3D structure prediction

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The molecules of the ribonucleic acid (RNA) perform a variety of vital roles in all living cells. Their biological function depends on their structure and dynamics, both of which are difficult to experimentally determine, but can be theoretically inferred based on the RNA sequence. We have developed a computational method for molecular simulations of RNA, named SimRNA.

SimRNA is based on a coarse-grained representation of a nucleotide chain, a statistically derived energy function, and Monte Carlo methods for sampling of the conformational space. The backbone of RNA chain is represented by P and C4' atoms, whereas nucleotide bases are represented by three atoms: N1-C2-C4 for pyrimidines and N9-C2-C6 for purines. Despite the bases being represented by only three atoms, other atoms can be implicitly taken into account in terms of the excluded volume. All base-base interactions were modeled using discrete three-dimensional grids built on local systems of coordinates.

All terms of the energy function used were derived from a manually curated database of crystal RNA structures, as a statistical potential. Sampling of the conformational space was accomplished by the use of the asymmetric Metropolis algorithm coupled with a dedicated set of moves. The algorithm was embedded in either a simulated annealing or replica exchange Monte Carlo method. Recent tests demonstrated that SimRNA is able to predict basic topologies of RNA molecules with sizes up to about 50 nucleotides, based on their sequences only, and larger molecules if supplied with appropriate distance restraints. The user can specify various types of restraints, including restraints on secondary structure, distance and position.

SimRNA can be used for systems composed of several chains of RNA. It is also able to fold/refine structures with irregular (non-helical) geometry of the backbone (RNA pseudo knots, coaxial stacking, bulges, etc.). As SimRNA is based on folding simulations, it also allows for examining folding pathways, getting an approximate view of the energy landscapes, and investigating of the thermodynamics of RNA systems.

SimRNA was incorporated in dedicated prediction pipelines and tested in worldwide blind predictions experiment: RNA-puzzles, providing one of the best predictions.

411 MASH-FRET: A user-friendly simulation and analyzing tool for single-molecule FRET videos.

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Single-molecule Förster resonance energy transfer (smFRET) is a powerful technique to probe biomolecular structure and dynamics. A popular implementation of smFRET consists in recording fluorescence intensity-time traces of surface- or vesicle immobilized, chromophore-tagged molecules, such as NAs or proteins (1).

We developed a MATLAB-based Multifunctional Analysis Software for Handling smFRET data (MASH-FRET) that allows to analyze and simulate camera-based smFRET videos (SMV) (2-5). Our software includes video processing to extract single-molecule fluorescence trajectories form SMVs, trace processing for molecular sorting and the trace correction, histogram analysis to extract thermodynamic parameters and transition analysis to extract kinetic parameters (2,4). Our software enables to simulate realistic SMV including different models for camera noise (3).The software is freely available for download on github: https://github.com/RNA-FRETools/MASH-FRET. Further we provide a comprehensive documentation including tutorials for analyzing smFRET videos and validating experimental results with the help of simulated data (https://rna-fretools.github.io/MASH-FRET/).

Here, we provide a presentation of our software package with a standard analyzing strategy for SMV. We further explain the basic concepts of smFRET and how to get the most out of your intensity-based smFRET data in terms of thermodynamics and kinetics.

Financial support by the Swiss National Science Foundation (RKOS), the UZH Forschungskredit (MCASH, DK, FDS, SLBK, SZP and RB) and the University of Zurich (RB and RKOS) is gratefully acknowledged.

(1) Börner et al. Coord. Chem. Rev. (2016) (2) Hadzic et al. Proc. SPIE (2016) (3) Börner et al. PONE (2018). (4) Hadzic el al. J. Phys. Chem. B. (2018) (5) König et al. PONE (2013)

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412 Developing an accurate all-atom fixed-charge force field for RNA with implicitly polarized charges

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Molecular dynamics (MD) simulations are powerful tools for modeling the structural ensembles of nucleic acids that mediate their biological functions. MD simulations generate hypotheses about molecular structure that can then be tested experimentally. The utility of computer simulations for biomolecules relies on the accuracy of the energy model, a parameterized function called a force field. Currently available fixed-charge force fields for RNAs can accurately describe A-form helices composed of canonical Watson-Crick base pairs. However, there is a consensus among the RNA simulation community that these force fields fail to model other common features of RNA tertiary structure, such as noncanonical base interactions in loop regions and the relative orientations of helices interrupted by bulge loops or asymmetric internal loops. The failures of current RNA force fields limit the applicability of MD simulations to provide useful information about the tertiary structures of RNAs. Thus, development of an accurate fixed-charge force field that is transferable to structurally diverse RNAs remains an open challenge.

We report a new parameter set for the Amber RNA force field using the *ff99* functional form and bonded terms. The nonbonded terms describing the electrostatic and van der Waals interactions are fit to quantum mechanical interaction energies, obtained using symmetry-adapted perturbation theory (SAPT), for a diverse set of nucleoside-nucleoside and nucleoside-phosphate dimers. Following the implicitly polarized charges (IPolQ) strategy used for the *ff15ipq* force field, we account for the influence of solvent in the SAPT calculations by including a field of point charges representing the time-averaged solvent density obtained from classical MD simulations of water and ions around a restrained RNA.

413 tRAX and tDRnamer: Tools for Analyzing and Visualizing tRNA and tRNA-derived RNA Expression

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Transfer RNAs (tRNAs) play an essential role in protein translation in all living cells. Additionally, multiple studies reveal that small RNAs derived from tRNAs (tDRs) can have diverse regulatory functions. Novel methods have been developed to greatly improve quantitation of mature tRNAs and tRNA-derived RNAs (tDRs) by employing specialized enzymatic treatments to circumvent common tRNA modifications that interfere with reverse transcription. However, analyzing tRNA and tDR sequencing data also presents special challenges to standard small RNA-seq analytic methods. Eukaryotic genomes typically have multiple identical copies of a large portion of tRNA genes, resulting in ambiguous mapping of sequencing reads for both mature tRNAs and especially tDRs. Moreover, post-transcriptional addition of the 3′ CCA and tRNA intron removal can result in read mapping failure or misalignment to genomic reference sequences. To overcome these problems, we developed tRAX (tRNA Analysis of eXpression), a tRNA/tDR sequencing data analysis pipeline that includes read alignment, abundance estimation, differential expression analysis, tDR classification, and RNA modification detection. tRAX produces numerous publication-quality visualizations such as read distributions among tRNAs and other small RNA types, expression distribution across each tRNA isotype, individual tRNA expression profiles, tDR type (5′ tDR, 3′ tDR, and others) comparison across samples, and mismatch incorporation charts for modification identification. To further aid recognition of structural/processing/functional relationships among a wide constellation of tDRs, we also developed tDRnamer in collaboration with other tRNA researchers to provide a consistent, uniform naming system integrating three parts: (i) the prefix "tDR", (ii) the mature tRNA positions from which the small RNA is derived, and (iii) the Genomic tRNA Database gene symbol. The web-based tDRnamer resource (http://trna.ucsc.edu/tDRnamer) also gives additional tDR annotation including length, secondary structure, and alignments with all possible source tRNAs and similar tDRs. Together, these new tools will equip RNA researchers with consistent, standardized analyses to better uncover the complex, multifunctional roles of tRNAs and tDRs.

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414 rnaSMART - tool for identification of Structural Motifs Across RNA Transcripts

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RNA is fundamental building element for all living cells. The functions of RNAs are in most cases directly connected to their secondary and tertiary structures. Detection of repeating motifs within RNA secondary structures allow identification of novel regulatory mechanisms and provides premise on their common functions. The problem of comparing RNA structures, to find local structural motifs, is non-trivial and difficult to calculate. For this reason, the currently available methods tend to operate on sets of RNAs expected to be related by sequence or function similarity.

Here, we present a novel tool, rnaSMART (tool for identification of Structural Motifs Across RNA Transcripts), designed to find local structural motifs in unrelated RNA molecules. rnaSMART employs analysis of RNA abstract shapes to find local motifs with similar structural topology. Next, the exact structural distance between motif members is estimated. Finally, sequence of single stranded regions is compared. In this way rnaSMART enables the flexible identification of local structural motifs with a similar secondary structure topology, using independent control of sequence and structure similarity. As the result, rnaSMART enable identification of motifs that may occur in only a few of the thousands of transcripts submitted to analysis as well as to find motifs repeatedly occurring within the same transcript.

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415 rG4-seeker enables high confidence identification of novel rG4 motifs from rG4-seq experiment via platform-specific noise modeling

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Emergence of RNA-seq has revolutionized the studying and understanding of transcriptome, and empowered many high-throughput RNA structural/regulatory element mapping platforms. However, subsequent bioinformatics search to retrieve elements-of-interest often pick up noise that impacts overall research interpretation and outcome. Nevertheless, noise is often considered normal consequences of biological variances and tolerated by conducting statistical tests with replicated experiments.

We have recently developed RNA G-quadruplex sequencing (rG4-seq) for transcriptome-wide mapping of RNA G-quadruplexes (rG4s) by exploiting their intrinsic reverse transcriptase-stalling (RTS) properties. RNA G-quadruplex secondary structures are proposed to play significant regulatory roles in transcriptional, post-transcriptional and translational processes. In this study, we investigated the context of non-biological platform-specific noise in rG4-seq and demonstrated how noise modeling could improve both sensitivity and specificity of rG4 detection in replicate-independent manner.

Through in-depth re-analysis of HeLa rG4-seq datasets, it was revealed that the RNA fragmentation process in rG4-seq chemistry is associated with a distinct distribution of background RTS signal, which contributed as the most significant source of noise. By modeling and thus eliminating the effect of noise in RTS measurements, an improved rG4 detection pipeline called rG4-seeker were formulated. In contrast to the original pipeline that achieved 12% FDR with a 4-replicate-combined analysis; the new implementation demonstrated significant improvements by enabling reliable single-replicate analysis at FDR <2% and recalling ~80% of rG4 motifs identified previously. Meanwhile, unrecalled rG4 motifs were found coincidentally mapped to transcript regions of significantly higher GC ratio, where RTS signals were likely compromised by sequencing bias and rendered inconclusive rG4 detection outcomes. Furthermore, with rG4-seeker we identified hundreds of novel rG4 that nucleotide sequence do not match existing motif definitions, where candidates were experimentally validated. The information provided new insights in interpreting the nucleotide sequence rules governing rG4 formation.

Employing rG4-seq analysis as a showcase, our research demonstrated how the understanding of platform-specific noise could help tailoring bioinformatic analysis for better interpretation of high-throughput RNA structural/regulatory element probing experiments, which bring promises to further elucidate the transcriptome-wide landscape of RNA regulations and interactions.

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416 CRISPR-Cas9 on-target activity is more efficient in absence of local off-targets and strong or weak binding energies

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The RNA-guided DNA endonuclease CRISPR-associated protein 9 (Cas9) is a precise and widely used genome-editing tool. Recent studies have shown that the ability of Cas9 to cleave a target DNA sequence largely depends on structural characteristics and sequence composition of the guide RNA (gRNA) being used. Based on this, several computational tools have been developed to facilitate researchers in the process of designing gRNAs that optimize Cas9 on-target activity, while minimizing the possibility of off-target edits. However, many aspects of the gRNA-DNA interaction remain unclear, and on-target activity predictions based on our current understanding of this complex lack accuracy.

Recently, it was shown that an energy-based model can be effectively used to identify Cas9 off-target bindings[1]. Following this concept, here we assess how the binding energies of nucleic acid duplexes formed in the Cas9-gRNA-DNA complex impact on Cas9 cleavage efficiency. Our results suggest that the gRNA-DNA binding energy and the DNA-DNA opening energy of efficient gRNAs fall into a restricted rages of values compared to inefficient ones, and that inefficient gRNAs tend to form more stable secondary structures. In addition to this, we examined the binding energy of potential sub-optimal gRNA-DNA interactions, that due to the presence of bulges and mismatches can lead to the formation of inactive local off-targets. For this, we extended RIsearch1[2] software to allow fast computation of sub-optimal gRNA-DNA binding energies. We observed that the presence of sub-optimal bindings with low free-energy negatively affects Cas9 cleavage activity. Together, our results show that the binding energies governing gRNA-DNA interactions are of fundamental importance for Cas9 activity and efficiency assessment.

[1] Alkan, F., Wenzel, A., Anthon, C., Havgaard, J. H. & Gorodkin, J. CRISPR-Cas9 off-targeting assessment with nucleic acid duplex energy parameters. Genome Biol. 19, 177 (2018).

[2] Wenzel, A., Akbaşli, E. & Gorodkin, J. RIsearch: fast RNA-RNA interaction search using a simplified nearest-neighbor energy model. Bioinformatics 28, 2738-2746 (2012).

417 SAXS data-driven modeling of RNA 3D structures

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The majority of known RNAs exert their cellular functions in complexes with other molecules. Many RNAs interact with small molecule ligands, which emphasizes their importance as potential therapeutic targets. However, in order to discern the molecular functions of such interactions, it is important to understand the 3D structure of RNA molecules, in the presence and in the absence of ligands. Due to the difficulties associated with experimental determination of high-resolution RNA structures, experimental data-aided computational modeling has become an important approach in generating high-quality theoretical models[1]. The inherent flexibility of RNA molecules allows it to sample a large conformational space. This hints at the fact that its 3D structure is best represented by an ensemble of atomistic structures rather than a single structural model.

The small angle X-ray scattering (SAXS) technique describes the distribution of electron density in a molecule and hence can be used to interpret the low-resolution envelope of a biomolecule. We have developed a computational workflow for SAXS data-driven modeling of RNA 3D structures and their ensembles. The workflow involves generation large sets of plausible conformations of target RNA with SimRNA (using a coarse-grained representation and a statistical potential to generate physically realistic structures)[2]. These decoys are scored against experimental SAXS data using CRYSOL[3,4], followed by clustering, ensemble optimization[5], and refinement with QRNAS. The workflow also allows the use of data from other sources, such as information about RNA secondary structure from computational predictions or from experimental probing[6].

Our method can also model 3D structures of RNA-protein and RNA-ligand complexes. In such cases, this method is used in conjunction with experimental restraints from other techniques. I will illustrate the applications of our approach with case studies involving RNA molecules of different size.

References : 1 Ponce-Salvatierra, A. et al. Biosci. Rep. 39, BSR20180430 (2019); 2 Boniecki, M. J. et al. Nucleic Acids Res. 44, e63 (2016); 3 Svergun, D. et al. J. Appl. Crystallogr. 28, 768-773 (1995); 4 Konarev, P. V. et al. J. Appl. Crystallogr. 39, 277-286 (2006); 5 Tria, G. et al. IUCrJ 2, 207-217 (2015); 6 Patel, T. R. et al. Methods 118-119, 146-162 (2017)

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418 Theoretical pKa determinations of modified pyrimidines using an implicit-explicit solvation model

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Nucleobase modifications play an important role in modulating the structural characteristics of RNA. Common examples of modifications include methylation of nucleobases, which changes the electronic structure and introduces non-polar character. Due to our interest in higher-order RNA structure and function, the specific characteristics of modifications need to be determined. One characteristic, the pKa value, plays an important role in determining the strength of hydrogen-bonding interactions by a modified nucleotide. Nucleobases contain one or more sites that can undergo protonation/deprotonation, and modifications to the nucleobase may result in changes to the pKa values. For RNA base pairs to form, they must displace waters in the first solvation sphere. A qualitative approximation may be achieved by combining implicit and explicit solvation to mimic the properties of the first solvation sphere. In this study, we used ab initio quantum mechanical calculations using a B3LYP/6-31+g(d,p) level of theory and SMD implicit solvation model with explicit water molecules to simulate the first solvation sphere. Calculations were performed using the Gaussian 09 program to predict pKa values for modified pyrimidine nucleobases and compared the results to experimentally determined pKa values. This approach gives improved theoretical pKa values for unmodified nucleosides and demonstrates that modifications have a wide range of effects on the pKa values of functionalized nucleobases. Understanding the pKa values of modified nucleobases will give insight into the specific structural and energetic impacts of these modifications.

References:

Thapa, B; Schlegel, H. B.; Calculations of pKa's and Redox Potentials of Nucleobases with Explicit Waters and Polarizable Continuum Solvation. J. Phys. Chem. A, 2015, 119 (21), pp 5134-5144

419 AGUTI - Annotation of Genomic and Transcriptomic Intervals

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In recent years, high throughput sequencing has emerged as one of the most popular technologies in RNA research. The multiplicity of developed protocols allow studies of almost every aspect of RNA biology. Obtained results are usually stored in the form of genomic coordinates or intervals. In order to retrieve information about their biological functions and origin, the most common approach is to annotate these regions with known genomic features. Currently available annotation tools are limited to report overlaps with genes and transcript, however in many applications a more detailed description of analyzed regions is desired, such as exons, UTRs, CDSs, etc.

Here, we present a tool developed to enable in-depth annotation of genomic interval data with known features from public databases in GTF/GFF format. It is able to assign intragenic regions from provided annotation (UTRs, CDS, exons etc.), calculate relative position within the gene or transcript (e.g. 5' part, middle, 3' part, whole), annotate non-standard, column-based input files and retrieve multiple annotations of single region. Additionally it allows to scan the contents of provided annotation files and list all feature types, their relationships and assigned attributes, that can be used in annotation process. Thus it is able to efficiently handle the complexity and inconsistency of GFF/GTF annotation formats, which currently cause annotation process to be challenging, time-consuming and in most cases requiring programming skills. Software is provided as a stand-alone tool and as a web server, allowing for interactive browsing and filtering of obtained results.

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420 Unknown RFAM Puzzles Project: RNA 3D structure prediction of the RFAM database

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RNA 3D structure is a key aspect in understanding the molecular functions. RNA-Puzzles is a community wide effort of blind assessment of RNA 3D structure prediction. In the past 7 years, it has demonstrated high accuracies in predicting 23 RNA structures, while 12 of the puzzles were related to RFAM families. However, among the 2791 RFAM families, only 91 families have experimentally solved structures. Computational prediction of unknown RNA structure may help our understanding of RNA structures in a fast and efficient way.

To understand the unknown RNA structure space, we organized the "Unknown RFAM Puzzles Project" since 2017, aiming to predict RNA families. We used R-scape to identify 20 RNA families that are likely to have stable structures. Till now, we have organized three rounds of prediction, while some of the RNAs have already been solved either by X-ray crystallography or CryoEM. Here, we show the first case that predicted RNA structure can be used for molecular replacement. The prediction results also demonstrate that the RNA structural rules may greatly improve the prediction accuracy. We also show a recursive improvement process in the prediction: first use the initial prediction to identify the potential long distance contacts; then, optimize the sequence alignment according to the predicted structure and infer the long distance contact; finally, use the confirmed contacts as constraints in structure prediction. In the prediction of SAM-IV riboswitch, our predicted result resembles SAM-I riboswitch and SAM-I/IV variant in a well aligned way. Besides, the SAM binding sites form the same shape as other SAM riboswitches, which validates the reliability of the prediction.

All the predicted and experimental data will be released to the RNA society and be available at: rnapuzzles.org. We hope the predicted RNA structure may give useful insights to our understanding of RNA structure.

421 Dual RNA-seq provides insight into the RNA biology of the neglected intracellular human pathogen *Orientia tsutsugamushi*

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Emerging and neglected infectious diseases are a major threat to public health. One example is scrub typhus, a bacterial infection caused by an intracellular pathogen *Orientia tsutsugamushi*, primarily in Southeast Asia. Diagnosis of the disease is difficult as the symptoms are non-specific, and mortality of patients can range from 6% to 24%. Understanding and combating these infectious diseases will require the development of new technologies to characterize their causative agents quickly and in a cost-effective manner.

Next generation sequencing is one such technology. RNA sequencing in particular can provide a snapshot of cellular behavior by capturing gene expression across the genome. The recently developed Dual RNA-seq protocol extends this method to simultaneous capture of host and pathogen gene expression. Applying Dual RNA-seq we can profile gene expression of both host and pathogen during the infection, as well as identify new non-coding transcripts that may play an important role in bacterial pathogenesis.

In this study, we have infected Human umbilical vein endothelial cells (HUVECs) with two *Orientia* strains, Karp and UT176 that differ in virulence and applied Dual RNA-seq. This has allowed us to explore unusual aspects of the RNA biology of *Orientia*. Examples include extensive genome reshuffling that has lead to isolated islands of conserved operons, strong expression of a circularly permuted two-piece transfer-messenger RNA (tmRNA), and widespread antisense regulation. Applying a simple machine learning approach, we evaluate the relationship between antisense transcription and protein expression, and provide evidence that antisense transcription plays a major regulatory role in this peculiar pathogen.

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422 A Tool for Quick Refinement of Nucleic Acid Structures using Amber Force-fields

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Computational models of RNA three-dimensional (3D) structures often present various inaccuracies caused by simplifications used in structure prediction methods, such as template-based modeling or coarse-grained simulations. To obtain a high-quality 3D model, the preliminary RNA structure needs to be refined, taking atomic interactions into account. The goal of the refinement is not only to improve the local quality of the model, but to bring it closer to the true structure (global minimum). We present QRNAS (Quick Refinement of Nucleic Acid Structures), a tool for fine-grained refinement of nucleic-acid structures, an extension of the AMBER1,2 simulation method, with additional restraints. QRNAS is capable of handling RNA, DNA, chimeras, and hybrids thereof, and enables modeling of nucleic-acids containing modified residues. It uses an explicit description of hydrogen bonds in addition to the electrostatic and van der Waals interactions used in AMBER. To enforce base coplanarity, Watson-Crick base pairs are restrained by both explicit hydrogen bonds and by applying an additional force for flattening. The backbone regularization feature in QRNAS can adjust the backbone atoms of each residue to a known conformation stored in an internal database. We demonstrate the ability of QRNAS to improve the quality of models generated with different methods. QRNAS was able to improve MolProbity3 scores of NMR structures, as well as of computational models generated in the course of RNA-Puzzles experiments4. A comparative analysis shows that QRNAS outperformed the available refinement tools for RNA in terms of improving the MolProbity scores. QRNAS can optimize the geometry, especially on the level of correctly modeled base-pairs, but the systematic improvement of RMSD to the reference structure should not be expected. The method has been integrated into a computational modeling workflow, enabling improved RNA 3D structure prediction. QRNAS can be downloaded from http://genesilico.pl/software/stand-alone/qrnas.

References

1 Banás, P. et al. J. Chem. Theory Comput. 6, 3836-3849 (2010)

2 Zgarbová, M. et al. J. Chem. Theory Comput. 7, 2886-2902 (2011)

3 Davis, I. W. et al. Nucleic Acids Res. 35, W375-83 (2007)

4 Miao, Z. et al. RNA 23, 655-672 (2017)

423 Comprehensive computational analysis reveals noncanonical reverse transcriptases in CPR bacteria

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Reverse transcriptases (RTs) are enzymes that polymerize DNA from RNA templates. RTs are usually thought to be viral and eukaryotic elements, but they are also present in bacteria. Bacterial RTs are seemed to be ancestors of eukaryotic RTs and several types are identified i.e. group II introns, retrons, CRISPR/Cas-associated RTs, diversity-generating retroelements, and Abi-like genes. Recently, several studies reported that there are multiple uncharacterized and highly diverse RT-like sequences in bacteria. However, their evolutionary relationships and the processes of diversification are not well understood. In this study, we collected myriad of RT-like sequences from prokaryotic and viral genomes and systematically characterized RT evolution. Using known RT sequences and domain profiles as queries, sequence similarity search was performed against 16,612 genomes in the database. After clustering similar sequences, we obtained 1,216 distinct RT sequences which have RT-related functional domains. Visualization of their sequence similarities by constructing networks showed that a recently reported bacterial group, candidate phyla radiation (CPR), have different RTs from the other bacteria. CPR bacteria have an extremely small genome and lack numerous biosynthetic pathways. These CPR RTs formed distinct groups on the sequence similarity network and were as short as approximately 120 amino acid residues compared to medians with the other bacterial RTs. Sequence alignments showed that some CPR bacteria have extremely short RTs, whose sequence lengths are shortened to about one third as compared to the other bacteria, suggesting these RTs are truncated sequences. However, we extracted upstream and downstream region sequences of the "truncated" RT sequences and revealed that RT-like sequences exist in those regions. The RT-like regions are present in the other reading frames implying the occurrence of ribosomal frameshifts during translation. These putative frameshift RTs are widely distributed in the CPR phylogeny, but this trend was not observed with DNA polymerases analyzed similarly for comparison. Based on these results, we will discuss the evolution and possible roles of RTs in bacteria, archaea, and viruses.

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424 Applying IntEREst R/Biocondcutor package to detect U12-type introns and carrying out differential Intron retention analysis

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Intron Exon Retention Estimator, IntEREst, is a freely available R/Biocondcutor software that is primarily used for detection and differential analysis of Intron retention (IR) events across various samples. IntEREst provides tools to analyse RNAseq data (i.e. Binary Alignmnet Map / BAM files) from the studied samples. IntEREst has also a separate module that can be used to identify and annotate U12- and U2-type introns within the genome.

Here, we use the latest IntEREst (1.8.0) to analyse the retention of U12-type and U2-type introns within the Human genome (i.e. hg38, using Ensembl 95). We have used an RNAseq dataset from Myelodysplastic Syndrome (MDS) patients and healthy individuals (Madan et al., 2015). A subset of these MDS patients feature mutations in the ZRSRS2 gene that is involved in the 3' splice site recognition of U12-type introns. We compare the IR changes of U12-type introns to those of the U2-type introns and eventually extract all the significantly retained U12-type and U2-type introns. Additionally, we also investigate cryptic U2-type splice site activation near the U12-type introns in MDS patients with ZRSR2 mutation.

IntEREst is available at:

https://bioconductor.org/packages/release/bioc/html/IntEREst.html

425 QM/MM Calculations on Protein-RNA Complexes: Understanding Limitations of Classical MD Simulations and Search for Reliable Cost-Effective QM Methods.

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Although atomistic explicit-solvent molecular dynamics (MD) is a popular tool to study protein-RNA recognition, satisfactory MD description of some systems is not always achieved. Unfortunately, it is often difficult to separate MD simulation instabilities primarily caused by the simple point-charge molecular mechanics (MM) force fields from problems related to the notorious uncertainties in the starting structures.

Here, we report a series of large-scale QM/MM calculations on the U1A protein-RNA complex, one of the most widely studied protein-RNA complexes, often used as a benchmark system. It possesses experimentally-well characterized, intricate protein-RNA interface, poorly reproduced by MD simulations. Our QM/MM calculations identify several H-bonds poorly described by the MM method, which are also the most problematic in MD simulations. Thus, the results suggest that the source of simulation instabilities for these parts of the U1A interface is the inferior accuracy of the MM potential energy surface. We suggest, that advanced QM/MM computations could be routinely used in MM-based MD simulations of protein-RNA complexes and other biomolecular systems to indirectly rationalize simulation problems. These conclusions are supported also by similar analysis of simple HutP protein-RNA interface, which is well-described by MD with exception of just one H-bond. More recently, we have applied the QM/MM calculations on RNA UUGC tetraloop.

In our study, computationally demanding meta-GGA density functional TPSS-D3(BJ)/def2-TZVP was used to describe the QM region. Because considerably faster methods would be needed to extend sampling and to study even larger systems, we also compared the results with low-cost QM/MM methods. The PBEh-3c and B97-3c density functional composite methods appear to be suitable for protein-RNA interfaces. In contrast, HF-3c and the tight-binding Hamiltonians DFTB3-D3 and GFN-xTB perform unsatisfactorily and do not provide any advantage over the MM description.

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426 A computational method for detecting novel classes of trans RNA-RNA interactions

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Many key mechanisms of gene regulation happen on RNA level. Key examples include miRNA-mRNA interactions, RNA editing and RNA splicing which play key functional roles. At the core of these interactions are trans RNA-RNA interactions, i.e. direct interactions between two RNAs. Compared to protein-protein interactions, very little is known about the universe of trans RNA-RNA interactions. Even the most recent experimental methods only give us a biased glimpse into all trans RNA-RNA interactions in a living cell. On the computational side, there already exist a few methods, yet these have a range of significant limitations. To overcome these challenges, we have developed a new computational method for detecting novel types of trans RNA-RNA interactions in an unbiased manner provided they have been conserved in evolution. Moreover, our method even extends to long transcripts which has been one major limitation of existing methods.

Our prediction method uses as input two multiple sequence alignments as well as a phylogenetic tree specifying the evolutionary relationship between the sequences. It is capable of predicting conserved RNA structure elements in both input alignments as well as trans interactions between them. We tested the method on a biologically diverse set of confirmed trans RNA-RNA interactions comprising sequences up to 3400 nt length and show how to address the conflict between known self-structure and known trans interactions.

427 cTRAP: identification of candidate causal perturbations from differential expression data

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The Connectivity Map (CMap) is a repository of transcriptomic signatures of thousands of genetic and pharmacological perturbations of human cells. Comparing differential gene expression profiles with those from CMap allows to infer candidate molecular causes for the observed differences, as well as compounds that may promote or revert them.

The CMap and LINCS Unified Environment (CLUE) was developed as a collection of user-friendly tools for the manipulation of CMap data and their integration with user-provided data. However, CLUE does not do so in an automated way, also limiting the maximum number of genes available to query CMap and expressing results' significance in a non-standard and difficult-to-interpret score.

We thus developed cTRAP, an R package that identifies potentially causal molecular perturbations by seamlessly comparing full user-provided differential gene expression results with those available from CMap. cTRAP also supports comparisons with gene expression/drug sensitivity associations derived from the NCI-60, the Cancer Therapeutics Response Portal and the Genomics of Drug Sensitivity in Cancer, to identify compounds that could target the phenotypes associated with the user-provided differential expression profiles. In cTRAP, similarity is measured by correlation or gene set enrichment score.

As a positive control, cTRAP was used to rank CMap's molecular perturbations based on their similarity to an ENCODE knockdown. Reassuringly, CMap's perturbation of the cognate gene was ranked amongst the top 6 most similar to ENCODE's for all included methods of comparison.

cTRAP is available in Bioconductor (bioconductor.org/packages/cTRAP). We are currently developing an easy-to-use visual interface to guide users through cTRAP's analyses.

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428 Kinetic Pathways of Metal Cation Binding to RNA – Accurate Exchange Rates and Atomistic Insights from MD Simulations

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Metal cations are essential for the folding and function of RNA. In particular, the exchange kinetics of metal cations and the lifetime of contact pairs determines the structural stability and folding times of RNA in solution.

Here, we apply molecular dynamics simulations to gain atomistic insights into the fundamental interplay of different mono- and divalent metal cations and RNA. Simulating cation binding with atomistic resolution is challenging since it involves slow processes such as partial ion dehydration to form inner-sphere configurations upon binding. In order to capture such rare events and to accurately determine cation exchange rates, we apply a combination of enhances sampling techniques including transition path and umbrella sampling.

Our results provide atomistic insights into the molecular pathways of metal cation binding to possible binding sites on RNA. We show, that the timescales involved cover more than eight orders of magnitude ranging from nanoseconds (Na^+) up to a hundred milliseconds (Mg^{2+}). In all cases, Mg^{2+} plays an exceptional role due to its high binding affinity and particularly slow exchange kinetics.

429 Autosomal monoallelic expression of protein-coding genes overlapping at 5 end for adenocarcinoma cell lines

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The presence of protein-coding genes located on opposite strands of DNA and sharing fragment of genomic sequence in a sense-antisense orientation (i.e. overlapping genes) was discovered in mammalian genomes over thirty years ago. It was proposed that such arrangement of genes may lead to the transcriptional interference and the depression of their expression levels. Our studies however, do not show such effect on genes overlapping at their 5' ends. To explain this phenomenon we investigated the possibility of autosomal monoallelic expression (MAE), i.e. expression of one gene from the paternal and another from the maternal chromosome, of involved genes. To identify MAE events we performed bioinformatic analyses of RNA sequencing (RNA-seq) and whole genome sequencing (WGS-seq) data for fourteen adenocarcinoma cell lines. In the analysis we utilised BWA-MEM aligner, STAR, Picard and Genome Analysis Toolkit (GATK).

To identify genes with autosomal monoallelic expression we investigated 287 pairs of genes overlapping at their 5' end in at least one library. Altogether 567 genes were analysed, GATK did not detect any SNPs for 308 (54,3%) genes and all of them had to be eliminated from further analysis. Remaining 259 genes were further processed and classified as monoallelic, biallelic or non-informative. For as many as 195 genes we detected a signal for monoallelic expression in at least one library. However, in majority of these cases the signal from other libraries was not informative (i.e. some SNPs demonstrated monoallelic and other biallelic expression). Nevertheless, we identified 15 pairs, in which both genes demonstrated only monoallelic expression in at least one library. Out of them, five pairs were selected for validation using RNA-FISH experiment.

Our results demonstrate that MAE might be considered as a possible mechanism that rescue overlapping genes from transcriptional interference.

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430 Building a More Sensitive RNA Homology Search and Alignment Tool Using Hidden Potts Models

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Current methods for RNA homology search and alignment use both primary sequence and RNA secondary structure conservation. These methods typically neglect pseudoknots and other non-nested interactions. It would be desirable to take these additional evolutionarily conserved interactions into account in RNA sequence alignment and homology search. Recent work in protein folding prediction has successfully used a class of statistical physics models called Potts models to capture complex pairwise correlation structure between sites in deep multiple sequence alignments. However, Potts models do not consider insertions and deletions, limiting their use for homology search. We are working on extending Potts models to the homology search and alignment problem by creating what we call a hidden Potts model (HPM) that couples a Potts emission process to a generative probability model of insertions and deletions. Our model is incompatible with polynomial-time dynamic programming sequence alignment algorithms, but we have developed an approximate alignment algorithm based on importance sampling by using simpler probabilistic profiles (HMMs and SCFGs) as proposal distributions. Benchmark comparisons show HPMs perform promisingly relative to current methods in remote homology search and are able to accurately align RNA structural elements including pseudoknots.

431 DesiRNA - method for RNA secondary structure-based sequence design

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Ribonucleic acid (RNA) molecules are master regulators of cells. They are involved in a variety of molecular processes: they transmit genetic information, they sense and communicate responses to cellular signals, and even catalyze chemical reactions. These functions of RNAs depend on its ability to assume one or more structures, which is encoded by the ribonucleotide sequence. One of the fundamental challenges of biology and chemistry is to design molecules that form desired structures and carry out desired functions. The computational design of RNA requires solving the so-called RNA inverse folding problem given a target structure, identify one or more sequences that fold into that structure (and do not fold into any other structure). Designing RNA sequences with specific folding properties and with desired functions has already proven useful in a number of applications in the areas such as the development of probes and sensors, molecular medicine, and material science. Nonetheless, RNA design is very difficult, especially for molecules with complex structures.

Currently, existing methods for RNA design methods exhibit many severe limitations. Typically, they do not check for the potential of the RNA sequence to oligomerize, and few methods allow for designing RNA molecules comprising several different chains or ones that fold into several alternative structures.

In this work, we present a program - DesiRNA, for designing RNA sequence based on the provided secondary structure and various additional constraints (sequence, stable motifs, prevented patterns). DesiRNA can design not only single molecules (taking into account the potential of RNA to oligomerize) but also oligomers; additionally, it allows to design molecules that fold into several alternative structures (e.g. riboswitches).

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432 Reference-free evaluation of RNA 3D models quality

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The knowledge about RNA 3D structures has been steadily growing over the years. This phenomenon enables to apply computational methods to learn from the data and to propose knowledge-based approaches for problems of RNA structure analysis. Especially in the field of RNA 3D structure prediction, there are currently several state-of-the-art tools available. They implement different approaches, each generating up to thousands of predictions per single target. It is difficult to assess even a single model as it requires a lot of background information to be gathered, understood and verified against available raw 3D coordinates. Faced with thousands of models generated with different tools, even the most advanced users are likely to have problems with the selection of native-like ones.

To aid the assessment of multiple RNA 3D models we propose a new computational tool. It does not require any reference 3D structure but can benefit from optional constraints: either direct information about base pairing or inferred one from Rfam seed alignment. Each input 3D model is analyzed in terms of its interaction network. Canonical and non-canonical base-pairs are extracted into a shared set. Then, a consensus-seeking procedure finds the subset which meets an agreement criterion. It is a parameter of the method with sensible defaults found in computational experiments. If reference 2D data is available, it influences the agreement by enforcing certain base pairs to be part of the solution and thus penalizing all models lacking it.

The consensus subset forms an interaction network i.e. a virtual reference structure. This enables to compare input 3D models and output assessment results of two kinds. First, a global ranking is computed and every model gets position according to Interaction Network Fidelity (INF) similarity score. Second, each base pair in every model can now be marked as True Positive (TP), True Negative (TN), False Positive (FP) or False Negative (FN). Additionally, our proposed tool is able to visualize the virtual reference structure and models. The graphical representation highlights the features of base pairs making it easier to assess models visually.

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433 Hunting for the identity of the missing metabolite of the Moco riboswitch

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Molybdenum cofactor (Moco) is an essential metabolite for almost all living organisms consisting of molybdenum(VI) coordinated to a tricyclic pyranopterin. Moco-dependent enzymes use of the metal redox properties to catalyze fundamental metabolic reactions. Moco is biosynthesized through a highly conserved 4-step pathway starting from GTP, which is consecutively converted into cPMP, MPT and MPT-AMP prior to the insertion of the molybdenum(VI) deriving from molybdate. This pathway involves a variety of different enzymes, whose expression seems to be controlled by an ncRNA regulatory element, the Moco riboswitch[1]. However, no evidence of the direct interaction between this RNA and Moco or any of its biosynthetic precursors could ever be observed. This is due to the scarce availability and high instability of Moco and its precursors, which share a peculiar organic oxygen-sensitive scaffold. However, protocols were established to isolate cPMP from bacteria and to obtain Moco inserted in a Moco carrier protein (MCP) from *Chlamydomonas reinhardtii*[2,3].

Our goal is to determinate the identity of the metabolite that causes a conformational change in the Moco riboswitch testing the possible *in vitro* interaction between the Moco riboswitch and all available metabolites along the Moco biosynthetic pathway including cPMP and Moco in the form of Moco-MCP. For this purpose, we performed native gel electrophoresis and different footprinting assays under strict oxygen-free conditions. We proved that GTP, molybdate and cPMP are not specific Moco riboswitch binder.

Instead, Moco loaded MCP causes conformational changes in the RNA structure. Although similar changes were also observed at very high apo-MCP concentrations, the Moco-loaded protein has a higher affinity to the RNA compared to the apo-MCP. Our findings suggest that Moco plays a role in the RNA-protein interaction enhancing the affinity between the two species and that MCP might not be only involved in the Moco transfer but it also has an active role in the RNA gene regulation.

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[1]Regulski *et al.*, *Mol.Microbiol.* **2008**, 68, 918.

[2]Santamaria-Araujo *et al.*, *JBIC*. **2012**, 17, 113.

[3]Fischer *et al.*, *J.Biol.Chem.* **2006**, 281, 30186.

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434 Characterization of orphan riboswitches using a genetic screen

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Bacteria use cis-encoded RNA structures called riboswitches to regulate genes in response to changing intracellular conditions. The study of these RNAs has enriched our knowledge of bacterial physiology and the capabilities of RNA structures. Riboswitches are found in the 5'-UTR of mRNAs where they modulate the expression of downstream genes in response to concentrations of small molecule ligands that stabilize the formation of regulatory structures. Hundreds of riboswitch families have been identified, yet most of them remain "orphans," without an assigned ligand (Weinberg et al., 2010, 2017). Several approaches have been used to identify the ligands of orphan riboswitches, but many rely upon a priori knowledge of the genes and metabolic pathways controlled by the riboswitch. Additionally, screens of compound libraries do not include the full breadth of metabolites that exist in cells, some of which may be unknown to science. To circumvent these issues, we have employed a mutagenesis screen in Bacillus subtilis to map mutations that alter the expression of orphan riboswitches. From the screen we can rationalize candidate ligands by considering the effect of each mutation on the intracellular environment.

We tested this approach using transposon mediated mutagenesis in Bacillus strains with various orphan riboswitch sequences fused to lacZ. Initial rounds of mutagenesis have yielded several insertions that alter the expression of the riboswitch-controlled reporter gene. These mutant libraries suggest several candidate ligands for the orphans tested and reveal surprising connections between different metabolic pathways. Ligands suggested by the screen will be tested for binding in vitro using SHAPE structure probing and isothermal titration calorimetry (ITC) experiments.

Weinberg, Z., Lünse, C. E., Corbino, K. A., Ames, T. D., Nelson, J. W., Roth, A., ... Breaker, R. R. (2017). Detection of 224 candidate structured RNAs by comparative analysis of specific subsets of intergenic regions. Nucleic Acids Research, 45(18), 10811-10823. http://doi.org/10.1093/nar/gkx699

Weinberg, Z., Wang, J. X., Bogue, J., Yang, J., Corbino, K., Moy, R. H., & Breaker, R. R. (2010). Comparative genomics reveals 104 candidate structured RNAs from bacteria, archaea, and their metagenomes. Genome Biology, 11(3), R31. http://doi.org/10.1186/gb-2010-11-3-r31

435 Biochemical validation of a second tetrahydrofolate riboswitch class in bacteria

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The *folE* RNA motif, which was uncovered by bioinformatic analyses of bacterial genomes, is present in various species of Alphaproteobacteria and exhibits various features that make it a strong riboswitch candidate. For example, representatives of this motif are commonly found upstream of *folE* genes, which code for GTP cyclohydrolase I, the first enzyme in the *de novo* folate biosynthesis pathway. Additional bioinformatics analyses allowed us to update substantially the consensus sequence and secondary structure model of the motif. Despite its simple architecture, biochemical analysis using in-line probing assays reveal that the motif selectively binds tetrahydrofolate (THF) and several of its close derivatives. The binding affinity is also sensitive to mutations at conserved nucleotides positions, which indicates that these nucleotides are critical for THF aptamer function. The *folE* motif has a consensus sequence and structure distinct from the previous validated class of THF riboswitches that are found in Gram-positive bacteria. Thus, *folE* motif RNAs are members of a novel riboswitch class that recognizes THF, making this the second distinct riboswitch class known to respond to this fundamental enzyme cofactor.

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436 Local-to-global signal transduction at the core of a Mn^{2+} sensing riboswitch

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The yybP-ykoY class of riboswitches regulates the expression of genes involved in Mn^{2+} homeostasis in bacteria through directly sensing the small Mn^{2+} ion. The riboswitch adopts an H-shaped structure with two docked stem-loops connected by a coaxially stacked 4-way junction. Loops L1 and L3 form the docking interface between the stems and contain two divalent metal ion binding sites. While one site allows binding of either Mg^{2+} or Mn^{2+}, the other site strongly favors binding of Mn2+. The ability of the RNA to selectively discriminate and respond to Mn^{2+} over Mg^{2+} in the cellular environment makes this riboswitch a particularly intriguing gene regulatory system. However, the mechanism of this exquisite metal ion sensing riboswitch and its role in regulating transcription are still poorly understood. Here, we have solved the structures of a Mn^{2+}-sensing riboswitch from *Xanthomonas oryzae* that revealed partially unfolded conformations in the ligand-sensing region. Molecular Dynamics (MD) simulations of the structures show a locally polymorphic environment of the two metal binding sites, suggesting that Mn^{2+} binding may impact the RNA through a local-to-global conformational cascade. Using single-molecule FRET, we observe a previously unknown undocked conformation that samples transient docked states in the presence of Mg^{2+}. By contrast, we directly demonstrate that a stable docked conformation is formed only upon the addition of sub-millimolar Mn^{2+}. Using single-molecule kinetics analysis of RNA transient structure (SiMKARTS) assay, we show that the presence of submillimolar Mn^{2+} leads to stabilization of P1.1 stem, which is responsible for disruption of terminator hairpin and promoting transcription readthrough. Overall, our work reveals that fast docking of the loops observed in the presence of Mg^{2+} pre-organizes the riboswitch into a compact global structure with a local binding site poised for Mn^{2+}. Once the site is occupied by Mn^{2+}, a stably folded conformation results that is not observed for other transition metal ions except Cd^{2+}. Such high sensitivity and cooperativity between Mn^{2+} and Mg^{2+} in folding of a riboswitch underscore the importance of the coupling of local with global structure of RNA in general, laying the foundation for amplifying small local difference into gene regulatory decisions.

437 Current Understanding of Pistol Ribozyme Structure and Mechanism

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Comparative genomic analysis revealed the identity of four novel classes of self-cleaving ribozymes termed twister, twister-sister (TS), pistol and hatchet, thus expanding the sequence space of catalytically active RNAs. One of them, the pistol motif is of particular interest because its strand scission rate is among the fastest of all nucleolytic RNAs. In this sense, applications capitalizing on high speed cleavage combined with small molecule binding aptamers have been presented recently. They concern the engineering of chemically regulated self-cleaving ribozymes (commonly referred to as aptazymes) that are an emerging class of genetic devices allowing for dynamic control of gene expression. Our recent crystal structure of the pre-catalytic state of the pistol ribozyme reveals the overall fold and provides insights into the architecture of the active site pocket. It shows guanosine (G40) and adenosine (A32) close to the G53-U54 cleavage site. While the N1 of G40 is in 3.4 Å distance to the G53 2'-OH group that attacks the scissile phosphate and hence suggests its direct role in general acid-base catalysis, the function of A32 is not clear. Here, we present evidence by atom-specific mutagenesis that neither N1 nor N3 base positions of A32 are involved in catalysis. By contrast, the ribose 2'-OH of A32 seems crucial for proper positioning of G40 via H-bond networks that involve G42 as a bridging unit between the A32 and G40. We also found that disruption of the inner-sphere coordination of the active site-Mg^{2+} cation to N7 of G33 renders the ribozyme inactive. A mechanistic proposal is suggested with A32 playing a structural and hydrated Mg^{2+} playing a catalytic role in phosphodiester cleavage of the pistol ribozyme.

Neuner S, Falschlunger C, Fuchs E, Himmelstoss M, Ren A, Patel DJ, Micura R. Atom-Specific Mutagenesis Reveals Structural and Catalytic Roles for an Active-Site Adenosine and Hydrated Mg^{2+} in Pistol Ribozymes. Angew. Chem. Int. Ed. 2017, 56, 15954-15958.

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438 Ribozyme-catalyzed site-specific labeling of RNA

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Site and sequence specific labeling of unmodified RNA molecules is a challenging task. A number of chemical and enzymatic tools have been developed for this purpose, however, many of these methods often lack versatility, sensitivity and/or simplicity of use. Development of alternative tools is still necessary for overcoming these limitations for applications in RNA imaging and labeling of native RNA protein complexes. Nucleic acid enzymes are a rather recent addition to the experimental repertoire for covalent RNA labeling. For example, in vitro labeling of RNA with DNA enzymes has been reported via formation of 2',5'-branched RNA, but ribozymes with similar activity for direct labeling of RNA are not yet known. Thus development of RNA catalysts with the ability to site-specifically label target RNA molecules has been the main objective of this study.

A partially structured random RNA library was used as the starting pool for in vitro selection of RNA labeling ribozymes. By implementing a stringent in vitro selection process using N6-modified ATP analogues as substrates, ribozymes were obtained with the ability to label target RNA molecules at specific internal positions. The most active variants of the evolved ribozymes demonstrate the desired flexibility with respect to labeling a wide variety of target sequences. The new RNA catalysts have also been shown to readily accept a number of ATP analogues with various biorthogonal functional groups and fluorophores as N6-modifications. These ribozymes therefore have proven to be valuable tools for site-specific RNA labeling in vitro, and similar ribozymes will have the potential to label cellular RNAs in vivo.

439 Fitness study of Azoarcus Group I mutants under a gradient of magnesium chloride concentration - Construct of Seascapes

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The RNA world hypothesis predicts that functional RNA molecules played a central role in the origins and early evolution of life. In order to critically evaluate this scenario, we need to understand the capacity of populations of functional RNA molecules to evolve specific adaptations. RNA fitness landscapes are one approach to understanding evolution that uses high-throughput sequencing to map genotype to fitness for vast libraries of sequences. However, fitness landscapes can change in response to environmental shifts, altering the peaks and valleys that determine evolutionary outcomes. Despite the importance of environmental gradients and fluctuations for life's origins, the relationship between RNA fitness landscapes and changes in the physio-chemical environment remains understudied. The first part of the study is to construct empirical fitness "Seascapes", using sequencing as a high-throughput assay of group I ribozyme catalytic activity. Instead of using throughput to analyze more sequences, we will direct some of the throughput to study the same sequences at different points across an environmental gradient (magnesium ion concentration). From this, we will be able to determine how fitness landscapes respond to the environmental change. We will use computational approaches to characterize evolutionary outcomes on these seascapes during constant and fluctuating environmental conditions. We predict that environmental fluctuations will facilitate evolution by maintaining genetic diversity that can fuel adaptation.

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440 Structure and ligand binding of the SAM-V and glutamine-II riboswitches

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Riboswitches are regulatory elements in mRNA that bind specific ligands usually leading to the stabilization of a conformation that results in a changed level of gene expression. We have determined crystal structures of two new riboswitches, that permit us to deduced how they bind their ligands with selectivity, and how they control translation.

SAM-V is one of the class of riboswitches that bind S-adenosylmethionine, regulating gene expression by controlling translation. We have solved the crystal structure of the metY SAM-V riboswitch bound to its SAM ligand at 2.5 Å resolution. The RNA folds as an H-type pseudoknot, with a major-groove triple helix in which resides the SAM ligand binding site. We propose a model in which SAM binding leads to the association of the triplex third strand that stabilizes a short helix and occludes the ribosome binding site. Thus, the new structure explains both ligand specificity and the mechanism of genetic control.

We have also solved the crystal structure of the glutamine-II riboswitch bound to its L-glutamine ligand at 2.2 Å resolution. We will discuss the structure and ligand binding of this riboswitch.

L. Huang, and D. M. J. Lilley: Structure and ligand binding of the SAM-V riboswitch. *Nucleic acids res.***46**, 6869-6879 (2018).

L. Huang, J. Wang and D. M. J. Lilley: Structure and ligand binding of the glutamine-II riboswitch (in preparation).

441 Can we predict a riboswitch activity with different ligands by calculations?

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Riboswitches are non-coding mRNA fragments, able to regulate gene expression upon binding small molecules. Predicting the mode of action of riboswitches with various ligands using computations remains an open question and would be of high value for riboswitch/ligand design purposes. It would also provide a unique tool for conditional gene expression.

Here, we investigate a small synthetic riboswitch N1 binding three different aminoglycoside ligands. Two of the ligands inhibit translation of a reporter fluorescent protein in a yeast system with riboswitch inserted to mRNA, while the third ligand does not disturb translation. The striking similarity between active and inactive ligands motivated us to take a closer look at the dynamics of the system at the atomic level of detail. We performed a series of molecular dynamics simulations and observed that using the replica-exchange method was essential to determine the key interactions responsible for subtle differences in ligand binding modes, and, consequently, intruding the translational machinery (Kulik et al. (2018), Nucleic Acids Research, 46 (19), 9960-9970). In this work, we explained the reasons behind the differences in activity of ligands at the atomic level and also why looking at the NMR structures only does not reveal those differences straight away. Interestingly, the same aminoglycoside ligands can bind to another RNA receptor, namely the ribosomal A-site, but are not discriminated by this receptor.

Overall, the enhanced sampling molecular dynamics simulations seem to be the only computational option at the moment to provide insight into the activity of riboswitches with different ligands, as other currently available methods, such as free energy calculations, frequently fail for ligands differing in the net charge. However, the system size is a serious limitation for this technique and further work on this topic is necessary. Estimating the experimental binding constants for this kind of system is yet another unresolved problem, which we aim to tackle in the future.

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442 Evidence that the *nadA* Motif is a Riboswitch for the Ubiquitous Enzyme Cofactor NAD^+

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The *nadA* motif is a riboswitch candidate present in various Acidobacteria species that was previously identified by bioinformatic analysis of bacterial DNA datasets. Over 100 unique representatives have been identified exclusively upstream of *nadA* genes, which code for an enzyme associated with the biosynthesis of the ubiquitous coenzyme NAD^+. The architecture of this motif indicates that it likely employs similar ligand-binding aptamer domains in tandem to control translation initiation. Biochemical analyses using in-line probing reveal that the first domain selectively binds ligands carrying an adenosine 5 ´ -diphosphate (5 ´ ADP) moiety, including NAD^+ and its reduced form NADH. Genetic analyses indicate that a *nadA* motif RNA suppresses gene expression when NAD^+ is abundant, and that both aptamer domains are required for maximal gene regulation. Additional studies with NAD^+ biosynthetic pathway knockout strains support these findings, where gene expression turns on under low NAD^+ levels compared to basal expression at high NAD^+ concentrations. Our analyses demonstrate the *nadA* motif is a riboswitch that recognizes NAD^+, however there are still lingering questions. For example, we have not observed selective binding of the nicotinamide moiety of NAD^+, or binding by the second putative aptamer *in vitro*, despite its sequence and structural similarity to the first aptamer.

443 Cellular Small Molecules Contribute to Twister Ribozyme Catalysis by Indirect Proton Transfer

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The number of self-cleaving small ribozymes has increased sharply in recent years. Advances have been made in describing these ribozymes in terms of four catalytic strategies: α (in-line attack), β (neutralization of the non-bridging oxygens), γ (activation of the nucleophile) and δ (stabilization of the leaving group). Current literature presents the rapid self-cleavage of the twister ribozyme in terms of all four of these classic catalytic strategies. Here, we describe a multi-channel mechanism for the twister ribozyme whereby at low pH conditions the N3 of A1 acts as a general acid for direct proton transfer while at neutral pH the N3 of A1 participates in a proton shuttle facilitated by small molecules catalyzing indirect proton transfer. At biological pH, the rate of the wild-type twister ribozyme is enhanced up to 5-fold in the presence of moderate buffer concentrations, similar to the 3 to 5-fold effects reported previously for buffer catalysis for protein enzymes. We observe this catalytic enhancement not only with standard laboratory buffers, but also with diverse biological small molecules, including imidazole, amino acids, and amino sugars. Brønsted plots suggest that small molecules assist in indirect proton transfer, most likely with δ catalysis. Additionally, greater than a hundred-fold small molecule rescue was found in the background of either a 3-deaza-A1 or an abasic A1 twister suggesting that proton transfer at this position, whether direct or indirect, contributes significantly to k_{obs}. Overall, cellular small molecules provide a simple way to enhance the limited functional diversity of RNA and have the potential to participate in the catalytic mechanisms of many ribozymes *in vivo* including large RNP machines such as the ribosome and spliceosome.

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444 How chemically functionalized ligands trigger engineered preQ$_1$ riboswitches in *E.coli*

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We utilized class-I and class-II preQ$_1$-sensing riboswitches as model systems to decipher the structure-activity relationship of rationally designed ligand derivatives *in vitro* and *in vivo*. We found that synthetic preQ$_1$ ligands with amino-modified side chains that protrude from the ligand-encapsulating binding pocket, and thereby potentially interact with the phosphate backbone in their protonated form, retain or even increase binding affinity for the riboswitches *in vitro*. They, however, led to significantly lower riboswitch activities in a reporter system *in vivo* in *E. coli*. Importantly, when we substituted the amino- by azido-modified side chains, the cellular activities of the ligands were restored for the class-I conditional gene expression system and even improved for the class-II counterpart. Kinetic analysis of ligand binding *in vitro* revealed enhanced on-rates for amino-modified derivatives while they were attenuated for azido-modified variants. This shows that neither high affinities nor fast on-rates are necessarily translated into efficient cellular activities. Taken together, our comprehensive study interconnects *in vitro* kinetics and *in vitro* thermodynamics of RNA-ligand binding with the ligands' *in vivo* performance and thereby encourages azido- rather than amino-functionalized design for enhanced cellular activity.

Eva Neuner, Marina Frener, Alexandra Lusser & Ronald Micura (2018), Superior cellular activities of azido- over amino-functionalized ligands for engineered preQ1 riboswitches in *E.coli*, RNA Biology, 15:10, 1376-1383.

445 Hatchet ribozyme structure and implications for cleavage mechanism

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Small self-cleaving ribozymes catalyze site-specific cleavage of their own phosphodiester backbone with implications for viral genome replication, pre-mRNA processing and alternative splicing. We report on the 2.1 Å crystal structure of the hatchet ribozyme product, which adopts a compact pseudo-symmetric dimeric scaffold, with each monomer stabilized by long-range interactions involving highly conserved nucleotides brought into close proximity of the scissile phosphate. Strikingly, the catalytic pocket contains a cavity capable of accommodating both the modeled scissile phosphate and its flanking 5′ nucleoside. The resulting modeled pre-catalytic conformation incorporates a splayed-apart alignment at the scissile phosphate, thereby providing structure-based insights into the in-line cleavage mechanism. We identify a guanosine lining the catalytic pocket positioned to contribute to cleavage chemistry. The functional relevance of structure-based insights into hatchet ribozyme catalysis is strongly supported by cleavage assays monitoring the impact of selected nucleobase and atom-specific mutations on ribozyme activity.

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446 Implementation of Fluorescent Cross Correlation Spectroscopy (FCCS) to Analyze Single Molecule Riboswitch Performance in Eukaryotic Cells.

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Synthetic RNA riboswitches have emerged as promising candidates for the realization of externally controlled genetic networks. The modular nature and portability of riboswitches offers unique opportunities for their rational design and application. However, previous studies regarding the efficacy of riboswitches have yielded inconsistent results. A fundamental understanding of what sets apart high and low performers is still needed for successful applications. This information has been difficult to obtain because investigating the behavior of RNA inside living cells is challenging, due to the fact that they are transient, unstable molecules that must fold into secondary and tertiary structures necessary for their action. Existing methods to study RNA such as qRTPCR, FISH, and SHAPEseq fall short as they alter the behavior of the RNA molecules being investigated or only quantify the presence and structure of RNA. This loses sight of important temporal and mechanistic contexts inside of living cells in which they function. I will overcome these challenges by implementing FCCS to measure single molecule riboswitch dynamics directly at the site of transcription in living cells. Data will be collected using a state of the art laser scanning fluorescence microscope capable of 2-photon excitation and 3D orbital tracking. This enables the collection of fluorescence time course data on a millisecond timescale for hours with minimal photo-bleaching. A previously developed β-globin RNA reporter system contains PP7 and MS2 bacteriophage hairpins in the first or second intron and final exon. Upon transcription, these hairpins are bound by fluorescently tagged phage coat proteins and enables the simultaneous quantification of transcription and splicing kinetics inside of living cells. Specifically, cross correlation analysis of intron and exon signals originating from active transcription sites and subsequent modeling will quantify the number of nascent RNA transcripts, rate of transcription, timing of transcript release and degrees of co-transcriptional splicing. FCCS data obtained across several RNA constructs will provide critical insight into the temporal and mechanistic behaviors that contribute to riboswitch performance. This project will not only aid in the design of improved synthetic RNA riboswitches, but will also introduce FCCS methodology as a tool to study synthetic RNA in-vivo.

447 An aminoacylation ribozyme evolved from a natural tRNA sensing riboswitch

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In the hypothesized RNA world, the early organisms used RNA as both genome storage and functional molecule. Some modern riboswitches are therefore considered the molecular fossils of ancient ribozymes existed in the RNA world. However, to the best of our knowledge, there has been no experimental evidence to support this hypothesis. Here we report a novel aminoacylation ribozyme, Tx2.1, evolved from partially randomized Bacillus subtilis glyQS T-box riboswitch which binds to a cognate uncharged tRNA. Tx2.1 recognizes the anticodon of substrate tRNA by base-pairing and conjugates an N-biotinyl-L-phenylalanine to the 3'-end of tRNA. Anticodon recognition by Tx2.1 can be easily engineered by simple compensatory mutations. Aminoacyl-tRNA prepared by Tx2.1 can be used for in vitro reconstituted translation system to produce peptides bearing non-canonical amino acid. Our results support the hypothesis that riboswitch may be a descendant from an ancient ribozyme and represent a potential use of Tx2.1 for translation of peptides containing nonproteinogenic amino acids.

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448 The asymmetry and cooperativity of tandem glycine riboswitch aptamers

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Glycine riboswitches are known to utilize both single aptamer and tandem aptamer architectures. The presence of a second binding site in glycine riboswitch tandem systems raises questions regarding the individual contributions each aptamer provides toward helical switching in the expression platform. To dissect these contributions, we characterized the effects of 684 single point mutants of the tandem glycine riboswitch from *Bacillus subtilis* both in the context of the wild-type construct and in the background of binding site mutations that selectively restrict ligand binding to either the first or second aptamer. We used a high-throughput assay that we recently developed, termed SMARTT (Sequencing-based Mutational Analysis of RNA Transcription Termination), to simultaneously monitor the *in vitro* transcription termination efficiencies of each mutant as a function of ligand concentration. Despite the known structural symmetry of tandem aptamers, the response to these mutations was frequently asymmetrical between the two aptamers. Mutations in the first aptamer often significantly weakened the $K_{1/2}$, while mutations in the second aptamer often improved the overall amplitude of response. These results indicate that the native fold of the second aptamer is suboptimal in the wild-type construct. This is in contrast to the tandem glycine riboswitch from *Vibrio cholerae*, which was previously shown to have preferential binding to its second aptamer. Our results indicate that helical switching is driven by ligand binding in the first aptamer for some tandem glycine riboswitch variants, while others rely more heavily on binding by the second aptamer. This dataset provided evidence that the ligand response of tandem glycine riboswitches does display some cooperativity. The reason this appears in some systems but not in others will be discussed.

449 *corA* : A new riboswitch in *Escherichia coli*

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Intracellular ions are essential for the survival of bacteria. Magnesium ions are very important for enzymes and gene regulation and is known to affect the virulence in bacteria such as *Salmonella enterica* (M. E. Maguire 2008). In *E. coli*, the MgtA membrane protein is involved in the transport of magnesium ions and is regulated by PhoP/PhoQ at the promoter level. The expression of *mgtA* is also controlled by a riboswitch mechanism, which is modulating the access of the Shine-Dalgarno sequence as a function of intracellular magnesium concentrations.

To characterize the effect of magnesium on gene expression, we have performed a ribosome profiling study. As expected, the ribosome occupancy of *mgtA*was reduced in presence of high magnesium concentrations. We also observed that the ribosome occupancy was reduced for the magnesium ions transporter *corA*. Surprisingly, inspection of 5' UTR*corA*sequence suggested the presence of a riboswitch similar to the one regulating mgtA. Using ß-galactosidase assays, we demonstrated that *corA* expression is repressed in the presence of high magnesium concentrations, consistent with a riboswitch regulation mechanism. We are currently investigating the effect of magnesium ions on *corA* RNA structure and determining how this could be involved in the riboswitch regulation. Together, our results show for the first time that *corA*is regulated by intracellular magnesium, thus suggesting that *corA* expression is central for bacterial homeostasis. Our results will be discussed in light of *corA* cellular function and its role in transport of other cations such as cobalt and nickel ions.

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450 One transcript, distinct stabilities: The methionine biosynthesis operon in *Staphylococcus aureus*

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N-formyl methionine is the universal N-terminal amino acid of prokaryotic proteins making methionine indispensable for bacterial growth. The common human pathogen *Staphylococcus aureus* is capable of synthesising methionine *de novo* and therefore to sustain in niches where the amino acid is lacking. Recently, we identified a unique hierarchical control pathway regulating *de novo* methionine biosynthesis in *S. aureus* involving stringent-response control in combination with a T-box riboswitch and RNA decay [1]. Riboswitches are *cis*-acting RNA regulatory elements, located in 5'-UTRs of genes. The T-box family of riboswitches represents transcription termination control systems which bind uncharged cognate tRNAs as effector molecules. The T-box riboswitch residing in the 5'-UTR of the *S. aureus metICFE-mdh* methionine biosynthesis operon specifically interacts with uncharged methionyl-tRNAs $tRNA^{Met}$. In addition to T-box riboswitch-mediated transcription control, the *met* leader/*metICFE-mdh* mRNA was shown to undergo processing and rapid degradation involving various RNases [1]. Here we demonstrate that stability of the *metICFE-mdh* mRNA varies over the length of the transcript with a longer lifespan towards the 3'-end of the transcript. Furthermore, we were able to determine the exact processing site of the *met* leader RNA and the processing region of the *metICFE-mdh* RNA, respectively by RACE approaches. RNases III and J1/J2 showed to be central to *met* leader and *metI* RNA processing and degradation, respectively. We solved the secondary structure of this exceptionally long (440 nt) T-box riboswitch RNA using in-line probing to confirm the presence of structures required for RNase III cleavage. The uncommon, immediate physical separation of the *met* leader RNA from the *metICFE-mdh* mRNA appears to be the driving force for *met* mRNA 5' destabilisation.

We hypothesise that targeted RNA decay represents another level in the hierarchical methionine biosynthesis control network influencing translational efficiency and adjusting the protein amounts of the distinct enzymes of the pathway to current requirements.

[1] Schoenfelder, S. M.K. et al. 2013. Methionine Biosynthesis in *Staphylococcus aureus* Is Tightly Controlled by a Hierarchical Network Involving an Initiator tRNA-Specific T-box Riboswitch. Plos Pathogens 9(9).

451 Expeditious Validation of Multiple Orphan Riboswitch Candidates

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Riboswitches are structured noncoding RNAs that function as cis-acting gene control elements, which are typically located in the 5′ UTRs of bacterial mRNAs. Riboswitches recognize a diverse collection of ligands, including numerous metabolites and ions, with very high specificity. At present, more than 40 riboswitch classes have been experimentally validated. However, many more orphan riboswitch candidates, discovered via bioinformatics, remain to be associated with their target ligands. Additional, rare, orphan riboswitch candidates are currently being discovered by presorting intergenic regions (IGRs) of each bacterial genome based on two characteristics: length and GC content. Long and GC-rich IGRs are more likely to carry novel riboswitch candidates. It is likely that novel biological processes remain to be discovered through the validation of orphan riboswitch candidates. To accelerate the pace of riboswitch experimental validation, we have established a streamlined analysis procedure to uncover evidence for riboswitch function. We present six orphan riboswitch candidates that are at various stages of this process. One candidate, algC, has experimental evidence demonstrating that it is a genetic control element and further efforts are underway to identify the ligand it recognizes.

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452 Successful design of highly active HDV ribozymes by a combined computational and experimental study

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Design of RNA sequences that adopt functional folds helps establish principles of RNA folding and develop applications in biotechnology. Inverse folding for RNAs, which employs computational design of sequences that adopt specific structures, can be utilized for unveiling RNA functions and developing genetic tools in synthetic biology. Although many algorithms for inverse RNA folding have been developed, the pseudoknot, which plays an essential role in folding of nearly all known ribozymes and riboswitches, is not addressed in most algorithms due to complexity. For the few algorithms that attempt to design pseudoknot-containing ribozymes, self-cleavage activity has not been tested. Herein, we design double-pseudoknot HDV ribozymes using an inverse RNA folding algorithm and test their kinetic mechanisms experimentally [1]. More than 90% of the positively designed ribozymes possess self-cleaving activity, whereas more than 70% of negatively designed ribozymes, which are predicted to fold to the necessary structure but with low fidelity, do not possess activity. Kinetic and mutation analyses reveal that our designed pseudoknotted RNAs cleave site-specifically and with the same mechanism as the WT ribozyme. In fact, most ribozymes react just 50- to 80-fold slower than the WT ribozyme. Structural comparison by in-line probing experiments between the designed and WT ribozyme reveals that rates in the designed ribozymes can be improved to near WT ribozyme by mutation of a junction. Thus, fast-cleaving functional ribozymes with multiple pseudoknots can be designed by a combination of computational and experimental studies. Our RNA design could be applied to other pseudoknot-containing ribozymes and riboswitches.

[1]Ryota Yamagami, Mohammad Kayedkhordeh, David H. Mathews, and Philip C. Bevilacqua, ***Nucleic Acids Res***., 2019, 47 (1), 29-42.

453 RNA methylation states in CD8+ T-lymphocyte differentiation

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N6-methyladenosine (m6A) is a regulatory element of mRNA stability and translation that plays a critical role in cell differentiation. Yet, there is limited insight into the temporal dynamics of m6A marks that impact cell fate transitions, and the regulatory mechanisms of this modification are unexplored. In response to T-cell receptor (TCR) activation, CD8+ T-lymphocytes undergo a rapid transition from a naïve, quiescent-like state into a proliferative effector state. Employing the iCLIP technique targeted to methylomes (miCLIP) in human primary CD8+ T-lymphocytes, we find m6A methylation signatures in mRNAs and non-coding RNAs that are either static or dynamic during CD8+ T-cell activation. In particular, we will present distinct hyper- and hypo-m6A profiles of early and late TCR-response genes that point to m6A regulation in effector and memory-precursor function(s). To gain an insight in the transcriptome turnover, we performed SLAM-Seq and will present the relationship between m6A signatures, mRNA half-lives and de novo transcription of module genes that define a naïve, day1- and day5- activated CD8+ T-cell state. We have m6A-profiled subpopulations of primary naïve, central-memory, effector-memory, and effector CD8+ T lymphocytes freshly isolated from human blood, and will discuss the potential role of m6A in CD8+ T-cell fate determination. On non-coding RNAs, the m6A profile shows known and newly identified m6A sites for tRNAs and their dynamics in CD8+ T-cell activation. Finally, from genetic models, we will address possible regulatory mechanisms of m6A marks in the CD8+ T-cell system. These findings raise hypotheses for CD8+ T-cell reprogramming via m6A control.

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454 Understanding the role of the m^{6}A methyltransferase METTL3 in breast cancer

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N6-methyladenosine (m^{6}A) is the most abundant internal modification in messenger RNA (mRNA) and long non-coding RNA (lncRNA), and plays important roles in RNA homeostasis. m^{6}A is a reversible modification dynamically regulated by a complex interplay of writers, readers and erasers. The core complex of the m^{6}A writer consists of the methyltransferase-like protein 3 and 14 (METTL3 and METTL14), which interacts with adaptor proteins WTAP, VIRMA, RBM15 and Zc3h13. Dysregulations of m^{6}A homeostasis has been shown to be involved in different types of cancer including glioblastoma, lung and breast cancer. In patients with breast cancer, the expression of METTL3, VIRMA and RBM15 is significantly upregulated, suggesting that m^{6}A dysregulation plays a central role in initiation and progression of breast tumorigenesis. To understand how METTL3 and aberrant m^{6}A-modified mRNA contribute to breast tumorigenesis, we characterized *METTL3* by generating *METTL3* knockdowns and attempted to knockout *METTL3* in breast cancer cells, and we studied METTL3 interactome in breast cancer cells.

455 Characterization of RNA post-transcriptional modifications in *Staphylococcus aureus* tRNAs during infection and antibiotic stresses

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S. aureus is a Gram - positive major human pathogen involved in a wide range of infectious diseases. The treatment of *S. aureus* infection is very challenging due to the emergence of multiple antibiotic resistant isolates. The high diversity of clinical symptoms caused by *S. aureus*, as well as its drug resistance mechanisms depend on the precise expression of numerous virulence factors and stress response pathways, which are tightly regulated at several levels (transcription, translation, mRNA decay).

RNA post-transcriptional modifications which can be modulated in response to adaptive processes could add an additional layer of control. Altering the chemical and physical properties of nucleotides, they affect RNA base - pairing formation, protein recognition, RNA structure and stability, mRNA translation and tRNA decoding properties. However, their impact in pathogenic bacteria during growth adaptation and infection has just started to be appreciated.

Using mass spectrometry and RNA sequencing methods, we are characterizing the tRNA-related modifications of S. aureus in various conditions mimicking stresses encountered during infections. The methods used and some preliminary data showing differences in tRNA modifications will be discussed.

Keywords : Staphylococcus aureus, Stress adaptation, Epitranscriptomics

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456 Exploring the role of 5 methyl cytosine modification in RNA metabolism of mitochondria

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RNA is the direct output of the genome and central to all life. Chemical modifications of RNA have recently attracted significant interest because of their impact on RNA fate and function, and their critical role in various human diseases, including cancer. RNA modifications are involved in both nuclear and cytoplasmic processes and are one of the major factors in regulating gene expression. They provide fast, efficient and dynamic means to regulate cellular protein content in response to a changing microenvironment. Out of 150 RNA modifications known so far, one of the most prominent is cytosine-5 methylation (m5C) which, among other enzymes, is catalyzed by NSUN2. NSUN2 is important for cell proliferation, differentiation, stress tolerance and has also been implicated in various neurological diseases and cancer. However, the precise role of NSUN2 in disease and development is still not well understood.

By performing a synthetic lethality screen using haploid human cell line, Hap1 cells, we identified a genetic interdependence between NSUN2 and mitochondrial function. This led to the question the role of RNA modification in mitochondrial translation. Mitochondria modulate their translation according to the metabolic status of the cell and maintain homeostasis. However, which factors coordinate the rate of mitochondrial protein synthesis are not known. Using U2OS and NCIH358 cancer cell line, I am investigating novel links between RNA modification and mitochondrial function, and testing if dynamic regulation of mitochondria based on cellular needs can be fine-tuned by RNA modifications. Since both RNA-modifying enzymes and mitochondria are associated with various human diseases, exploring this novel genetic interaction between NSUN2 and mitochondria might help unravel novel therapeutic targets.

457 Analysis of cap-associated adenosine modifications in eukaryote mRNA

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In eukaryotes, transcripts that are synthesised by RNA polymerase II (pol II) are co-transcriptionally modified. One of the co-transcriptional modifications is the addition of the 7-methyl guanosine cap to the 5' end. If no further modifications are added, this cap structure is referred to as cap 0; this is found in plants and yeast. Further modifications take place in higher eukaryotes; the ribose of the first nucleotide can be methylated at the 2' position to give cap 1, if the ribose of the next nucleotide is also methylated in the same position this gives cap 2. If the cap 1 methylation occurs on an adenosine, the nucleotide itself can be methylated to form 2'-O-dimethyl adenosine (m6Am).

The methylases responsible for the formation of cap1, cap2, and m6Am have now all been identified and roles in RNA stability, translatability and the innate immune response have been reported.

Cap adjacent nucleotides and methylation can be detected and quantified with Thin Layer Chromatography (TLC). Levels of the modifications vary in characteristic ways between species and in different organs with high levels being found in the brain.

This robust methodology is very sensitive and can detect cap adjacent modifications on low levels of input RNA and structures associated with transcripts from individual genes can be assayed. Such assays reveal that a combination of different starting nucleotides and different methylations can give rise to a population of gene transcripts with alternative 5' structures.

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458 Epitranscriptomic regulation of HIV-1 gene expression by m^5C and the novel m^5C reader MBD2

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How the covalent modification of individual mRNA nucleotides, termed epitranscriptomic modification, alters mRNA function remains unclear. One road block has been the difficulty of quantifying epitranscriptomic modifications. Using purified HIV-1 genomic RNA, we show that this RNA bears more epitranscriptomic modifications than cellular mRNAs, with 2'O-methyl and 5-methylcytosine (m^5C) modifications being particularly high. The primary writer for m^5C on HIV-1 RNAs was identified as NSUN2 and inactivation of NSUN2 inhibits HIV-1 gene expression without affecting HIV-1 RNA levels. We identify MBD2 as an m^5C reader and demonstrate that MBD2 has a higher affinity for m^5C modified RNAs. Loss of MBD2 function phenocopies loss of NSUN2 in that both reduce HIV-1 gene expression and perturb alternative splicing of HIV-1 RNAs. These data identify m^5C as a post-transcriptional regulator of both mRNA splicing and function.

459 Rational design of novel H/ACA snoRNAs for targeted pseudouridylation

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H/ACA snoRNAs are a class of non-coding RNA responsible for directing site-specific isomerization of uridine to pseudouridine in cellular RNAs. This ubiquitous modification occurs both in coding and non-coding RNAs and is one of the most abundant post-transcriptional RNA modifications. Furthermore, pseudouridylation tends to occur in functionally important regions of RNA, such as the peptidyl transferase center of 25S rRNA or the branch site recognition sequence of U2 snRNA. In eukaryotes and archaea, many pseudouridines are introduced by H/ACA small nucleolar ribonucleoprotein (snoRNP) complexes. Although the Cbf5/dyskerin protein is the catalytic subunit in H/ACA snoRNPs, an H/ACA guide RNA is required to recognize the target site in a sequence-specific manner. H/ACA guide RNAs typically share a common two hairpin structure with each hairpin harboring an internal loop called a pseudouridylation pocket that binds target RNAs in a bipartite manner.

Here, we have investigated the structure-function relationship of H/ACA guide RNAs with the goal of developing strategies for designing novel, highly active H/ACA guide RNAs. First, we established and applied rational design principles to generate a number of novel H/ACA guide RNAs. For comparison, and to refine features of active H/ACA guide RNAs, we also generated guide RNA variants designed to exhibit sub-optimal pairing with target RNA. Second, all designed H/ACA guide RNAs were quantitatively assessed for the ability to direct pseudouridylation in novel substrates using a highly active reconstituted Saccharomyces cerevisiae H/ACA snoRNP in vitro system. Altogether, the results not only refine our understanding of the base-pairing requirements between H/ACA guide and target RNAs, but the data also reveal important H/ACA guide RNA structural features that need to be considered when designing novel guide RNAs. Our findings open new avenues for identifying guide-substrate RNA combinations in vivo and for designing novel and active H/ACA guide RNAs.

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460 Role of N6-methyladenosine in the metabolism of circular RNAs

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Circular RNAs (circRNAs) are covalently closed, single-stranded transcripts produced by exon back-splicing circularization, wherein a downstream 5′ splice site (splice donor) is joined to an upstream 3′ splice site (splice acceptor). Circular RNAs have been shown to be abundant and evolutionarily conserved but their function is still largely unknown1. Expression profiling of circRNAs during in vitro differentiation of murine and human myoblasts allowed the identification of conserved species regulated in myogenesis. Upon an RNAi-based circRNA functional screening, we focused on circ-ZNF609, which affects myoblast proliferation when knocked-down. Circ-ZNF609 contains an open reading frame created upon circularization. We demonstrated that circ-ZNF609 is translated in a splicing-dependent and cap-independent manner from two different START codons, providing an example of a protein-coding circRNA in eukaryotes. N6-methyladenosine (m6A) is the most abundant mRNA modification. It occurs preferentially at a consensus motif (RRACH) and it is involved in every step of RNA metabolism. Circ-ZNF609 contains several RRACH motives both in its untranslated and coding region with different functions in circ-ZNF609 metabolism. In this regard, we are currently focusing on the possible regulatory role of m6A in circ-ZNF609 producing vectors able to overexpress the wild type sequence of circ-ZNF609 or mutants which are abolished for the m6A modification. Furthermore, we are investigating the role of readers known to be involved in m6A-mediated translation or splicing and the writers involved in circRNA methylation through a knock-down based approach. This study could increase the knowledge in the fields of both circRNAs epitranscriptome, biogenesis and cap-independent m6A-mediated translation.

461 p.p1 {margin: 0.0px 0.0px 0.0px 0.0px; font: 12.0px Times} mRNA methylation complex components and methylation outcomes in a model plant system.

Alexander Bridgen[1], Cameron Grundy[1], Samuel Chak[1], Doha Albalawi[1], Mi Zhang[1], Kamil Ruzicka[2], Yka Helariutta[4], Zsuzsanna Bodi[1], Gordon Simpson[3], Rupert Fray[1]

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N6-methyladenosine (m^6A) is a ubiquitous base modification found internally in the mRNA of most Eukaryotes. In the model plant *Arabidopsis thaliana*, levels of methylation equivalent to at least 50% of transcripts carrying m^6A are common, but methylation levels vary between transcripts, tissue types and developmental stage. Methylation is not distributed evenly across mRNAs - most m^6A is found close to the stop codon with some transcripts also showing an additional smaller peak near the start of the message.

We have identified a core set of mRNA m^6A writer proteins. The components required for m^6A in Arabidopsis include the methylases MTA and MTB and their interacting partners FIP37, VIRILIZER and the E3 ubiquitin ligase HAKAI. With the exception of the E3 ligase, these m6A writer components are essential during embryonic development, and appear to be well conserved between plants and mammals. Whilst complete knockout of the plant methylase is embryonic-lethal, a set of hypomorphic mutants with reduced m^6A levels has been generated. These plants show altered growth patterns, changed cell identities and perturbed hormone responses. Using these mutants and the wild type plants we are using immunoprecipitation sequencing approaches (MeRIPSeq) to study the topology of the epitranscriptome.

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462 The human Pus10 produces pseudouridine 54 in select tRNAs where its recognition sequence contains a modified residue

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The nearly conserved U54 of tRNA is mostly converted to a version of ribothymidine (T) in Bacteria and eukaryotes and to a version of pseudouridine (Ψ) in Archaea. Similarly, the conserved U55 is nearly always modified to Ψ55 in all organisms. Orthologs of TrmA and TruB that produce T54 and Ψ55, respectively, in Bacteria and eukaryotes are absent in Archaea. Pus10 produces both Ψ54 and Ψ55 in Archaea. Pus10 orthologs are found in nearly all sequenced archaeal and most eukaryal genomes, but not in yeast and bacteria. This coincides with the presence of Ψ54 in most archaeal tRNAs and some animal tRNAs, but its absence from yeast and bacteria. Moreover, Ψ54 is found in several tRNAs that function as primers for retroviral DNA synthesis.

We recently showed (Deogharia et al. 2019. RNA 25:336) that human Pus10 can produce Ψ54 in select tRNAs, including $tRNA^{Lys3}$, the primer for HIV reverse transcriptase. This synthase activity of Pus10 is restricted to the cytoplasm and is distinct from nuclear Pus10, which is known to be involved in apoptosis. Previous crystal structure-based modeling suggested that Pus10 binds the tRNA in the TΨC and acceptor arms. We showed that the sequence GUUCAm^1AAUC (m^1A is 1-methyladenosine) at position 53-61 of the TΨC arm of tRNA along with a stable acceptor stem results in maximum Ψ54 synthase activity. This recognition sequence is unique for a Ψ synthase in that it contains another modification. In addition to Ψ54, SF9 cells-derived recombinant human Pus10 can also generate Ψ55, even in tRNAs that do not contain the Ψ54 synthase recognition sequence. This activity may be redundant with that of TruB.

463 Withdrawn

464 Novel analogues of N3-CMC with enhanced stability for analysis of pseudouridine in cellular RNA

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Pseudouridine formation and ribose methylation are the two most abundant post-transcriptional modifications in cellular RNA. Pseudouridine is found in all major classes of RNA and was found to impact the structure and function of cellular machines, such as the ribosome and the spliceosome. Recent findings of widespread pseudouridylation of eukaryotic mRNA has challenged existing methods for mapping and quantitation based on selective chemical labeling by the carbodiimide CMC. In one strategy, an azido-analogue of CMC was used to enrich for pseudouridylated RNA by biotin-conjugation via click-chemistry and subsequent streptavidin-based pull-down. However, this compound was difficult to synthesize and was highly unstable. We present the synthesis of new derivatives with much improved chemical properties, purity, stability and thus convenience of handling. We provide assays of the compounds by gel-shift experiments and demonstrate their use in standard experiments for detection and enrichment of pseudouridylated cellular RNA.

465 A simple screening system for inhibitors of m6A-regulatory enzymes

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N^6-methyladenosine (m6A) is the most abundantinternal RNA modification, which is mainly found at the RRACH sequence (R: G/A, H: U/A/C). The levels of m6A methylation are controlled by m6A regulatory enzymes, such as FTO and ALKBH5 demethylases or METTL3/METTL14 methyltransferases. The modification regulates various physiological processes including differentiation, tumorigenesis, and virus infection. Therefore, m6A modification can be a new therapeutic target andnew screening methods are required for screening inhibitors of m6A-regulatory enzymes.

MazF is a bacterial toxin involved in growth regulation responded to stress.MazF cleaves single-strand RNA at ACA triplet in a sequence-specific manner.We clarified that MazF is an m6A-sensitive endoribonuclease. By using this m6A-sensitivity, MazF was used to detect both demethylation and methylation activity of m6A-regulatory enzymes. The cleavage of RNA fragment by MazF can be easily detected by FRET-based plate assay. Obtained z'-factors indicated the applicability of this method to inhibitor screening of m6A-regulatory enzymes.

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466 Protein-mediated enrichment and characterization of 5′ end of RNA polymerase III transcripts

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RNA polymerase III (Pol III) is a specialized nuclear enzyme that produces small non-coding RNAs, including transfer RNAs, the 5S ribosomal RNA and the U6 spliceosomal RNA. It is generally accepted that the majority of Pol III transcripts have either triphosphate or monophosphate 5' ends. However, some RNA pol III transcripts are post-transcriptionally modified to generate unusual cap-like structure at their 5' end (*x*ppp5'N, wherein *x* has a non-nucleotide structure such as a methyl group). The detection of 5' terminal nucleotides is typically achieved through a laborious and time-consuming protocol, which includes pre-incubation of cells with radioactive ^{32}P-orthophosphate, fractionation of RNA, digestion to ribonucleotides with a non-specific nuclease, thin-layer chromatography (TLC), and comparative analysis of autoradiograms using known ribonucleotides markers. Other approaches involve the treatment of fractionated RNA with 5' specific enzymes, such as RNA 5′ polyphosphatase and 5′ phosphate-dependent exonuclease. Here, we present a platform for the rapid and unambiguously identification of 5' ends of cellular RNA by mass spectrometry (MS). In this platform, a target RNA is first hybridized to a probe. This probe:RNA duplex is then enriched through binding to the viral protein p19. The p19-bound RNA is partially digested with single-stranded specific ribonuclease to produce a blunted probe:RNA duplex. Finally, the duplex is analyzed by MS, either directly or after digestion to single nucleosides. We demonstrate the potential of this approach by detecting the 5' terminal nucleotides of Pol III transcripts 5S, 7SK and 7SL.

467 Increased endosomal microautophagy suppresses CNS defects caused by loss of ADAR RNA editing in *Drosophila*

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Inosine generated by hydrolytic deamination of adenosine catalysed by members of the ADAR family of RNA editing enzymes (Adenosine deaminases acting on RNA), is one of the most abundant modified bases in mammalian transcripts. Editing occurs site-specifically within coding sequences at intron-exon hairpins as well as promiscuously within longer RNA duplexes formed by Alu and other repetitive elements in introns, in mature mRNA 3' UTR regions and in noncoding RNAs. Mutations in human *ADAR1* cause Aicardi-Goutieres Syndrome, a rare childhood genetic autoimmune encephalopathy that mimics congenital virus infection with increased levels of Type1 interferon (IFN). *Adar1* mutant phenotypes reveal an evolutionarily conserved role for inosine, in self versus non-self discrimination between host RNAs and those of viruses and other parasites. On the other hand ADAR RNA editing of adenosine to inosine likely also mark as self very many host dsRNAs containing repetitive sequences, helping the innate immune system to tolerate these.

Drosophila has a single *Adar* gene encoding the orthologue of vertebrate ADAR2 which edits nervous system transcripts; hundreds of CNS transcripts are edited site-specifically in *Drosophila*. *Adar* mutant flies show reduced viability, uncoordinated locomotion and age-dependent neurodegeneration. We observe aberrant autophagy with enlarged vacuoles, insufficient canonical autophagy suppressible by reduced *Tor* gene dosage and aberrant induction of innate immune transcripts in *Drosophila Adar* mutant heads. *Adar* mutant phenotypes are suppressed by overexpression of the Hsc70-4 chaperone protein that targets proteins containing the KFERQ pentapeptide sequence for endosomal microautophagy (eMI); eMI is particularly important at synapses. Since vertebrate ADAR2 regulates synaptic plasticity by editing glutamate receptors, we believe *Drosophila Adar* also regulates synaptic plasticity, perhaps especially during sleep. A role for ADAR RNA editing in synaptic plasticity and in learning may explain the independent dramatic evolutionary increases in site-specific editing events in advanced insects and molluscs.

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468 HBV hijacks TENT4 to stabilize its RNA via mixed tailing

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Hepatitis B virus (HBV) is a major global health challenge with about 250 million infected individuals and almost 1 million deaths per year. Here we discover that HBV exploits host terminal nucleotidyltransferase TENT4 proteins (TENT4A and TENT4B) to modify HBV RNAs to add mixed tails and enhance viral gene expression. Analysis of RNA tail by TAIL-seq reveals that the HBV transcripts are guanylated much more frequently than cellular mRNAs, which reflects the activity of TENT4. Knockdown of TENT4 results in decrease of tail length, mixed tailing frequency, abundance and half-life of HBV RNAs. By fCLIP-seq, we further find that TENT4 binds to the HBV posttranscriptional regulatory element (PRE) which has been widely used to enhance ectopic gene expression. Consistently, purified TENT4 generates longer mixed tails on RNA with PRE in vitro. A single PRE sufficiently enhances the stability of the reporter mRNA by TENT4-dependent tailing. Thus, HBV RNAs recruit TENT4 through PRE to gain mixed tails which in turn stabilize HBV RNAs. Our study uncovers a key host factor for HBV and demonstrates the importance of mixed tailing in targeted mRNA stabilization.

469 Surveying the landscape of tRNA modifications by combined tRNA sequencing and RNA mass spectrometry

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tRNAs are heavily decorated with post-transcriptional modifications (tRNA modification) that optimize their function. Some modifications are broadly conserved in the three domains of life whereas other modifications are only found in a narrow range of organisms. Profiling an organism's tRNA modification is highly labor intensive and has been accomplished for few species. Thus, a high throughput method to map tRNA modifications would be valuable.

Here, we took advantage of deep sequencing to map tRNA modifications in *Escherichia coli* and *Vibrio cholerae*. In E. coli, where modifications have been well-characterized, we found that more than half of the modified tRNA nucleosides resulted in either premature termination or incorporation of mismatched bases during reverse transcription. We used these RT-derived signatures present in the sequences of cDNAs to indirectly detect many sites of E. coli tRNA modification. tRNA modifications have not been characterized in *V. cholerae*, though many of its modification enzymes are conserved with those of E. coli. RT-derived signatures present in sequences of V. cholerae's tRNAs enabled detection of the predicted conserved modifications. Furthermore, subsequent sequencing-based and chemical analyses of tRNAs isolated from mutant *V. cholerae* strains lacking particular modification enzymes enabled validation of the presence of modifications predicted by tRNA sequencing.

We identified RT signatures in *V. cholerae* tRNAs that were not observed in E. coli's tRNAs, suggesting that *V. cholerae* has species specific modifications. RNA mass spectrometry of V. cholerae tRNAs enabled determination of the mass values of the modified nucleosides. Two of the modifications identified in *V. cholerae* appear to be novel because their mass values do not correspond to known modifications. We also observed differences in the RT signatures of modification in different growth conditions, e.g., log vs stationary phase, suggesting that growth conditions modulate the abundance and/or chemical composition of tRNA modifications.

Collectively, our findings indicate that combining tRNA sequencing with RNA mass spectrometry offers a robust approach for mapping and characterizing tRNA modifications. This approach enables a high throughput means to catalogue modification sites in diverse organisms, track how environmental conditions modulate tRNA modification and provides clues for detection of novel modifications.

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470 A chemical toolbox to study NAD-capped RNAs

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NAD is an enzymatic co-factor with multiple biological functions. Recently, it has been discovered that NAD serves also as a 5'-end-modifying molecular tag in some bacterial and eukaryotic RNAs. The biological roles of this new, fascinating RNA modification remain unraveled, but it has been established that both in bacteria as well as in higher organisms NAD-linked RNAs are targeted by specific hydrolytic enzymes that contribute to RNA turnover. Here, we have designed a set of phosphate-modified NAD analogues that enable synthesis of NAD-linked RNAs with altered susceptibility to selected deNADding enzymes. We report the synthesis and stereochemical characterization of these novel NAD analogues and demonstrate that they are efficiently incorporated into RNA using in vitro transcription method to produce NAD-linked RNAs. The new molecular tools reported here can be applied to study enzymatic deNADding at the molecular level and may provide new insights into the biological roles of NAD-linked RNAs.

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471 RiboMeth-seq analysis of diffuse large B-cell lymphoma cell lines and patient samples

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Cancer cells are addicted to ribosome biogenesis and high levels of translation. Thus, differential inhibition of cancer cells can be achieved by targeting e.g. pol I transcription, pre-rRNA processing or structural differences in ribosomes in cancer cells compared to normal cells. Structural differences comprise post-transcriptional rRNA modifications and the protein composition of the ribosomes. Ribosomal RNA is a well-known drug target in combatting infections and in several cases, post-transcriptional modifications are implicated in target discrimination of drugs. Thus, an alternative to inhibition of rRNA synthesis in cancer would be to target mature ribosomes based on differences in their rRNA modification pattern. We developed a sequencing-based method, RiboMeth-seq, for profiling the ~110 2'-O-Me sites in human rRNA and previously showed distinct profiles in HeLa cervical and HCT116 colon carcinoma cancer cell lines. Here, we focus on cell lines and patient samples from diffuse large B-cell lymphoma (DLBCL), the most common and aggressive non-Hodgkin's lymphoma in adulthood accounting for 30-40% of diagnoses. Treatment of DLBCL is complicated by the fact that it is a heterogeneous diagnostic category comprising different subtypes that probably originate from B-cells at different stages of differentiation.

We demonstrate pronounced hypomethylation at several sites in patient-derived DLBCL cell lines as also noted for other cancer cell lines. The extent of hypomethylation appeared more severe in two representatives of activated B-cell like DLBCL (ABC-DLBCL) compared to two representatives of germinal center B-cell like DLBCL (GBC-DLBCL). Thus, the extent of hypomethylation in cell lines correlates with clinical aggressiveness of the two subtypes. In a comparison of 17 tumor samples from patients to control samples from benign reactive lymph nodes, a distinct pattern of hypo- and hypermethylation was noted at a small fraction of sites clustering in the 5' domain of the SSU and domain VI of the LSU. A correlation with clinical parameters will be presented and the applicability of RiboMeth-seq to diagnosis and for predicting sites for drug targeting will be discussed.

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472 Global reprogramming of poly-(A) tail metabolism in macrophage immune response

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Macrophages are among the most important of the innate immune systems that protect the host from various sources of harm like infection. However, uncontrolled production of pro-inflammatory cytokines by macrophages can cause chronic inflammation in neighboring healthy tissues, which is associated with various inflammatory diseases such as arthritis, and Alzheimer's disease. Understanding the molecular mechanisms controlling macrophage immune response is important because of its potential as a therapeutic target, Previously, it has been reported that transcripts of pro-inflammatory cytokine gene TNF-a underwent an increase in translation and RNA stability upon macrophage activation by lipopolysaccharide (LPS), which was concurrent with increased poly-(A) tail length. However, the transcriptome-wide landscape of poly-(A) tail metabolism reprogramming in macrophage immune response remains largely unknown. We previously developed Tail-end displacement sequencing (TED-seq), a sequencing method that snapshots poly(A) tail length of cellular RNA population at a high resolution and transcriptome-wide. Using this technique, we profiled poly-(A) tail length for 6,120 genes in human macrophage cells activated by LPS. Comparison of TED-seq data between the unstimulated and LPS-treated conditions showed that poly-(A) tail metabolism was unchanged for most of the genes, but altered for a small fraction of genes (7%) upon LPS stimulation in macrophages. These genes were enriched with functions related to RNA binding and innate immune response, suggesting the importance of reprogramming poly(A) tail metabolism in macrophage immune response. Clustering genes based on our time-course TED-seq data revealed that many individual genes had distinct patterns of temporal poly-(A) tail regulation. Although our data showed a positive correlation with Δ poly-(A) tail length and ΔRNA between the unstimulated and the LPS-stimulated macrophage cells, a significant number of individual genes were found to have poly-(A) tail length change regardless of RNA abundance, which suggests that poly(A) tail metabolism can be involved in controlling RNA lifecycles other than RNA level.

473 Chemical synthesis of oligoribonucleotides comprising the trypanosomic cap-4

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Nature's invention of 5' caps was one key feature for the evolution to eukaryotes. A structural characteristics of 5' caps is methylation, with this feature already present in early eukaryotes such as trypanosoma. While the common cap0 (m7GpppN) shows a rather simple methylation pattern, the trypanosoma cap4 displays seven distinguished additional methylations within the first four nucleosides. The study of essential biological functions mediated by these unique structural features of the cap4 and thereby of the metabolism of an important class of human pathogenic parasites is hindered by the lack of reliable preparation methods. Here, we present the synthesis of custom-made nucleoside phosphoramidite building blocks for m62Am and m3Um, their incorporation into short RNAs, the efficient construction of the 5' to 5' triphosphate bridge to guanosine using a solid phase approach, the selective enzymatic methylation on position N7 of the inverted guanosine, and enzymatic ligation to generate trypanosomatid mRNA in the 10 to 40 nt range. This study introduces a reliable synthetic strategy to the much-needed cap4 RNA probes, using a combination of chemical and enzymatic steps.

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474 Profiling snoRNA Essentiality in Lung Cancer via a Targeted CRISPR Knockout Screen

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Small nucleolar RNAs (snoRNAs) are a class of evolutionarily conserved non-coding RNAs responsible for guiding the chemical modification of ribosomal and spliceosomal small nuclear RNAs with pseudouridine and 2′-O-methyl ribose. Individual snoRNAs have been suggested to act as oncogenes in diverse cancer types, with overexpression driving hyperproliferation, increased cell migration, and metastasis. However, the mechanism by which these snoRNAs exert their effects are currently unknown. It is also unclear which snoRNAs are necessary for cancer cell proliferation. To address these questions, we performed a CRISPR-Cas9-based genetic screen to systematically profile the essentiality of all annotated snoRNAs in the H460 human non-small cell lung cancer (NSCLC) cell line. In contrast to budding yeast, which tolerates deletion of most individual snoRNAs, our screen identified dozens of snoRNAs whose depletion leads to significant defects in cellular proliferation similar in magnitude to that of essential protein-coding genes. Low-throughput knockout of individual snoRNA hits from the screen showed six- to twelve-fold reductions in proliferation, validating the requirement of these individual snoRNAs for cell viability. Intriguingly, several snoRNAs identified as essential are members of paralogous families predicted to guide the same modification site on ribosomal and/or spliceosomal RNAs. These data suggest that individual snoRNA family members may function outside of their canonical targets, potentially guiding modification of mRNA or other RNA classes. Ongoing efforts aim to identify the targets and elucidate the molecular mechanisms underlying the requirement for certain snoRNAs in lung cancer cells

475 A high resolution A-to-I editing map in the mouse identifies editing events controlled by pre-mRNA splicing

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Pre-mRNA-splicing and adenosine to inosine (A-to-I) editing occur mostly co-transcriptionally. During A-to-I editing a genomically encoded adenosine is deaminated to inosine by adenosine deaminases acting on RNA (ADARs). A-to-I editing affects various cellular processes ranging from the recoding of transcripts to masking RNAs to the innate immune system. ADARs bind and edit double-stranded RNAs. Editing-competent stems are frequently formed between exons and introns. Consistently, studies using reporter assays have shown previously that splicing rates can affect editing levels.

Here we use nascent RNA-seq and identify ~90,000 novel A-to-I editing events in mouse brain transcriptome. The majority of novel sites are located in intronic regions. Unlike previously assumed, we show that both ADAR1 and ADAR2 can edit repeat elements and regular transcripts to the same extent. In agreement with the notion that RNA editing levels are influenced by the stability of exon-intron boundaries we find that inhibition of splicing primarily increases editing levels at hundreds of sites, suggesting that reduced splicing efficiency can lead to an increased exposure of intronic and exonic sequences to ADAR enzymes. Lack of splicing factors NOVA1 or NOVA2 changes global editing levels, demonstrating that alternative splicing factors can modulate RNA editing. Finally, we show that intron retention rates correlate with editing levels across different brain tissues. We therefore demonstrate that splicing efficiency is a major factor controlling tissue-specific differences in editing levels.

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476 Detection of 5-carboxylcytosine in RNA

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5-carboxylcytosine (5caC) is a recently identified rare RNA modification. In murine RNA, 5caC mostly occurs in messenger RNA, although its function and location in sequence is still unknown. In DNA, 5caC is known to be formed through an active demethylation pathway, in which 5-methylcytosine (5mC) is oxidized by TET enzymes to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine and finally to 5caC. There is in vitro evidence that TET enzymes are able to perform the oxidation of 5mC in RNA as well, suggesting that RNA may share the same demethylation mechanism. Although functions of 5mC and its oxidized forms in RNA are still largely unknown, it is thought that they might affect RNA stability and translation. For example, it has been demonstrated that 5-hydroxymethylcytosine can favor mRNA translation in *Drosophila*. Low amounts of 5caC hampers its detection in nucleic acids and thus, the functions and distribution of 5caC in RNA remains largely unexplored. We searched for possibilities to detect 5caC containing RNA using affinity enrichment and chemical derivatization methods.

477 Modulation of Serotonin 2C Receptor RNA Editing by Alterations in Energy Balance

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The 2C-subtype of serotonin receptor ($5HT_{2C}$) has been implicated in a number of human psychiatric and behavioral disorders, including major depressive disorder, dysthymia, obsessive-compulsive disorder, anxiety, and schizophrenia. Despite these numerous roles in behavior, the best characterized function for this receptor involves an anorexigenic response mediated by $5HT_{2C}$ receptors expressed in pro-opiomelanocortin (POMC)-producing cells in the arcuate nucleus of the hypothalamus to maintain energy homeostasis. Transcripts encoding the $5HT_{2C}$ receptor can be differentially modified by adenosine-to-inosine (A-to-I) RNA editing, generating up to 24 protein isoforms that differ in G-protein coupling efficacy and constitutive activity. Widespread disruption of normal $5HT_{2C}$ RNA processing patterns alters feeding and energy homeostasis in mice. Previous attempts to examine dynamic changes in RNA editing profiles from bulk tissue samples have largely proven futile, as many of these studies relied upon population-averaged assays that failed to capture the functional and transcriptional heterogeneity of unique subpopulations within complex neuronal networks. As the hypothalamus comprises a number of distinct $5HT_{2C}$-expressing cell populations, not all of which are involved in the regulation of energy balance, ensemble measurements have the potential to mask biologically meaningful changes in $5HT_{2C}$ RNA processing occurring in small neuronal subpopulations. Accordingly, we hypothesize that experience-dependent alterations in $5HT_{2C}$ RNA processing occur only within those $5HT_{2C}$-expressing cells directly involved in modulating relevant responses. To investigate whether chronic manipulation of energy balance can dynamically modify $5HT_{2C}$ RNA processing profiles as part of a cellular strategy to refine serotonergic signaling we have used a genetically-engineered mouse model to enrich for $5HT_{2C}$ RNAs expressed selectively in POMC neurons. Here we describe the effect of metabolic perturbations such as high-fat diet, calorie restriction, and exercise on $5HT_{2C}$ RNA editing, specifically in POMC neurons. These studies provide insights into the interplay between $5HT_{2C}$ RNA editing and energy homeostasis.

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478 Theoretical pKa Calculations of Modified Purine Nucleobases using Explicit Waters and a Polarizable Continuum Model

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There are numerous modified nucleobases, which are believed to play roles in fine-tuning structure and function of various RNA molecules. Modifications of nucleobases have a wide range of effects including participating in regulation of expression and response to stress in the cell. Despite a growing wealth of knowledge, specific details about modifications are still not known. The pKa value is a defining characteristic of nucleobases, which is used to understand base-pairing energetics of modified nucleobases. Purine nucleobases and their modifications contain a minimum of two protonation/deprotonation sites that have unique pKa values. Modifications on nucleobases affect the pKa values, resulting in a change of nucleobase behavior. In this study, we determined the pKa values of 19 modified purine nucleobases by performing calculations with the Gaussian 16 program using the B3LYP/6-31+G(d,p) method with a combination of implicit-explicit solvation. This method involved the use of explicit water molecules forming hydrogen bonds with the nucleobases surrounded by an implicit solvation field. The pKa values were calculated from the change in the free energy between protonated and deprotonated forms of the nucleobases and the energy of the solvated proton. By using this approach, we have determined that a single modification can change the pKa value of protonation/deprotonation site on a purine nucleobase by as much as 4.0 units when compared to the unmodified purine nucleobase.

479 Role of the RNA modification 5-methylcytosine on ribozyme catalytic activity

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RNA modifications are present in all three branches of life and are concentrated on non-coding RNAs. Despite their widespread presence, the functional role of RNA modifications remains poorly understood largely because only a few such RNA modifications and target RNAs have been studied. The most famous example is tRNA, where correct codon recognition and endonuclease cleavage are regulated by the RNA modification 5-methylcytosine (5mC). 5mC is a cytosine modification on the 5-carbon position, facing away from the Watson-Crick interface and therefore not affecting base-pairing. The functional understanding of 5mC on RNA is largely limited to tRNA biology. In order to gain a better general understanding of the function of 5mC on RNA we require additional models of 5mC modified RNA.

We have identified RNase:MRP as a novel 5mC modified RNA. RNase:MRP is an essential catalytic RNA required for both the degradation of Cyclin B mRNA during the cell cycle and the correct processing of rRNA. RNase:MRP exhibits unusual features for a modified RNA. First, the modification is concentrated in a region of 6 successive cytosines, in contrast to the singly modified cytosines in tRNAs. Secondly RNase:MRP shows a high degree of modification where all 6 cytosines are modified almost completely with 5mC. Both the high density and the high degree of modification, in combination with the location of the modification in a catalytically active region, create a powerful new system to address the role of 5mC on RNA.

To study the relationship between RNA function and RNA modification, and assess the phenotypic effects in vivo we have introduced mutations in the 5mC modified region of Drosophila melanogaster RNase:MRP. Currently we are developing tools to add and remove 5mC in a site specific manner, and aim to find the RNA methyl transferase responsible for depositing 5mC on RNase:MRP RNA.

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480 Non-canonical poly(A) polymerase TENT5C regulates immunoglobulins expression and B cells differentiation

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Cytoplasmic polyadenylation was mainly studied in the context of gametogenesis and local protein synthesis in neurons. In both instances, it is restricted to deadenylated dormant mRNAs. We have recently described a novel family of non-canonical cytoplasmic poly(A) polymerases TENT5 (FAM46). One of the members, TENT5C, is an endoplasmic reticulum-associated ncPAP with broad mRNA substrate specificity which has been shown as growth suppressor of multiple myeloma a cancer of terminally differentiated B cell.

Herein, we show that TENT5C expression is highly upregulated in naïve B-cells upon stimulation of specific Toll-like receptors (TLR) and its deficiency accelerates naïve B lymphocytes growth and differentiation into CD138+ positive plasma cells in vitro. In agreement with this TENT5C KO mice have increased spleen mass and an elevated amount of spleen-residing plasma cells.

To define endogenous TENT5C mRNA substrates in responding B cells we applied in-lab modified Nanopore direct RNA-sequencing approach and measured global poly(A) tails length in WT and TENT5C KO primary cells. This allowed us to define immunoglobulins (Ig) mRNAs as primary TENT5C substrates as its deficiency leads to decreased median poly(A) tail lengths of those mRNAs from 83 to 67 nucleotides. Further analyses revealed that such relatively minor changes in poly(A) tail lengths lead to reduced Ig mRNA steady-state level and decreased protein production efficiency. Consequently, KO B-cells stimulated in vitro as well as bone marrow and splenic plasma cells from TENT5 KO mice secrete less immunoglobulins and subsequently have reduced ER size and ER-stress level.

Summing up, our findings increase knowledge about B cell physiology and reveal first ncPAP enzyme directly involved in immunoglobulins mRNAs polyadenylation; thus TENT5C is a new regulator of the humoral immune response. More broadly speaking our results change the view on cytoplasmic polyadenylation which according to our data is not restricted to deadenylated stored mRNA.

481 Effects of A-to-I editing on RNA structure, dynamics and molecular recognition

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The conversion of adenosine to inosine (A-to-I editing) in RNA provides an important post-transcriptional regulation mechanism involved in various cellular processes. A specific set of enzymes called Adenosine Deaminases Acting on RNA (ADARs) catalyses the deamination of the exocyclic N6 amino group in adenine to form the non-canonical residue inosine, thereby increasing transcript diversity as well modulating a variety of cellular events, such as alternative splicing and the innate immune response.[1]

However, the structural consequences that result from A-to-I editing are poorly understood and not well studied . In the context of double-stranded RNA (dsRNA), A-to-I editing causes deviations from A-form geometry, as well as internal dynamics. Furthermore, a pronounced thermodynamic instability of IU basepairs has been reported.[2]

We employ site-specific isotope labelling with a 20 nt long dsRNA containing a hyper-edited motif with four IU mismatches to perform NMR studies that aim to further elucidate the conformation, as well as the dynamics of IU basepairs at atomic resolution. Intitially, we focus on the hydrogen bond patterns of the IU basepair in comparison to AU and GU basepairs also using recently developed solution-state NMR methods.[3] NMR will then be used to determine the solution structure of the inosine-containing model RNA and characterise its conformational dynamics from ns to μs timescales. We are also studying how proteins specifically recognise inosine-containing transcripts in different aspects of gene regulation.

References

[1]Eisenberg, E., and Levanon, E.Y., A-to-I RNA editing-immune protector and transcriptome diversifier. Nature Reviews Genetics (2018): 1.

[2]Dallmann, A., et al., Site‐Specific Isotope‐Labeling of Inosine Phosphoramidites and NMR Analysis of an Inosine‐Containing RNA Duplex. Chemistry-A European Journal 22.43 (2016): 15350-15359.

[3]Dallmann, A., et al. Efficient detection of hydrogen bonds in dynamic regions of RNA by sensitivity‐optimized NMR pulse sequences. Angewandte Chemie International Edition 52.40 (2013): 10487-10490.

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482 ADAR1 regulates early T cell development via MDA5-dependent and -independent pathways

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ADAR1 is an RNA editing enzyme that converts adenosine to inosine in double-stranded RNA (dsRNA) and is highly expressed in thymus. We have recently reportedthat deletion of *Adar1* specifically from T cells at the double-positive (DP: $CD4^+CD8^+$) stage in mice impairs negative selection, which results in abnormal thymic T cell maturation and autoimmunity without reduction in the number of thymocytes. These abnormalities are rescued by concurrent deletion of MDA5, a cytosolic dsRNA sensor, which suggests that ADAR1-mediated RNA editing is required for the establishment of self-tolerance during the late stage of T cell development by preventing MDA5 sensing of endogenous dsRNA as non-self. However, the role of ADAR1 at the early stage of thymic T cell development remains unknown. Here, we demonstrate that the mutant mice in which *Adar1* is deleted at the double-negative (DN: $CD4^-CD8^-$) stage reveal severe thymic atrophy with excessive apoptosis and loss of the expression of T cell receptors (TCRs), which inhibit the DN3-to-DN4 transition. Intriguingly, concurrent deletion of MDA5 ameliorates apoptosis but not restores TCR expression. In contrast, forced expression of TCRs on thymocytes rescues transition from DN3 to DN4, revealing the contribution of ADAR1 on early T cell development in a MDA5-dependent and -independent manners. In accordance, we find that TCR transgene and concurrent knockout of MDA5 synergistically ameliorates the defects of T cell development in mice lacking ADAR1 expression at DN stage. These findings suggest that early stage of thymic T cell development requires not only ADAR1-mediated prevention of MDA5 activation to avoid excess apoptosis but also MDA5 pathway-independent ADAR1 function underlying the expression of TCRs.

483 Bacterial ribosomal RNA modification - functional orphans

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Ribosomes contain post-transcriptional modifications around the functional centers in its RNA component. In spite of that, single omission of nucleotide modification does not exhibit significant phenotypic effects in most cases1. Even deletion of multiple genes responsible for rRNA modification has little phenotype. An example is deletion of all 7 rRNA pseudouridine synthase genes in E. coli without significant defect in translation process2. This lack of phenotype has complicated functional analysis of rRNA modifications.

Here we use an alternative approach. We have constructed an E. coli strain lacking 10 genes encoding enzymes that modify 23S rRNA around the peptidyltransferase center. This strain (del10) is able to grow slowly only under optimal conditions. Reintroducing individual modifications allows to evaluate their role in vivo and in vitro. In the absence of other modification each single modification contributes significantly to cell growth and to ribosome functioning. The del10 strain contains large fraction of free ribosome subunits suggesting ribosome assembly defect. Ribosome large subunit activation assay was devised to define ribosome assembly status of free 50S subunits. It was possible to activate ribosome large subunit precursor particles lacking specific modifications in vitro. Ribosome large subunit catalytic activity was determined by rapid peptide bond formation assay using puromycin as an acceptor. The results demonstrate that specific rRNA modifications are needed for efficient ribosome subunit assembly. An overlapping set of modifications strongly stimulate peptide bond formation on the ribosomes. The results have implications to the evolutionary origin of rRNA modifications, traits that often possess only negligible phenotypic effect.

1. Sergiev PV, Aleksashin NA, Chugunova AA, Polikanov YS, Dontsova OA. (2018) Structural and evolutionary insights into ribosomal RNA methylation. Nat Chem Biol. 14, 226-235. Review.

2. O'Connor, M., Leppik, M. and Remme J. (2018) Pseudouridine-Free Escherichia coli Ribosomes. J. Bacteriol. 200:e00540-17.

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484 Loss of 5-methylcytosine (m5C) alters the biogenesis of non-coding Vault-derived small RNAs to coordinate human epidermal differentiation

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The presence and absence of RNA modifications regulates RNA metabolism by modulating the specific binding of writer, reader, and eraser proteins. For most RNA modifications, including 5-methylcytosine (m5C), it is however largely unknown how they recruit or repel RNA-binding proteins. Here, we set out to decipher the consequences of m5C deposition into the highly abundant non-coding vault RNA VTRNA1.1. We reveal that methylation of cytosine 69 in VTRNA1.1 occurred frequently in human cells, was exclusively mediated by NSUN2, and determined the processing of VTRNA1.1 into small-vault RNAs (svRNAs). Using quantitative mass spectrometry, we identified the serine/arginine rich splicing factor 2 (SRSF2) as a novel VTRNA1.1-binding protein that counteracted VTRNA1.1 processing by binding the non-methylated form with higher affinity. We further reveal that both NSUN2 and SRSF2 are required to orchestrate the production of distinct svRNAs via a nuclear RNA processing machinery. Using skin cells as a model system, we identified a functional role of svRNAs in regulating the epidermal differentiation programme. In summary, our data reveal a direct role for m5C in the processing of VTRNA1.1, a pathway that is regulated by SRSF2 and crucial for efficient cellular differentiation.

485 Multispecies conservation of tRNA modification mediated oxidative stress responses

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Transfer RNAs (tRNAs) are richly decorated with evolutionary conserved post-transcriptional nucleoside modifications that are essential for rapid adaptation to environmental stresses. Previous studies on selected microbes have shown that oxidative stress-induced changes in tRNA modification profiles favour the translation of stress response pathway proteins. However, it is not known whether tRNA modifications in evolutionary distinct species will display similar changes in response to identical stress conditions. Considering the significant conservation between nucleoside modifications and their biosynthesis among unicellular organisms, we hypothesize that key tRNA modifications might undergo comparable changes in abundance with similar functional implications, such as facilitating the expression of stress response proteins. To this end, we use mass spectrometry to quantitatively determine the temporal dynamics of the complete tRNA modificome following induction of oxidative stress in representative unicellular model organisms from prokaryota, archaea, and eukaryota. The follow-up correlation of nucleoside modification and translation changes will create a detailed characterization of tRNA-mediated translational responses and identify key regulatory modifications on specific tRNA species. This study constitutes the first consolidated approach towards multispecies tRNA modificome characterization spanning all kingdoms of life, expanding our understanding of the evolutionary interplay between tRNA modification and translational regulation.

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486 Hierarchical modification of tRNA by TrmA, TrmB, and TruB

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Approximately 10% of the nucleosides within tRNA are post-transcriptionally modified by dedicated enzymes costing all cells significant energy. These modifications play a variety of roles during translation and increase tRNA stability within the cell. Each tRNA is differently modified; however, every *Escherichia coli* tRNA contains 5-methyluridine (m5U) 54 and pseudouridine (Ψ) 55. These modifications are introduced by the enzymes TrmA and TruB, respectively. Additionally, several tRNAs contain 7-methylguanosine (m7G) 46, which is catalyzed by TrmB.

Despite the abundance and importance of tRNA modifications, we are still lacking an understanding of how the actions of several tRNA modification enzymes are orchestrated during tRNA biogenesis. We hypothesize that these enzymes are likely to modify tRNA in a preferential order rather than a stochastic fashion. To test this, we prepared *in vitro* transcribed tRNAs that contain only one modification using purified TrmA, TruB, or TrmB modification enzymes. The single-modified tRNAs were systematically used in tRNA binding and modification assays with the other enzymes in comparison to unmodified tRNAs. Here, we report that TruB prefers to bind and modify unmodified tRNA over tRNAs containing already other modifications. In contrast, TrmA prefers to bind tRNA that already contains the Ψ55 modification. Unlike TruB and TrmA that universally modify all tRNAs, the methyltransferase TrmB does not have a preference for either modified or unmodified tRNA.

Taken together, our results suggest that TrmA and TruB likely act relatively early during tRNA maturation, whereas TrmB is likely to act later in this process. Next, we will expand this research to other tRNA modification enzymes to provide insight into the orchestration of several enzymes on a common substrate. Thus, this research lays the foundation to uncover how and why tRNAs are so highly modified.

487 Withdrawn

488 Translation and stability studies of mRNAs with enzymatically modified 3' ends

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Structures present on both ends of mature eukaryotic mRNAs are essential for the functionality of this molecule. N7-methylguanosine bound to the first transcribed nucleotide by the 5′-5′ triphosphate bridge is called cap structure. It is responsible for the protection of the 5′ end from nucleolytic degradation, but also for mRNA synthesis, nucleocytosolic transport and efficient translation. On the other hand, 3′ end of mRNA is decorated with a sequence of adenosines, serving as a binding platform for proteins stabilizing mRNA and forming translation initiation complex. Selected chemical modifications incorporated into 5′ cap structure improve its resistance against decapping machinery. Moreover, translation efficiency of mRNA capped with modified structures is also increased [1], [2]. Based on that we assume similar properties could be achieved by the incorporation of ribonucleotide analogues into untranslated mRNA regions, such as poly(A) tails.

The aim of this research is to set-up the enzymatic methods which allow for incorporation of ribonucleotide analogues into mRNA untranslated regions. We biochemically characterized the properties of modified transcripts according to changes in the stability and influence on translation efficiency. We also investigated RNA polymerases substrate preferences with regard to various ATP analogues.

References:
[1] Kowalska et al., Nucleic Acid Research 2014, 42 (16), 10245-10264.
[2] Su et al., RNA 2011, 17: 978-988.

489 mRNA cap 2′-*O*-ribose methylation regulates translation required for reward learning in *Drosophila*

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Modifications of nucleotides in mRNA expands the repertoire of post-transcriptional regulation and adds a novel dimension due the potential for dynamic and epigenetic control. Dynamic methylation in mRNA occurs at cap adjacent nucleotides on the ribose and internally most frequently on adenosine (m6A), cytidine (m5C) and on the ribose. While recent technological progress has allowed for determining topographic maps of modifications, it remains largely unknown how mRNA modifications impact on gene expression and what their biological functions are.

Here, we use a Drosophila model to determine the function of cap methyl transferases (CMTrs) that methylate the first and second cap adjecant nucleotides on the ribose. Intruiguingly, CMTrs act redundantly in 2'-O-ribose methylation and flies with a knockout of all CMTRs are viable. However, these flies display a reduced tolerance against heat-stress and have learning disabilities. Since a hallmark of learning and memory involves translational regulation, we determined the impact of 2'-O-ribose methylation on translation by monitoring puromycin incorporation and by polysome profiling. We identified specific sets of genes whose expression is under control of cap 2'-O-ribose methylation. Since CMTrs localize to sites of transcription, cap 2'-O-ribose methylation provides a novel mechanism to adjust protein production at the very beginning of gene expression.

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490 NAD-RNA and its function in virus-infected cells

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In recent years, only a few new RNA modifications have been discovered, one of them is nicotinamide-adenine dinucleotide (NAD) found in prokaryotes, Saccharomyces cerevisiae, and human cells. It was proved that NAD is covalently bound to the small regulatory RNA and short fragments of processed mRNA in prokaryotic cells. In Saccharomyces and human cells, NAD was found to be attached to a broad spectrum of mRNA and snoRNA.

Nevertheless, its function remains unknown. Thought the experiments showed that the presence of NAD at the 5' end of the RNA increased its stability in prokaryotes, in human cells the NAD-RNA was degraded more rapidly compared to RNA without a classical cap. We envisaged that the viruses, thanks to their simple intrinsic structure, could serve as suitable model systems for the understanding of the roles of various RNA modifications such as NAD. To reveal the role of NAD-RNA, we focus on small RNA isolated from the cells infected by two types of viruses: HBV and HIV1.

Infected and non-infected cells were harvested and fractions of short RNA were isolated. Afterwards, we applied the NAD captureSeq protocol on isolated RNA to identify the RNA molecules with attached NAD.

491 Alkylation Repair And Demethylation Of RNA By The ALKBH3/ASCC Complex

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Alkylation chemotherapy is one of the most widely used forms of systemic therapy for cancer. Yet its efficacy is limited by cytotoxicity to rapidly dividing normal cells, risks of secondary tumors, and the development of resistance. Tumor suppressor ALKBH3 is overexpressed in many cancer cells with correlation to poor prognosis and invasiveness. Relevant to its potential as a drug target, it's knockdown or that of its associated helicase partner ASCC in cancer cells reduces cell proliferation, increases apoptosis, and increases alkylation sensitivity. ALKBH3 is a direct damage reversal dealkylase that works on RNA and single-stranded DNA with ASCC, a multifunctional, heterotrimeric helicase complex consisting of ASCC1, ASCC2, and ASCC3 subunits. ALKBH3 can be considered an eraser of endogenous RNA methylation, as it demethylates 1mA, 3mC, and 6mA (m6A) and has been shown to demethylate 1mA in RNA *in vivo*. Intriguingly, the ASCC interactome includes RNA Pol II transcription machinery, transcription termination factors, polyadenylation factors, splicing factors (confirmed in cells), and DNA repair proteins. Inhibition of RNA Pol II elongation similarly reduced ASCC foci formation. Furthermore, pre-incubation of permeabilized cells with RNase reduces these foci. These results suggest that ALKBH3-ASCC is recruited to active transcription sites during alkylation damage. These data, combined with ALKBH3 specificity towards methylated RNAs, imply a persuasive role of ALKBH3-ASCC in RNA repair and regulation. Despite our knowledge of its enzymatic activity, little is known about the substrates of the ALKBH3-ASCC complex in tumor cells, the mechanism by which this repair complex is recruited to sites of damage, how their activities are regulated, and how these ALKBH3-ASCC processes interface with other classic repair pathways. ASCC1 contains a hnRNP K-homology (KH) domain that is a known sequence-specific ssRNA binding motif with a preference for the tetranucleotide sequence C/U-A/C-A/C-C/Uand an RNA ligase-like domain. We hypothesize that ASCC1 targets ALKBH3-ASCC to specific RNA sites and aids demethylation by the ALKBH3-ASCC eraser complex. To understand how the ALKBH3/ASCC complex functions to dealkylate RNA, we have solved the crystal structure of ASCC1 at 2.8 Å resolution and initiated functional studies. Results from these ongoing studies will be presented.

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492 Phylogenetic reconstruction of human RNA methyltransferases

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Background: RNA methyltransferases (RNMTs) play an important role in functional regulation of RNAs, and have thus attracted an increasing interest as potential drug targets. The overall structure of RNMTs is conserved (Rossman fold or SPOUT domain), binding the S-adenosylmethionine cofactor (SAM) as a methyl group donor to the substrate. Although SAM-binding sites are localised at the same position of the fold, the chemistry of the SAM-binding interaction, as well as the SAM-binding residues show an important variation across RNMTs.

Methods: Here we aimed to infer the evolutionary relationships of RNMTs in our genome. By mining public databases, we collected and collated structural and sequence information for human RNMTs to create three datasets for phylogenetic reconstruction: (i) a structural alignment of the conserved Rossman fold SAM-binding domain; (ii) a multiple sequence alignment based on the full RNMT protein sequences; and (iii) a combined structural and sequence-based seed alignment of the binding domain across enzymes. We next used Maximum Likelihood reconstruction to build phylogenies and assessed statistical support of each node using rapid bootstrapping as implemented in RAxML v8.2.

Results: Phylogenetic analyses yielded different tree topologies depending on the dataset used. All phylogenetic trees showed minimal statistical support for the interrelationships of RNMTs, which most likely stems from their extreme evolutionary divergence. Our results unequivocally support that RNMTs are highly variable in both structure and sequence, despite their conserved SAM-binding domain.

Conclusions: To elucidate the phylogenetic affinities of human RNMTs, we assembled a comprehensive up-to-date phylogenetic dataset for RNMTs, accommodating both structural and sequence data. Our study suggests that RNMTs do not share a single origin in the genome, but they may have evolved multiple times independently.

493 Regulation of HIV-1 gene expression by post-transcriptional acetylation of cytidine in viral RNA transcripts

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As obligate parasites, viruses need to navigate a variety of cellular regulatory systems while infecting and replicating in the host cell. Post-transcriptional modifications have recently emerged as an important layer of regulation of viral RNA function. For example, our lab and others have shown that the RNA modification N^6-methyladenosine (m^6A) can enhance the replication of multiple viruses in *cis*, including Human Immunodeficiency virus 1 (HIV-1), Influenza A virus, SV40 and Kaposi's sarcoma-associated herpesvirus (KSHV). Recent reports have revealed the presence of another RNA modification, N^4-acetylcytidine (ac4C) on cellular mRNAs and have shown that ac4C can enhance mRNA translation. Here, we demonstrate that ac4C is present at multiple sites on HIV-1 mRNAs and on the viral genomic RNA. A prominent ac4C site was found on the R region of the long terminal repeats (LTR), which when on the 5' end contains thetrans-activation response (TAR) hairpin that is essential for viral transcription activation; or on the 3' end forms part of the 3'UTR of all viral transcripts, potentially regulating RNA stability or translation. Furthermore, we have found that varying the expression level of the ac4C acetyltransferase NAT10 leads to a corresponding effect on viral infection, an effect that is dependent on the NAT10 RNA binding domain. Our data suggest that HIV-1 has evolved to incorporate ac4C in essential viral gene coding regions and regulatory RNA structures, and that NAT10-dependent ac4C addition enhances HIV-1 replication. We will present the latest developments from this project addressing how ac4C regulates HIV-1 infection, providing us with new potential points of intervention.

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494 Widespread Adenosine-to-Inosine editing of vascular microRNAs alters target gene selection and function during ischemia and angiogenesis

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Adenosine-to-inosine- (A-to-I) editing in the seed sequence of microRNAs can alter their function via altered target gene selection. We recently demonstrated that increased A-to-I-editing of microRNA-487b during ischemia enhances angiogenesis. We examined whether other vascular microRNAs undergo A-to-I seed-editing and how this changes their function. By re-analysing public RNA-seq datasets, we identified a total of 35 vascular microRNAs that are A-to-I-edited in a tissue-dependent manner.

We quantified total expression and A-to-I-editing of the primary microRNAs (pri-miRNA) of these 35 microRNAs in primary human vascular endothelial cells and fibroblasts cultured under normoxic or ischemic conditions. We found that the pri-miRNAs of 10 of the selected vasoactive microRNAs were edited in human vascular cells and that all these editing sites were located in the seed. Pri-miRNA editing and expression levels were cell-type specific and consistently increased under ischemia.

Next unedited versus edited mature microRNA expression was specifically quantified for the highest expressed microRNAs, miR-376a-3p, miR-376c-3p, miR-381-3p, miR-411-5p. For each of these, we found that the edited mature microRNA was expressed and increased under ischemic culture conditions. Consistently, expression of both A-to-I-editing enzymes ADAR1 and ADAR2 increased during ischemia. Mechanistically, in vitro knockdown experiments showed that microRNA editing was only dependent on ADAR1, but both ADARs affect total miRNA biogenesis.

We found that microRNA editing was also significantly upregulated in a mouse ischemia model and in human cardiovascular disease. Specifically, edited mature microRNA expression in vascular tissue increased by at least 5-fold in both patients with intermittent peripheral artery disease (n=6; mild ischemia) and end-stage peripheral artery disease (n=8; critical ischemia) compared to vascular tissue from patients with coronary artery disease patients (n=8; normoxic tissue). Each editing event was predicted to induce a shift in target gene selection, which we validated through luciferase reporter gene assays. Furthermore, we demonstrated editing-specific regulation of endogenous mRNA expression through in vitro microRNA overexpression studies. Finally, we confirmed that for 3 of the 4 microRNAs, editing enhanced in vitro angiogenesis

Our results demonstrate that seed sequence A-to-I-editing of vascular microRNAs is significantly induced by ischemia. The subsequent shift in target gene enhances the microRNA's function in angiogenesis.

495 Elucidation of ADAR1 functions in the Immune Response by using interactome data

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Adenosine deaminases acting on dsRNA (ADARs) are essential for a normal embryonic development and have a role in preventing innate immune response to endogenous dsRNA. ADARs deaminate adenosine to inosine by hydrolytic deamination, known as A-to-I editing. Our group was the first to demonstrate that this editing event in endogenous dsRNA prevents the interferon (IFN) signalling cascades from dsRNA sensors in the cytoplasm: RIG-I and MDA5. In accordance, mice lacking *Adar1* die at the embryonal stage with heightened levels of type-I IFN and widespread apoptosis. In humans, mutations in *ADAR1* cause the autoimmune disorder Aicardi Goutières syndrome-AGS. Most of the AGS mutations were shown to reduce editing activity of the protein, except ADAR1 D1113H. This mutation is in the deaminase domain and it can lead to the perturbation in protein-protein interactions.

To address this, we looked at the ADAR1 interactome under different conditions. For this goals we have prepared a tetracycline-inducible HE239T stable cell line, expressing both isoforms of ADAR1. These proteins were tagged with Strep-tag or BirA at either N- or C-terminus. To further elucidate biological functions of ADAR1p150, cells were treated with type I IFN. In addition, we have induced immune response in HeLa cell line and performed co-IP of ADAR1 to look at the protein complexes formed at the endogenous level. Taken together, until now we have a comprehensive data set of ADAR1 protein complexes with or without induction of immune response; both with IFN and HMW Poly I:C. Our data are consistent between all sets and in agreement with all published interacting proteins of ADARs. In addition to the different protein complexes found to interact with ADAR1 upon immune response, we have discovered that tags at the different terminus influence protein stability and interactions.

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496 Revealing where ribosomal RNA gets oxidized via Mass Spectrometry-generated maps

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Previous research found that ribosomal RNA (rRNA) is oxidized under physiologically relevant conditions of oxidative stress, such as in aging and disease. These oxidative lesions such as 8-oxo-G, 8-oxo-A, 5-HO-C and 5-HOU have been shown to impair translation, by interfering with structural integrity and molecular mechanisms within the ribosome. However, the effect of any rRNA oxidation heavily depends on the exact location of the oxidative lesions within the ribosome [1].

We utilized liquid chromatography-tandem mass spectrometry (LC-MS/MS), as previously established to localize post-transcriptional RNA modifications [2]. These methods and tools were adapted it to identify oxidative lesions in the rRNA in a sequence-dependent manner. We established a workflow for the creation of rRNA oxidation maps, including sample preparation, LC-MS/MS approach, and data analysis. This work resulted in single-nucleotide resolution, qualitative maps of different oxidation states of the *E. coli* ribosome, showing which regions and sites of the rRNA are accessible and vulnerable to oxidation *in vivo*.

Understanding where oxidative lesions accumulate in the rRNA deepens our molecular understanding of how translation fails in age-related diseases. Furthermore, this method is useful for other researchers in need for positional information of oxidation, such as those studying oxidized mRNA, ncRNA, or rRNA of eukaryotes.

[1] J. Willi et al. (2018) "Oxidative stress damages rRNA inside the ribosome and differentially affects the catalytic center" Nucleic Acids Res 46(4):1945-1957

[2] P. A. Lobue et al. (2019) "Improved application of RNAModMapper - An RNA modification mapping software tool - For analysis of liquid chromatography tandem mass spectrometry (LC-MS/MS) data"Methods 156:128-138

497 Identification and Characterization of Pseudouridine Synthases

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Throughout all domains of life, pseudouridine (Y) modifications of tRNAs, rRNAs and snRNAs are among the most abundant RNA modifications. While the function of Ymodifications in other RNA classes has been extensively studied, little is known about the role of Yin mRNA. However, latest findings that alterations in mRNA modifications are associated with multiple human diseases and cancers, together with the therapeutic use of Yand other mRNA modifications recently put the spotlight on the role of modifications in mRNA. In our lab, we concentrate on the identification and characterization of pseudouridine synthases (PUS) and their role in pseudouridinylation of mRNA. For this purpose, we designed, expressed, and purified multiple archaeal and eukaryotic PUS enzymes. We find that while some PUS variants show a high substrate specificity for tRNAs and other known natural targets, several of our PUS enzymes are able to efficiently introduce Yinto various mRNA templates in vitro. We are in the process of determining the substrate features that define PUS binding sites on mRNA. Our research is aimed at understanding the role of YmRNA modifications and the enzymes that are involved in writing the modification.

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498 From retrotransposon genome structure to function: Probing the structure of Ty1 genomic RNA in distinct biological states using SHAPE

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Long terminal repeat (LTR) retrotransposons and retroviruses show pronounced similarity in genome organization and replication strategy. They replicate via an RNA intermediate and insert their double-stranded cDNA copy into the host genome. LTR retrotransposons encode two partially overlapping ORFs: *GAG* and *POL*, but they lack of *ENV* gene required for infectivity.

On the way to understanding the function and replication of LTR retroelements, it is important to examine their RNA structure and RNA-protein interactions. To date, most research focused on the structure of retroviral RNAs, however much less is known about retrotransposon RNA structure. The Ty1 is the most abundant mobile genetic element in the Saccharomyces cerevisiae reference genome and can be considered as a model of retrotransposon and retroviral life cycle. Ty1 exploits cellular machinery to transcribe their integrated DNA copy, and Ty1 transcripts synthetized by RNA polymerase II are capped and polyadenylated. After export from the host nucleus Ty1 RNA plays a dual role in replication. It serves as a template for proteins translation as well as RNA genome, which is ultimately packed into virus-like particles (VLPs) and reverse transcribed. Despite the fact that Ty1 transposition is a rare event, Ty1 gRNA compromises up to~10% of polyadenylated mRNA in haploid S. cerevisiae. This is probably the consequence of the unusually long half-life of Ty1 gRNA compared to yeast mRNAs.

Here we present the structure of the entire Ty1 RNA genome (5.6 kb) at single nucleotide resolution using selective 2'-hydroxyl acylation analyzed by primer extension (SHAPE), high-throughput RNA analysis technology. Similar to HIV-1, Ty1 gRNA is A-rich (35.5%) and SHAPE analysis revealed that more than 50% of nucleotides remain unpaired. Comparison of Ty1 RNA structures in distinct biological states and cellular compartments allowed us to characterize structural motifs critical for Ty1 RNA journey in the cell, translation, packaging or reverse transcription. Based on SHAPE reactivity alteration we identified potential Gag and cellular proteins binding sites in Ty1 RNA.

This study was supported by the National Science Centre, Poland (project no 2016/22/E/NZ3/00426).

499 Nucleobase carbonyl groups are poor Mg^{2+} binders but excellent monovalent ion binders — Simple stereochemical rules stress the presence of K^+ at the rRNA decoding center

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Precise knowledge of Mg^{2+} binding site properties is vital for our understanding of nucleic acid systems. Unfortunately, the PDB, the main source of Mg^{2+} binding sites, contains a substantial number of assignment issues that blur our understanding of the functions of these ions. Here, we surveyed nucleic acid X-ray structures with resolutions ≤ 2.9 Å to classify the Mg^{2+} inner-sphere binding patterns to nucleotide carbonyl, ribose hydroxyl, cyclic ether, and phosphodiester oxygen atoms. We derived a set of "prior-knowledge" nucleobase Mg^{2+} binding sites and report that crystallographic examples of trustworthy Mg^{2+} binding sites are fewer than expected since many of those are associated with misidentified Na^+/K^+. We also emphasize that binding of Na^+ and K^+ to nucleic acids is much more frequent than anticipated. Overall, we provide evidence derived from X-ray structures that nucleobases are poor inner-sphere binders for Mg^{2+} but good binders for monovalent ions. Based on strict stereochemical criteria, we propose an extended set of guidelines designed to help in the assignment and validation of ions directly contacting nucleobase and ribose atoms. When borderline Mg^{2+} stereochemistry is observed, alternative placement of Na^+, K^+, or Ca^{2+} must be considered. Among numerous examples, we describe how the application of simple stereochemical rules helps to question the common Mg^{2+} assignment for the ion located at the rRNA decoding center proximal to G518 and stress the presence of K^+. Although, at first glance, such assignment issues seem unimportant, it must be envisaged that the Mg^{2+} stabilization effect is much greater than that of K^+. Henceforth, a site modeled with one or the other ion behaves differently and affect our perception of the energetics and dynamics of the ribosomal decoding center.

- Auffinger P, D'Ascenzo L, Ennifar E. 2016. Sodium and potassium interactions with nucleic acids. Met. Ions Life Sci. 16: 167-201.
- Leonarski F, D'Ascenzo L, Auffinger P. 2017. Mg^{2+}ions: do they bind to nucleobase nitrogens? Nucleic Acids Res. 45: 987-1004.
- Leonarski F, D'Ascenzo L, Auffinger P. 2018. Nucleobase carbonyl groups are poor Mg^{2+}inner-sphere binders but excellent monovalent ion binders - A critical PDB survey. RNA. 25: 173-192.

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500 Investigating the structure, conformational landscape, and inhibition of the MALAT1 ENE triplex

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An intriguing new class of intramolecular RNA triple helices protects the 3′ end of lncRNA transcripts through a polyA-independent mechanism. High nuclear abundance of the 7-kb oncogenic lncRNA MALAT1 transcript is maintained by an unusually long ENE triplex (element for nuclear expression) containing 10 base triples. Disruption of the ENE triplex downregulates MALAT1 levels and associated oncogenic pathways. Though the ENE triplex has been identified as a potential drug target, the functional protective mechanism and potential modes of triplex inhibition are not well understood.

Within this 94-nt RNA motif, the triplex is positioned between two duplexes resulting in an elongated rod-like structure comprising 29 base stacking interactions. We investigate the roles of these peripheral duplexes and a presumed single-stranded linker to support triplex-mediated protection from degradation. Our results demonstrate that the duplex regions finely tune triplex conformational dynamics to regulate the protective mechanism. We also identify a helical structure within the linker and present data supporting its role in triplex stability and protection. Towards therapeutic intervention targeting this ENE triplex, we performed molecular dynamics simulations to uncover molecular details of putative inhibitory conformations. Finally, our experimental data elucidate diverse structural changes within the triplex induced by bioactive small molecule inhibitors. Overall, our results comprehensively reveal structural and functional hot spots within this triplex that may aid in ongoing efforts to target this triplex for therapeutic development.

501 The role of conformational dynamics in miRNA targeting

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MicroRNAs (miRNAs) regulate messenger RNAs (mRNA) translation and stability. Currently, the main parameter explaining target selection and repression efficiency is the base-pairing between the miRNA and mRNA in the seed region.

Here we show how the intrinsic flexibility of the central, unpaired region of the miRNA-mRNA complex can contribute to target repression.

Using NMR spectroscopy, biochemistry and molecular simulations we describe a single base-pair conformational switch, that increases seed length and promotes co-axial stacking between seed and 3'-supplementary stem, for a prototypical bulged miRNA-mRNA pair. In cells, structure-informed mutations that trap the switch in its excited state, significantly enhance target repression.

We propose that this switch, toggles between an initial "screening" state, characterized by a weak seed pairing, and an "active" state where the seed is stabilized and extended. During the transition, the 3'-supplementary helix re-orients from the N-PAZ towards the N-PIWI interface in human Argonaute 2 (hAgo2), such that a conformation, reminiscent of the one observed for fully base paired DNA guide-target duplexes in prokaryotic Agos, becomes favoured.

Our observations tie together the current understanding of the step-wise miRNA targeting process and unveil the role of the RNA duplex in hAgo2 beyond the seed.

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502 Bridging Solution Experiments and Molecular Simulations to Characterize RNA Folding Dynamics

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RNA molecules are highly dynamic systems and typically realize their specific biological functions through interactions with peculiar molecular partners by adopting a well-defined structural organization. At the microscopic level, the characterization of the mechanistic rearrangements through which they achieve specific tertiary structures thus becomes of central interest to understand their function. In such a scenario, experimental techniques such as fluorescence spectroscopy and small-angle x-ray scattering (SAXS) are the well suited to obtain structural insights. However, since their outcome data are generated as time and ensemble averages and provide low resolution information, achieving an unambiguous interpretation is not always straightforward. As such, integrating the available data with an atomic-level outlook, as provided by molecular simulations, can be of striking support.

In order to properly associate with the ribosomal protein L11 and thus exert its role in protein translation, the GTPase-associated center (GAC), a 58-nucleotide RNA in the 23S ribosomal subunit, must adopt a complex tertiary structure. Recent SAXS experiments conducted on GAC have highlighted its structural flexibility in response to ions of different nature in the buffer solution.[1] In particular, the experimental data identified different degrees of structural compactness, which clearly indicate the existence of peculiar tertiary arrangements, resulting from the presence of the different ions. In order to move a step forward and achieve a comprehensive structural characterization of GAC, we took advantage of molecular dynamics (MD) simulations. Specifically, we used MD simulations and enhanced sampling methods to generate conformations with a broad range of compactness, so as to be able to reproduce experimental spectra associated with diverse ionic conditions. We then integrated simulation and SAXS data to eventually characterize the structural dynamics of GAC at atomistic level.

(1) Welty, R., Pabit, S.A., Katz, A.M., Calvey, G.D., Pollack, L., Hall, K.B. *RNA*, **2018**, 24(12), 1828-38.

503 Sugar puckering resolves kinetic heterogeneity of exon recognition by a group II intron

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Metal ions are essential to mediate specific RNA-RNA interactions. These tertiary contacts stabilize the RNA fold and facilitate RNA catalysis such as splicing where non-coding sequences (introns) are removed from the precursor mRNA. In a complementary way, RNA-DNA interactions assisted by proteins allow mobile introns to reintegrate into new DNA sites. Numerous smFRET studies showed kinetic heterogeneity to be intrinsic to many RNA systems (1). Therein, partial or insufficient occupation of Mg^{2+} binding sites has been discussed as major source of inhomogeneous kinetics.

Here, we use smFRET in combination with global hidden Markov modeling (HMM) and molecular dynamics (MD) as a hybrid approach. In our comparative study, we look at an obligate tertiary contact common to all classes of group II introns: the exon and intron binding site 1 (EBS1/(d)IBS1) with known NMR structure (2). Single-molecule detection gives us access to different subpopulations which show the same FRET efficiency but differ kinetically. A characteristic of such "degenerate" FRET states is their multi-exponentiality. Here, we fully resolve for the first time a degenerated, heterogeneous nucleic acid system using a global HMM on an ensemble of single-molecule FRET trajectories. Interestingly and in contrast to the RNA-RNA contact, the RNA-DNA contact displays homogenous unbinding kinetics. Our all-atom MD simulations show the structural origin of the observed kinetic heterogeneity in a uniform and hybrid duplex. In this way, we found that fast sugar puckering in the heteroduplex relieves molecular strain at the binding interface, which in turn makes the RNA-DNA contact more labile and kinetically homogenous (3).

In the context of group II introns, our study suggests a possible coevolution of intron-encoded proteins to stabilize labile RNA-DNA contacts in the event of an intron invasion. Such cooperative binding, where RNA and proteins act in concert, might be a general mechanism to overcome weak interactions under physiological ionic conditions, thus, at low concentration of divalent metal ions.

Financial support by the Swiss National Science Foundation (RKOS) and the UZH Forschungskredit (FDS and RB) is gratefully acknowledged.

(1) Börner etal. Coord. Chem. Rev. (2016). (2) Kruschel etal. RNA (2014). (3) Steffen and Börner etal. (2019) submitted.

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504 On the role of metal cations in RNA stability and folding: The interplay of binding affinity and kinetics

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Metal cations are essential for RNA stability, folding, and function. Resolving the role of metal cations is challenging experimentally since the resolution is typically insufficient to characterize the exact interactions. Here, we apply molecular dynamics simulations and enhanced sampling techniques in order to contribute atomistic insights into the interactions of metal cations and RNA. For the three most common ion binding sites on RNA, we calculate the binding affinities and exchange rates of eight different mono- and divalent metal cations. Our results show that the different ion binding sites are highly selective: In binding sites on the backbone preferentially bind metal cations with high charge density (such as Mg^{2+}) while the ion binding sites on the nucleobases preferentially bind cations with low charge density (such as Cs^{+}). In addition, our results reveal that the exchange rates for different cations cover more than nine orders of magnitude where the fastest exchange is observed for Cs^{+} (hundreds of picoseconds) and the slowest exchange for Mg^{2+} (hundreds of milliseconds). The different binding affinity and different exchange rates have important consequences for the mechanical properties of RNA or the assembly of individual RNA molecules. In particular, the end-to-end distance, the twisting angle or the RNA-RNA interactions can be controlled by adjusting the salt concentration or the type of counter-ion present in solution.

505 A structure-function analysis of FLT-3 RNA seeking therapeutic targets

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Fms-related Tyrosine kinase 3 (FLT3) is a membrane bound receptor tyrosine kinase that plays a key role in haematopoiesis and is strongly implicated in the development of acute myeloid leukaemia (AML), with around 15-35% of AML patients carrying mutations in this gene. Chemotherapeutic therapies targeting the FLT3 protein exist, however, drug resistance can be acquired through mutations in the amino acid sequence. An alternative target for therapy could be the FLT3 mRNA, which contains conserved, alternative, secondary structures of predicted high stability within its 5' untranslated and N-terminal coding regions, that are likely required for transcription or translational control. We are investigating these structures in vitro using selective 2'-hydroxyl acylation analysed by primer extension (SHAPE) chemistry to visualise the different conformers. We will follow this with analyses aimed at elucidating the functions of each structure. Ultimately, we plan to develop future therapeutic strategies for AML targeting these structures.

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506 RNA Structural Characterization by Small Angle X-ray Scattering

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Small-angle X-ray scattering (SAXS) is a powerful tool for structural characterization of macromolecules including RNA. It allows studying the structure and dynamics of biomacromolecules and their complexes in solution, near physiological environments. SAXS is particularly well suited for RNA because the contrast between RNA and buffer is high due to the electron-rich phosphate backbone in RNA. SAXS could provide information about the molecular size and global shape that outlines backbone topologies. The low-resolution models generated from SAXS data can be compared with homologous crystal or NMR structures. In addition, all-atom models can also be filtered and refined against SAXS data. Furthermore, SAXS data can be used to validate cryo-EM structure and find missing fragments. Lastly, SAXS can also be used to study structure-function relationship and conformation space of RNA. The SAXS Core Facility of Center for Cancer Research (CCR) of National Cancer Institute opens to all intramural and extramural research communities free of charge. The mission of the SAXS Core Facility is to provide support to user communities including expertise in experimental design, data collection, processing, analysis and interpretation. This presentation gives introduction to the SAXS Core Facility of CCR and highlights recent scientific achievements in RNA structure characterization produced by the SAXS Core users.

SAXS Core website: https://ccr.cancer.gov/Structural-Biophysics-Laboratory/SAXS-Core-Facility

507 Visualizing the long non-coding subgenomic flavivirus RNAs in solution

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Most mosquito-borne flaviviruses such as Zika virus (ZIKV), Dengue virus (DENV) and West Nile virus (WNV) produce an abundance of long non-coding subgenomic RNAs (known as sfRNAs) in infected cells that link to viral pathogenicity and immune evasion. Until now, structural characterization of these sfRNAs remains limited. Here, in combination with small angle x-ray scattering (SAXS) and computational modeling, we studied the tertiary structures of individual and combined subdomains and visualized the accessible 3D conformational spaces of complete sfRNAs from DENV2, ZIKV and WNV, respectively, in solution. The individual xrRNA1s and xrRNA2s adopt similar structures in solution as the crystal structure of ZIKV xrRNA1, the xrRNA12s form compact structures with reduced flexibility. While the DB12 of DENV2 is extended, the DB12s of ZIKV and WNV are compact due to the formation of intertwined double pseudoknots in proximity. All 3'SLs share similar rod-like structures. The complete sfRNAs are extended and sample a large conformational space in solution, which may enable their roles in modulating several cellular pathways by interacting with diverse viral and host proteins.

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508 Retrovirus-like transposons genome packaging: Gag function and structural determinants of Ty1 RNA dimerization.

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During replication of retrovirus-like transposons (ReLTs), their proteins and genomic RNA (gRNA) assemble into virus-like particles (VLPs) that are not infectious but functionally related to retroviral virions. Both virions and VLPs contain gRNA in a dimeric form. RNA dimerization is the non-covalent process by which retroelements carry two copies of gRNA into the virion/VLP. Genomic RNA dimerization is believed to be a crucial step of retroviral replication that affects translation of viral proteins, selective packaging of the genome, viron assembly and reverse transcription. Contrary to the infectious retroelements, genome dimerization and packaging processes in ReLTs are poorly understood.

Using structural, mutational and functional analyses, we explored RNA dimerization of Ty1 retrotransposon and interactions with Gag, critical for packaging. Retrotransposon Ty1 of the budding yeast *Saccharomyces cerevisiae* is structurally and mechanistically representative of the ancient family of Ty1/*copia* retrovirus-like elements. We show that, like retroviruses, Ty1 Gag promotes formation of RNA dimers. Applying the SHAPE method (selective 2′-hydroxyl acylation analyzed by primer extension) we map the secondary structure of the Ty1 RNA in dimeric state and identified potential intermolecular interactions sites. Our data inform a model in which two intermolecular PAL1/PAL2 interactions in the 5′ UTR of Ty1 RNA are critical for dimer maintenance and stability *in vitro*. Mutations that disrupt PAL1 - PAL2 complementarity significantly reduced *in vitro* dimerization of Ty1 RNA and Ty1 mobility in yeast. Our results support the critical role of Ty1 Gag not only in RNA dimerization but also in annealing of the initiator $tRNA_i^{Met}$ required for priming reverse transcription. Moreover we propose that the 5′ pseudoknot in Ty1 RNA constitutes a major Gag-binding site. However, the relationship between the pseudoknot and RNA dimerization appears to be indirect. These results expand our understanding of the genome dimerization and packaging strategies utilized by ReLTs.

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509 Exploration of sharply-bent states in RNA two-way junctions using disulfide conformational capture

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Two-way junctions provide a structural context for the formation of tertiary contacts in structured RNAs. Formation of some RNA structures requires sharp bending of two-way junctions into conformations that are less stable than more extended conformations of these junctions. Here, we examined the relative frequency of sharply bent conformations in a variety of RNA two-helix junctions by using a disulfide crosslinking conformational capture technique. We investigated the impact of junction topology (S_xS_y) and ion concentration on bendability and on the conformational preferences of sharply bent states. We find that increasing monovalent ion concentration increases the frequency of sharply bent states, as revealed by the rate of disulfide crosslinking. On the other hand, for some topologies, Mg^{2+} reduces the frequency of sharply bent states, even in high Na^{+} backgrounds. This result is consistent with previous findings demonstrating Mg^{2+}-induced helix stacking of HIV-TAR RNA bulge variants. Interestingly, this Mg^{2+}-dependent stiffening is not observed for the Tetrahymena group I intron internal loop junction J5/5a (S_5S_4), which adopts a highly bent structure in the folded intron. We also monitored conformational preferences within the ensemble of sharply bent states by altering the sites of crosslinking in the helical segments, enabling the capture of distinct bent states. We observe a range of nearly an order of magnitude in the frequency of different bent states, showing that two-way junctions exhibit conformational preferences in sharp bending far from the ground state. The existence of pronounced energy minima far from the ground state gives nature an opportunity to fine tune junctions that must bend far from their globally preferred conformation.

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510 The large Stokes shift fluorogen-activating RNA aptamer *Chili*

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Fluorogen-activating RNA aptamers (FLAPs) have emerged as powerful tools for tagging and visualizing RNA in vitro and in vivo. These artificial functional RNAs form specific, non-covalent complexes with conditionally fluorescent chromophores that are non-emissive while free in solution but show strongly enhanced fluorescence emission in the bound state. A prominent class of fluorogenic aptamers known as mimics of fluorescent proteins, named Spinach, Broccoli, and Corn, were evolved by Jaffrey and coworkers to bind fluorinated analogs of the GFP chromophore 4-hydroxybenzylidene imidazolone (HBI).

An RNA aptamer named Chili mimics large Stokes shift (LSS) fluorescent proteins by inducing highly Stokes-shifted emission from several new green and red HBI analogs that are non-fluorescent when free in solution. The RNA binds the ligands in the protonated phenol form and exploits excited state proton transfer pathways to enable a more than 350-fold enhanced fluorescence emission from the phenolate form of the HBI chromophore. The ligands feature a cationic aromatic side chain for increased RNA affinity and reduced magnesium dependence. Oxidative functionalization of the C2 position of the imidazolone yields an extended positively charged chromophore, which binds to the Chili aptamer with a low-nanomolar KD and exhibits highly red-shifted fluorescence emission at 592 nm. Detailed analyses of the ligand binding site will reveal the structural basis of the proton relay in the RNA responsible for the large apparent Stokes shift of more than 120 nm and are expected to guide the engineering of even brighter LSS aptamer variants and chromophores for applications in vitro and in cells.

511 Structural insights into synthetic ligands targeting A-A pairs in disease-related CAG RNA repeats

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The trinucleotide repeat expansion disorders (TREDs) constitute of a group of more than forty hereditary neurodegenerative human diseases associated with abnormal expansion of repeated sequences, such as CAG repeats. The pathogenic factor is a transcribed RNA or protein whose function in the cell is compromised. The disorders are progressive and incurable. Consequently, many ongoing studies are oriented at developing therapies.

We have analyzed crystal structures of RNA containing CAG repeats in complex with synthetic cyclic mismatch-binding ligands (CMBLs). The models show well-defined interactions between the molecules in which the CMBLs mimic nucleobases as they form pseudo-canonical base pairs with adenosine residues and engage in extensive stacking interactions with neighboring nucleotides. The binding of ligands is associated with major structural changes of the CAG repeats, which is consistent with results of biochemical studies. The results constitute an early characterization of the first lead compounds in the search for therapy against TREDs. The crystallographic data indicate how the compounds could be further refined in future biomedical studies.

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512 Secondary structure of genomic RNA of influenza A virus as an aid in antisense oligonucleotide design.

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Influenza A virus still remain an important factor in the morbidity and mortality of people around the world. WHO notifies that seasonal flu is responsible for up to 650000 deaths yearly. IAV is an enveloped virus with segmented (-) ssRNA genome. All 8 RNA segments are coated with nucleoprotein (NP) and interacts with heterotrimeric polymerase proteins forms ribonucleoproteins. It is known that function of RNA is strictly connected with their secondary and tertiary structure. The function of influenza genomic RNA (vRNA) is limited but most recent studies shows new insight into vRNP complex. Cryo-electron microscopy shows a new look at global 3D vRNP structure. Furthermore high-throughput sequencing of RNA with cross-linking and immunoprecipitation (HITS-CLIP and PAR-CLIP) indicated the profile of NP binding. It shows, that there are region of RNA free of NP, which can form secondary structure and play distinct role on different stages of influenza life cycle. Bioinformatics analysis demonstrated that RNA of IAV can form conserved structural motifs across both (-) and (+) strands. Multiple proposed conserved motifs were confirmed experimentally and several were confirmed as functional by additional experiments. Also, secondary structures of entire segments 7 and 8 vRNAs were experimentally determined in vitro. Herein we show the secondary structure of segment 5 vRNA and the potential of vRNA as antisense oligonucleotides (ASO) target. The secondary structure of segment 5 vRNA was determined based on chemical mapping data, free energy minimization and structure-sequence conservation analysis for type A influenza. In the presented secondary structure model, average base pairs conservation was 87% among all influenza A vRNA sequences in influenza database. Segment vRNA5 has circular folding and contains previously described panhandle motif which is highly conserved. Two other conserved motifs proposed in earlier bioinformatics analysis were confirmed and new are identified. Based on proposed secondary structure and isoenergetic microarray mapping, the antisense oligonucleotides were design. Five ASO inhibit influenza virus life cycle in MDCK cell line more than 40%, one of them lowered virus titer by ~90%. These results showed the importance of secondary structure of vRNA for virus function, as well as new targets for antisense therapeutics.

513 m^{6}A slows down RNA hybridization kinetics from site-specific measurements of NMR relaxation dispersion

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*N*6-methyl adenosine (m^{6}A) is an abundant epitranscriptomic modification found in a wide variety of coding and non-coding RNAs and is proposed to play critical roles in virtually all aspects of RNA metabolism. While it is thought to primarily function by recruiting specific reader proteins, studies also show that it can alter RNA-protein interactions through thermodynamic destabilization of RNA helical elements. Here, by combining NMR relaxation dispersion with optimal melting experiments, we developed a non-invasive approach for measuring nucleic acid hybridization kinetics with site-specific resolution. The methodology is demonstrated on two different DNA duplexes for which two-state behavior was observed across different sites along the DNA duplex. Application of the methodology to two RNA duplexes reveals that while m^{6}A increases the dissociation rate by 1.6-1.8 fold, it decreases the rate of association by an even greater amount of 2.8-3.4 fold. We propose that slow isomerization of the methyl group in m^{6}A between *syn*and *anti*conformations is responsible for the slower association rates providing a potentially new kinetic mechanisms for m^{6}A-specific functions that involve the slowing down of RNA conformational transitions involving base pairing. The new NMR methodology opens the door for site-specifically characterizing hybridization kinetics in a manner that can also resolve potential intermediates.

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514 Structure-based mutational analysis of the twister-sister ribozyme and implications on the cleavage mechanism

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The twister-sister RNA motif belongs to a group of four recently discovered[1] self-cleaving ribozymes that catalyze cleavage of the intramolecular phosphodiester bond in a site-specific manner.[2] The discovery of the new ribozymes has sparked a widespread interest towards an in-depth understanding of the cleavage mechanism of these catalytic RNAs. Here, we present structure-guided mutational analyses based on our previously solved crystal structure of the 4-way junctional twister-sister ribozyme.[3] Eleven conserved and spatially separated loop nucleotides are brought into close proximity at the C-A cleavage site. Comprehensive studies including cleavage assays on key base substitutions, different ribose mutations, and Mn2+ for Mg2+ replacements in the twister-sister construct, have revealed that the interactions between a guanine (i.e. G5) and a hydrated Mg2+ with the non-bridging phosphate oxygens at the cleavage site are important for the cleavage activity.

To gain further insight into the cleavage mechanism of the twister-sister ribozyme, in particular the involvement of G5, mutational studies with chemically modified guanosines are envisaged. To this end, we have focused on 3-deaza-guanine because of the shifted pKa value of its N1-H group compared to the native counterpart. Due to the expected altered interaction between NH-1 of G5 with the non-bridging oxygen of the scissile phosphate, a decrease in cleavage rate is expected, and should provide evidence for the mechanistic impact of G5 on phosphodiester cleavage. Here, we will delineate as well a novel synthetic path towards a 3-deaza-guanosine phosphoramidite for RNA solid-phase synthesis.[4]

References:

[1] Z. Weinberg, P. B. Kim, T. H. Chen, S. Li, K. A. Harris, C. E. Lünse and R. R. Breaker, Nature Chemical Biology 2011, 11, 606-610. [2] R. M. Jimenez, J. A. Polanco, and A. Luptak, Trends in Biochemical Sciences 2015, 40, 648-661. [3] L. Zheng, E. Mairhofer, Y. Zhang, M. Teplova, J. Ma, D.J. Patel, R. Micura and A. Ren, Nature Communications 2017, 8, 1180. [4] E. Mairhofer, L. Flemmich, C. Kreutz and R. Micura, Organic Letters, in revision.

515 Nucleotide level resolution of RNA folding interactions within peptide based complex coacervates

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Understanding how life arose is one of the great questions facing humanity. The RNA World Hypothesis partially addresses this by providing a genetic component as well as an enzymatic one, but does not provide for how RNA-compatible cells arose. A potential RNA-compatible protocell model that has recently garnered attention is complex coacervates. Coacervates are molecule-rich droplets formed via liquid-liquid phase separations (LLPS) primarily by electrostatic interaction of polycationic and polyanionic polymers leading to a reduced water content inside of the droplets. Coacervates have been demonstrated to form with both biotic and abiotic polymers and have also been shown to strongly partition nucleic acids, amino acids and metal ions. As protocells, they have been mostly studied from a physical/chemical point of view; however, there has been little characterization of them with regard to whether RNA catalysis and replication could occur within them.

Herein I describe our early efforts toward characterizing RNA folding interactions at the nucleotide level within complex coacervates made from lysine homopolymers and aspartic acid homopolymers, as well as lysine homopolymers and ATP. Nucleotide-level resolution of the folding status of the model RNA, $tRNA^{phe}$ from *S. cerevisiae*, was assessed by in-line probing. Through this method, a variety of additional coacervates made from both lysine and aspartic acid homopolymers of different lengths were also investigated. Some coacervates promoted native folding of the tRNA, while others led to strand-specific denaturation. The effects on folding also differed based on polymer length and identity. These studies serve as a proof-of-concept that nucleotide-level resolution of RNA folding states within complex coacervates can be obtained and that this technique should be applicable to other functional RNAs including ribozymes.

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516 Structural investigation of RNA-cleaving deoxyribozymes

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DNA enzymes, also known as deoxyribozymes, are synthetic single-stranded DNA molecules able to catalyze chemical reactions. There are two main reasons for studying deoxyribozymes - their practical value in various applications, and the understanding of basic properties - such as folding and catalysis - of a biopolymer that is of central importance for life. Compared to ribozymes, the DNA enzymes have a higher potential value as tools for industrial or therapeutic applications, owing to their easier and more cost-effective synthesis, as well as the higher stability.

The first crystal structure of a deoxyribozyme demonstrated that DNA possesses the intrinsic ability to adopt complex tertiary folds that support catalysis and unveiled the active site of a DNA enzyme in the post-catalytic state (Ponce Salvatierra et al). The second reported crystal structure of an RNA-cleavage deoxyribozyme (H. Liu et al.) further supports the intrinsic properties of DNA strands to adopt complex folds that support catalytic roles.

Although these structures represent a breakthrough in the field, they are insufficient to derive a clear picture of the specific features of deoxyribozymes, with respect to folding and catalytic mechanism. Therefore, we decided to investigate additional RNA-cleaving deoxyribozyme. Some of them are also of interest as tools for the selective inactivation of genes. Here, we show the synthesis, crystallization and preliminary crystallographic analysis of one such deoxyribozyme in complex with the substrate in the pre-catalytic state of reaction.

517 Investigating molecular determinants for *htrA* RNA thermometer behavior

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RNA thermometers are mRNA elements that regulate gene expression through temperature-dependent structural change. They commonly adopt conformations which restrict access to the ribosome-binding site at the non-permissive temperature. A very short RNA thermometer is found in the 5′ UTR of the mRNA for the protease HtrA, an enzyme critical for virulence of pathogenic *Salmonella* strains. At low temperatures, this thermometer forms a hairpin with a stretch of four uridine nucleotides opposing the Shine-Dalgarno (SD) sequence, making it a member of the fourU class of thermometers. A single-nucleotide bulge divides the hairpin into a lower stem (which contains the fourU motif and SD sequence) and an upper stem (which is topped by an unusual five-nucleotide loop).

We have used SHAPE (Selective 2′-Hydroxyl Acylation analyzed by Primer Extension) assays to characterize the *htrA* thermometer of *S. enterica*. Our results reveal that this thermometer melts in the physiological temperature regime (~ 36 ˚C), consistent with infection of a warm-blooded host. Melting curves for individual nucleotides in the stem suggest that the thermometer unfolds in a concerted fashion, with nucleotides in both the upper and lower stems gaining flexibility at a common transition temperature. Removal of the single-nucleotide bulge shifts the melting transition upward by ~ 14 ˚C, well outside of the physiological range.

We then performed detailed SHAPE analysis on a number of mutant sequences designed to examine the molecular determinants for thermometer behavior. We initially probed the role of the three G•U base pairs in the thermometer stem, two involving the SD region and the third immediately above the C bulge. Each of these mutations affected the melting temperature of nucleotides throughout the hairpin, suggesting communication between the two stems of the thermometer. We next examined the loop and the bulge regions of the thermometer. Our studies suggest that these features are also important in fine-tuning the melting temperature of the *htrA* RNA thermometer to the physiological range. Here, we present results from these investigations.

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518 RNA base pairing complexity in living cells visualized by correlated chemical probing

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Accurately determining RNA structure and dynamics is essential for understanding RNA mechanisms and biological function. However, strategies for determining RNA structure *in vivo* are limited. Chemical probing experiments do not observe base pairs *directly* and hence cannot unambiguously define global RNA structure or resolve structural dynamics. Newer duplex detection methods are laborious and have low resolution and poorly benchmarked accuracy. Here we convert the common reagent dimethyl sulfate (DMS) into a useful probe of all four RNA nucleotides. Building on this advance, we introduce a new strategy, PAIR-MaP, that harnesses correlated chemical probing to simultaneously map local RNA structure and directly detect base pairs in a single straightforward probing experiment. Extensive benchmarking on complex endogenous RNAs from *E. coli* and human cells demonstrates that PAIR-MaP has excellent specificity (>90%) and sensitivity (~45%) for directly detecting RNA duplexes in cells. PAIR-MaP visualizes long-range helices and pseudoknots, resolves alternative pairing interactions of structurally dynamic RNAs, and enables highly accurate structure modeling, including of RNAs containing multiple pseudoknots and extensively bound by proteins. Application of PAIR-MaP to human RNase MRP and two bacterial mRNA 5'-UTRs reveals new functionally important and complex structures undetectable by conventional analyses. PAIR-MaP is a powerful, experimentally concise, and broadly applicable strategy for directly visualizing RNA base pairs and dynamics in cells.

519 Preselection for Monomers Increases the Phenotypic Potential of Random Pools: Application to *In Vitro* Selection of Nanoluciferase Aptamers

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The in vitro selection of functional RNAs, aptamers and ribozymes, critically depends on the design and diversity of the starting pools. Various computational and experimental efforts have been extended towards studying the influence of starting pools on selection outcomes, including the length and structure of the random region. A key experiment remains – an experimental measurement of functional potential of randomized pools. We hypothesize that the enrichment of monomers from a starting random RNA pool can increase the phenotypic potential and subsequently improve a selection's outcome. To test this hypothesis, we performed an in vitro selection of RNA aptamers for the Nanoluciferase enzyme from a starting pool comprised of both the a pre-selected structured pool, and a random pool. Our studies show that after 7 rounds of selection, 7 of the top 8 clusters originated from the structured pool, while only one cluster enriched from the random pool. Our selection yielded a conserved stem-loop motif that was selected from the structured pool and was shown to bind to the target protein. In conclusion, our results indicate that enriched folded monomers in a starting pool can improve a selection's outcome to yield functional RNAs.

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520 Crystallization of human and chimp CPEB3 ribozymes

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Ribozymes are catalytic active RNA molecules that are present in all three kingdoms of life. As such they catalyze several crucial reactions like phosphoryl transfer or protein synthesis that are essential for all organisms. Their functional diversity is closely related to their complex three-dimensional (3D) structures, which they can adopt. In 1998, the first crystal structure of the Hepatitis delta virus (HDV) ribozyme was published revealing its complex native fold in a nested double pseudoknot [1]. Until 2006, the HDV ribozyme was considered to be the only representative of a small ribozyme with such a sophisticated fold. However, a genome-wide search identified the human cytoplasmic polyadenylation element-binding protein 3 (hCPEB3) ribozyme as the first HDV-like ribozyme [2]. Since that day, the HDV-like ribozyme family has largely expanded [3]. All members of HDV-like family have several features in common: (i) a double pseudoknot structure (ii) catalytic cleavage activity at 5'-end of the ribozyme and (iii) the presence of a cysteine (C75 – according to HDV nomenclature) in the catalytic core.

To expand the knowledge on these structures, we want to use X-ray crystallography to solve the structures of two representatives from the CPEB3 ribozyme family: the *Homo sapiens*and the *Pan troglodytes*(chimpanzee) ribozymes. Although the two ribozymes differ by only one nucleotide, the cleavage rate is for the chimpanzee 7 fold faster as for the human. Based on the published HDV structures, our main crystallization strategy is to introduce the U1A protein RNA-binding domain (RBD)as a crystallization module. Our results confirm as that the introduction of the RBD into catalytically dispensable segment of the ribozyme does not disturb the catalytic activity of the latest (co-transcriptional cleavage). Moreover, the binding of the U1A protein to engineered ribozymes was confirmed by band shift essay.

[1] Ferré-D'Amaré A. R. & Doudna J.A. Nature, 1998, vol 395, pp.567-574
[2] Salehi-Ashtiani, K. A Genomewide Search for Ribozymes Reveals an HDV-Like Sequence in the Human CPEB3 Gene. Science, 2006, 313, 1788–1792.
[3] Webb, C.H. et al. (2009) Widespread occurrence of self-cleaving ribozymes. Science, 2009, 326, 953

521 Network families of recurrent long-range interactions modules in RNA structures reveal embedded hierarchies

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The complexity of RNA 3D structures is achieved by large amount of nucleotide base pairs combinations. They form complex structures that are modulated by sophisticated interactions of various kinds. These structures are essential for molecular functions as ligand or protein binding.

The ensemble of configurations of the distinct structural elements, hairpins, bulges, interior loops and multi-loops, have been much analyzed and catalogued through known 3D structures. The next layer of complexity arises from the sets of highly organized long-range tertiary interactions that connect those local sub-structures. While some sets of interconnected sub-structures have been extensively described, like pseudo-knots and A-minor interactions, much is left to understand about their general topology.

To fill this gap in the understanding of the variety of functional structures, we developed a de novo data-driven approach to extract automatically from large data set of full RNA 3D structures the recurrent interaction networks containing long-range contacts. Our methodology enables us for the first time to detect the interaction networks connecting distinct components of the RNA structure, highlighting their diversity and conservation through non-related functional RNAs.

We model them as graphs to perform pairwise comparisons of all RNA structures available and to extract recurrent interaction networks and modules. Our analysis yields a complete catalogue of RNA 3D modules available from the structures in the Protein Data Bank and reveals the intricate hierarchical organization of the RNA interaction networks and modules. We assembled our results in an online database (http://carnaval.lri.fr)[1], which can be downloaded and will be regularly updated.

Additionally, we provide a tool that allows users to submit a novel RNA structure and automatically identify whether that novel structure contains previously observed recurrent interaction networks.

[1] V. Reinharz, A. Soulé, E. Westhof, J. Waldispühl and A. Denise. Mining for recurrent long-range interactions in RNA structures reveals embedded hierarchies in network families. Nucleic Acids Research, 46 (8), 3841-3851

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522 Structural and functional analysis of SINEUPs antisense non-coding RNA for mRNA-specific translation up-regulation

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SINEUPs are antisense non-coding RNA (ncRNA) which positively regulate the target mRNA protein expression at the post-transcription level. The binding domain (BD) of SINEUPs is complementary to the 5′ end of the target protein-coding mRNA and provides specificity while the non-overlapping effector domain (ED) enables the translation enhancement activity. The action of SINEUPs ED is mediated by an embedded SINE (short interspersed nuclear element) repeat. However, SINEUPs had proven efficient against a variety of targets in a wide range of human and mouse cell lines and also in *in vivo* system [Carrieri *et al.*, Nature 2012, 491 (7424); Zucchelli *et al.*, Front Cell Neurosci. 2015, 9:174; Indrieri *et al.*, Sci Rep. 2016, 6:27315], much has yet to be explored to improve the understanding of their mechanism of action and functional features.

Here, we present our latest work on key sequence and structural features of SINEUPs. Previously, we identified more than 30 natural antisense transcripts (NATs) in mouse FANTOM3 cDNA dataset as potential SINEUP candidates [Carrieri *et al.*, Nature 2012]. We report the functional validation of a number of diverse SINE sequences isolated from some of these NATs which were tested in synthetic SINEUP-GFP in human cells. We found that despite high sequence variation, these SINE sequences up-regulate GFP translation suggesting the abundance of SINEUPs in nature and possible involvement of RNA structure in SINEUP function. To further investigate the RNA secondary structure of SINEUPs inside cells, we performed modified icSHAPE (*in vivo* click selective 2′-hydroxyl acylation and profiling experiment). We found that local short motifs of partially conserved sequence and their combinations rather than a conserved global structure are important for SINEUPs function. The data indicates combinatorial nature of RNA motifs and possible involvement of RNA-binding proteins, higher order structures, and surrounding sequence elements in SINEUPs-mediated gene regulation.

523 Structural variants of RNA Mango enable split aptamer designs for RNA-RNA interactions

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RNA Mango I is a bright and genetically encodable aptamer-fluorophore complex. It can be useful both in vivo and in vitro as it is non-toxic and cell membrane permeable. RNA Mango I has a very high affinity for thiazole orange derivatives, TO1-biotin (TO1-B) and TO3-B, and has a Kd in the low nanomolar range (approximately 3nM). TO1-B is a dim fluorescent molecule by itself, but becomes strongly fluorescent when complexed with the RNA aptamer and demonstrates a fluorescent enhancement of approximately 1,100x. Mango I is only suitable as a hairpin replacement in its wild-type form. This limitation is due to the simplicity of the Mango structure as it contains a single stem. We added additional stems in different places around the G-quadruplex to increase the versatility of the Mango structure. We then split the active core of the molecule and demonstrated that it can fluoresce when both split strands are present. It can be used for applications such as the measurement or detection of non-hairpin structures and RNA-RNA interactions. We have created a more complex and brighter version of the RNA Mango I by adding additional structures and permutating them about the G-quadruplex.

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524 Withdrawn

525 Let it go: Kinetics of exon unbinding in group II introns by single-molecule FRET and molecular dynamics

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Formation of stable RNA tertiary contacts is inextricably linked to metal cations to compensate the electrostatic stress and guide RNA folding. Self-splicing group II introns establish long-range tertiary interactions between domain 1 and the upstream exon to accurately position the 5'-splice site within the ribozyme's active core.[1] Here we use single-molecule FRET and computer simulations to monitor the kinetics of exon recognition and release in response to K^+ and Mg^{2+}.[2] We find that exon unbinding rates are heterogeneous as a result of degeneracy in the FRET states which in turn originates from the presence or absence of specifically coordinated Mg^{2+} ions.[2-5] Metal ion binding locks the RNA tertiary contact in a rigid conformation. Molecular dynamics simulation show that strain on the sugar phosphate backbone is alleviated when the exonic strand is changed from RNA to DNA thereby promoting exon release. Understanding the kinetic coupling of tertiary contact formation, splicing and retrotransposition will be key to a dynamic picture of group II intron catalysis.

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[1] Pyle, *Annu. Rev. Biophys.* (2016), *45*, 183-205 ; [2] Steffen et al., *submitted* (2019); [3] Kowerko et al., *PNAS* (2015), *112*, 3403-3408; [4] Börner et al., *Coord. Chem. Rev.* (2016), *327-328*, 123-142; [5] Kruschel et al., *RNA* (2014), *20*, 295-307.

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526 Single-molecule FRET imaging of riboswitch transcriptional complexes

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Cotranscriptional RNA folding is crucial for the timely and selective control of biological processes such as riboswitch gene regulation. Due to the transient nature of cotranscriptional events, the characterization of implicated RNA structures during transcription elongation has remained challenging. Here, a method allowing single-molecule Fluorescence Resonance Energy Transfer (smFRET) analysis of cotranscriptional RNA folding within Escherichia coli transcription elongation complexes is described. The method allows the site-specific incorporation of a fluorescent trinucleotide and of an azido-uridine analog that is coupled to a fluorescent alkyne via a copper-free cycloaddition reaction. It was successfully used to structurally characterize nascent transcripts of the thiamin pyrophosphate (TPP)-sensing tbpA riboswitch. The results obtained at different positions along the transcription pathway show that TPP sensing is efficiently performed within a narrow transcriptional window, where the RNAP assists metabolite binding by directing nascent transcript folding. The results obtained using actively transcribing complexes support the importance of the transcriptional window for efficient binding. Overall, these results demonstrate that functional elongation complexes can be observed in smFRET to study cotranscriptional folding events implicated in RNA structures.

527 A Workflow to Determine RNA Structures Using Cryo-EM

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Single particle electron cryo-microscopy (cryo-EM) is a rapid growing technique to determine near-atomic resolution structures, however, isolated small RNAs has not been a focus of cryo-EM as in the case of RNA-protein complexes, likely due to the intrinsic heterogeneity that presents great challenges in high resolution structure determination. To date, there are only two maps of RNA-only structures with resolutions better than 10 Å in EM Data Bank (EMDB). We have developed an efficient workflow to determine small RNA-only structures using single particle cryo-EM with Volta phase plate (VPP), and resolved various small RNAs ranging from 40 to 130 kDa to subnanometer and near-atomic resolutions.

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528 RNA structure maps across mammalian cellular compartments

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RNA structure is intimately connected to each step of gene expression. Recent advances have enabled transcriptome-wide maps of RNA secondary structure, termed RNA structuromes. However, previous whole-cell analyses lacked the resolution to unravel the landscape and also the regulatory mechanisms of RNA structural changes across subcellular compartments. Here we reveal the RNA structuromes in three compartments — chromatin, nucleoplasm and cytoplasm in human and mouse cells. The cytotopic structuromes substantially expand RNA structural information, and enable detailed investigation of the central role of RNA structure in linking transcription, translation, and RNA decay. We develop a resource to visualize the interplay of RNA-protein interactions, RNA modifications, and RNA structure, and predict both direct and indirect reader proteins of RNA modifications. We validate the novel role of the RNA binding protein LIN28A as an N6-methyladenosine modification "anti-reader". Our results highlight the dynamic nature of RNA structures and its functional significance in gene regulation.

529 Exact distance measurement and spatial sampling in RNA

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RNA does not only translate the genetic code into proteins, but also carries out important cellular functions. As is the case for proteins, understanding such functions requires knowledge of the structure and dynamics at atomic resolution. Almost half of the published RNA structures have been solved by NMR. However, as a result of severe resonance overlap and low proton density, high-resolution RNA structures are rarely obtained from NOE data alone. Instead, additional semi-empirical restraints and labor-intensive techniques are required for structural averages, while there are only a few experimentally derived ensembles representing dynamics.

In a string of recent studies, we have replaced the standard NOE-based procedure for macromolecular structure determination by an approach that employs tight averaged distance restraints derived from exact NOEs (eNOEs) [Nichols et al. 2017].So far, we have used the approach to calculate multi-state structural ensembles of various proteins of sizes [Nichols et al. 2017]. Here, we present an application of the eNOE approach to nucleic acids.

We show that our eNOE-based structure-determination protocol is able to define a 14-mer UUCG tetraloop structure at high resolution without other restraints [Nichols et al., 2018]. Additionally, we explore the potential to elucidate spatial sampling of the RNA. First, we use the eNOEs to calculate a two-state structure. Second, we demonstrate that a molecular dynamics trajectory generated without experimental restraints features a distinct and previously unknown low-populated state that improves the agreement with the eNOEs. The eNOE protocol may open an avenue to obtain high-resolution structures of small RNA of unprecedented accuracy with moderate experimental efforts.

P. Nichols, A. Born, M.A. Henen, D. Strotz, J. Orts, S. Olsson, P. Güntert, C. Chi, B. Vögeli, *The exact NOE: Recent advances*, 2017, **Molecules**22, 1176

P. Nichols, A. Born, M.A. Henen, D. Strotz, P. Güntert, B. Vögeli, *High-resolution small RNA structures from exact nuclear Overhauser enhancement measurements without additional restraints*, 2018, **Comm Biol**1, 61

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530 Highly accurate RNA structure prediction adapted to design challenges in synthetic biology

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Typical RNA 3D structure prediction methods rely on fragments from previously solved structures for sampling or on coarse-grained potentials for scoring. Both types of methods provide invaluable accelerations but can face limitations in prediction accuracy, particularly but not exclusively on problems with features with no structural precedent. To address these shortcomings, we have developed an all-atom method for RNA 3D structure prediction that makes no use of fragments. The stepwise Monte Carlo (SWM) algorithm has achieved the first atomic-accuracy prediction of a complex RNA fold, the Zika xrRNA, as well as a prediction accurate enough to be used for an otherwise intractable crystallographic molecular replacement. Now, we have generalized the algorithm to sample sequence and structure concurrently, permitting the design of re-architected ribosomes capable of supporting life.

531 GTP-binding RNA-aptamers display surprisingly intricate tertiary structures

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Aptamers are single-stranded nucleic acids selected in vitro for high-affinity binding to a wide range of small molecule ligands. Structural studies of RNA-aptamers in complex with their cognate ligands highlight the diverse folds of those systems.

An in vitro selection using the nucleoside triphosphate GTP as ligand yielded 11 structurally and sequentially very diverse classes of RNA aptamers.[1] We initiated structural studies by NMR spectroscopy of a choice of these aptamers in complex with GTP to gain insight into the structural diversity of GTP recognition. Recently, we published the NMR solution structure of the 34 nucleotide long GTP class II aptamer, which comprises a surprisingly complex tertiary structure, that is stabilized by intricate hydrogen bonding interactions and a protonated adenosine (A) with a highly shifted pK_a.[2]

The GTP-binding aptamer 9-12 has a length of 39 nucleotides and binds GTP with high affinity with a K_D of 250 nM (pH 6.3, 2 mM Mg^{2+}).[1] Secondary structure predictions suggest two helical regions that form a three-way junction with the lower stem. So far, our NMR-studies contradict these predictions. The recognition of GTP is identical to the GTP Cl II aptamer, where the ligand is bound in an intermolecular Watson-Crick (WC) base-pair with a conserved cytidine (C) and an A which interacts with its WC-edge with the sugar edge of GTP.[2] However, the tertiary structure of the 9-12 aptamer is very different and even more complex than the tertiary structure of the GTP class II aptamer despite their very similar size.

We will discuss NMR approaches that will facilitate the delineation of complicated RNA-tertiary structures and support our structural investigations in this system.

Literature:

[1] Carothers, J. M.; Oestreich, S. C.; Davis, J. H.; Szostak, J. W. J. Am. Chem. Soc., 2004, 126, 5130-5137

[2] Wolter, A. C.; Weickhmann, A. K.; Nasiri, A. H.; Hantke, K.; Ohlenschläger, O.; Wunderlich, C. H.; Kreutz, C.; Duchardt-Ferner, E.; Wöhnert, J. Angew. Chem. Int. Ed. Engl. 2017, 56, 1, 401-404

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532 Defining the architecture of the influenza RNA genome by RNA-RNA-seq

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Influenza viruses are responsible for recurrent epidemics and occasional pandemics, and are a major burden on public health worldwide. Their segmented genome, composed of 8 negative sense viral RNAs (vRNAs), complicates virus assembly but offers evolutionary advantages by enabling reassortment. Current evidence suggests that influenza vRNAs are organized during assembly into a supramolecular complex. However, its molecular details are poorly understood.

Our goal is to define the quaternary RNA architecture of the genome in virions by identifying sites of interaction between the 8 vRNAs. To this end, we have developed RNA-RNA-seq, which can measure direct (RNA-RNA) and indirect (protein-mediated) interactions without being limited by specific protein or RNA baits. RNA-RNA-seq includes the following steps: fragmentation, adaptor ligation, selection, reverse transcription, amplification and sequencing. We use on-bead ligation to avoid 2D-gel isolation, add specific adaptors to clearly demark the boundary between interacting RNAs and enrich for RNA duplexes to avoid wasting sequencing depth. We will use this method to define the architecture of the influenza genome in viral particles with the ultimate goal of assessing the impact of genome architecture on influenza evolution by genetic reassortment.

533 Design artificial protein factors to specifically edit RNAs

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Human genes are extensively regulated in RNA processing stages through various mechanisms, including alternative splicing, RNA editing, alternative polyadenylation, etc. The RNA processing is tightly regulated inside cells, and the abnormality in RNA processing is closely related to a wide variety of human diseases. Here, we have developed artificial RNA editase by combining a designer PUF domain with different activity domains of ADARs that catalyze adenosine-to-inosine (A-to-I) nucleotide editing, referred to as PUF-ADAR RNA Sequence Editors (PARSE), which can specifically recognize RNA substrates and efficiently catalyze adenosine deamination edit near the cognate binding sites. As a proof of concept, we used this system to edit a premature termination codon pre-introduced in the GFP transcript, resulting in the restoration of normal GFP protein. We further engineer PARSE to efficiently rescue some disease-relevant mutations at RNA level with robust activity in cultured cells. In addition, we have developed another artificial RNA editase by combining a designer PUF domain with APOBEC3A that catalyze cytosine-to-uracil (C-to-U) nucleotide editing, named artificial Apobec3A-PUF RNA Sequence Editor (APRSE), can specifically recognize RNA substrates and efficiently catalyze cytosine deamination edit downstream of the cognate binding sites. Since artificial PUF-factors are simple enzymes without any RNA components and consist of sequences from endogenous human proteins, these engineered proteins may be an easier and more practical alternative for CRISPR-Cas system when applied in gene therapy.

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534 Splicing buffers suboptimal codon usage in human cells

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Although multiple studies have addressed the effects of codon usage on gene expression, such studies were typically performed in unspliced model genes. In the human genome, most genes undergo splicing and patterns of codon usage are splicing-dependent: guanine and cytosine (GC) content is highest within single-exon genes and within first exons of multi-exon genes. Intrigued by this observation, we measured the effects of splicing on expression in a panel of synonymous variants of GFP and mKate2 reporter genes that varied in nucleotide composition. We found that splicing promotes the expression of adenine and thymine (AT)-rich variants by increasing their steady-state protein and mRNA levels, in part through promoting cytoplasmic localization of mRNA. Splicing had little or no effect on the expression of GC-rich variants. In the absence of splicing, high GC content at the 5' end, but not at the 3' end of the coding sequence positively correlated with expression. Among endogenous human protein-coding transcripts, GC content has a more positive effect on various expression measures of unspliced, relative to spliced mRNAs. We propose that splicing promotes the expression of AT-rich genes, leading to selective pressure for the retention of introns in the human genome.

535 The Search for RNA Aptamers that Selectively Bind Monosaccharides

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Many RNA aptamers with a wide variety of specifications have been developed by employing various directed evolution methods. However, the selection of high-affinity RNA aptamers for monomeric sugar molecules has remained elusive. Furthermore, there are no reports of naturally occurring RNA aptamers for unmodified monosaccharides, despite the fact that there are several prominent sugar metabolites (e.g. glucose, fructose, mannose, ribose) that are ubiquitous in all species. The development of aptamers for specific sugars could enable the development of advanced molecular technologies, including biosensors or certain RNA-based therapeutics. In the current study, our general aim is to create RNA aptamers that can selectively bind to target sugar monomers, dimers, and polymers, from a collection including glucose, lactose, cellulose, agarose, maltose and mannose. We have incorporated a rapid method for RNA amplification called self-sustained sequence replication (3SR) to generate copies of aptamer RNAs isolated during each round of in vitro selection by affinity elution. Initialresults demonstrate that RNA populations after 15 rounds (G15) bind to chromatographic matrices containing glucose-agarose, lactose-agarose, and cellulose. Our next objectives are to identify aptamers that specifically bind to mono- and disaccharides with high affinity.

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536 Extensive self-targeting by CRISPR-Cas systems in the plant pathogen *Xanthomonas albilineans*

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CRISPR-Cas systems are RNA-directed adaptive immune systems in bacteria and archaea that defend against invading plasmids and bacteriophages. Genetic information of invaders is collected and incorporated as spacers in CRISPR arrays. As targeting relies on spacers, self-targeting spacers are expected to lead to autoimmunity and cell death. Nevertheless, there exist examples of strains with genome-targeting spacers. *Xanthomonas albilineans* is a unique example with over 20 self-targeting spacers, the largest content reported to date. The bacterium also harbors two CRISPR-Cas systems, a type I-C and a type I-F system and all *cas* genes required for these systems are encoded within *X. albilineans* genome. Type I systems encode a multi-protein effector complex called Cascade that is sufficient for target binding and, after recruiting Cas3, degrades its target. We used a cell-free transcription-translation system (TXTL) to characterize *X. albilineans* CRISPR-Cas systems and to address the function of these genome-targeting spacers.

We found that both systems could yield target DNA binding as well as cleavage activity. Furthermore, 5'CC and 5'TTC for I-F and I-C system, respectively, are functional PAMs. These PAMs are also associated with genome-targeting spacers, suggesting extensive self-targeting. Anti-CRISPR proteins encoded in *X. albilineans* genome could prevent the bacterium from lethal self-targeting. Supporting this hypothesis, we could identify ~15 putative anti-CRISPR proteins encoded within *X. albilineans* genome or plasmids. These proteins could serve as regulators and prevent cell-death. With this, a new alternative function for CRISPR-Cas systems as gene-regulating system could be discovered and new information about the evolution of CRISPR-Cas systems can be gathered.

537 Outcomes of genome targeting in bacteria vary between CRISPR nucleases and strains

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The discovery of CRISPR-Cas immune systems in bacteria and archaea has led to many applications based on programmable DNA and RNA targeting with each system's RNA-guided nucleases. Based on the observation that genome targeting in bacteria leads to cell death, these nucleases have been used to eradicate cells that failed to undergo recombineering as part of gene editing or exhibit virulence or pathogenicity as part of programmable antimicrobials. Previous studies have used individual single-effector nucleases in single strains for these purposes. However, how these nucleases compare across strains remains to be explored. In this study, we compared three single-effector nucleases with different targeting mechanisms, type II-A Cas9, type V-A Cas12a and type VI-A Cas13a, in different bacteria. We initially tested the genome-targeting activity of these nucleases in Escherichia coli MG1655. We found that each nuclease yielded widely varying reductions in the transformation efficiency of a targeting versus non-targeting guide RNA plasmid, with Cas12a yielding the largest reduction. We also identified different mechanisms of escape from targeting, including repair of cleaved DNA by RecA for Cas9, delayed cell death or recombination within the guide RNA for Cas12a, and reduced expression of the target RNA for Cas13a. Next, when evaluating the outcome of genome targeting with Cas12a in different strains of enteric bacteria, we observed two different trends across the strains when targeting essential and non-essential genes. Finally, we identified distinct means to counter the survival mechanisms, thereby enhancing cell death and reducing escape. These results show that the outcomes of genome targeting with Cas single-effector nucleases in bacteria can vary between nucleases and strains, with implications for the use of CRISPR for genome editing and programmable antimicrobials in bacteria.

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538 A Deep Learning Assisted Screen for RNA Localization

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RNA localization, the precise trafficking of transcripts to specific cell compartments, is a finely-tuned and essential cellular process. RNA localization allows for spatial restriction of gene expression, enhanced temporal resolution of gene expression, and localized translation. Though RNA localization was initially thought to be restricted to a few specialized transcripts, up to 70% of eukaryotic transcripts show specific localization patterns. Nonetheless, the mechanism by which the destination of a transcript is encoded remains largely unknown. Specific 'localization elements' necessary and sufficient for localization have been identified in the 3' untranslated region (3' UTR) of transcripts. To date, a limitation on fully understanding the cis-regulatory logic of the 3' UTR in RNA localization has been a lack of large scale, high-throughput data. To address this, we are developing a paired imaging and in situ sequencing methodology to assay RNA localization in human cells. This represents a novel sequencing framework to pair RNA localization data with 3' UTR sequence. Furthermore, we propose a deep learning framework to identify sequence features that predict observed localization outcomes, with the goal of determining non-canonical sequence motifs with critical roles in RNA localization. This informatic approach provides a potential avenue to identify novel signatures of localization fate that can be empirically validated.

539 Identification of a localized RNA-based mechanism that regulates cell-matrix interactions

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Diverse pathologies in numerous organ systems are associated with fibrosis. Fibrosis is characterized by tissue stiffening that occurs when cells are unable to appropriately sense and respond to changes in the extracellular matrix (ECM). Therefore, to treat or prevent pathologies related to fibrosis, it is critical to understand the mechanisms that govern cell-ECM interactions. Our lab has recently identified that, in response to changes in ECM stiffness, Argonaute 2-miRNA complexes regulate cytoskeletal, actin, and extracellular matrix (CAM) transcripts [1]. Disruption of this regulatory mechanism resulted in hyper-adhesive, hyper-contractile phenotypes in multiple systems in vitro and increased tissue stiffness during tissue regeneration in vivo. It remains unclear, however, how changes in ECM stiffness initiate RNA-based regulatory mechanisms to control cell behavior.

Here we hypothesize that the ECM could modulate cell phenotypes by driving translation of CAM genes locally at focal adhesions. Focal adhesions are protein complexes located at the ECM-cell membrane interface that mediate signaling pathways in response to the ECM. Using two diverse adherent cells type, human dermal fibroblasts and endothelial cells, we mechanically isolated focal adhesions from the rest of the cell bodies and analyzed genome-wide mRNAs, miRNAs, RNA translation levels, and proteins. Our results will define the road map of local proteins and translated RNAs at the focal adhesion. Furthermore, we will identify multiple miRNAs and RNA binding proteins that regulate the function of CAM genes in response to changes in matrix stiffness. Taken together, we will identify mechanisms of local RNA translation that govern cell-matrix interactions. These findings will provide new insight into RNA-based regulatory mechanisms that will help elucidate the cause of fibrosis.

[1] Moro, Albertomaria, et al. "microRNA-dependent regulation of biomechanical genes establishes tissue stiffness homeostasis." Nature Cell Biology (2019): 1.

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540 mRNAs and Mitochondria co-transport to distant neuronal sites

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Mitochondria are enriched in neuronal areas with high energy demands, such as synaptic nerve terminals and axon branches, and have essential roles in neuronal function and maintenance. To respond to local needs, it is suggested that distant-site mitochondria rely on local translation of nuclear-encoded mRNAs.

Here we show by biochemical fractionation that mRNAs of nuclear-encoded mitochondrial genes are associated with mitochondria, in both neural-like cell line and motor neuron axons. Furthermore, by applying the MS2 mRNA visualization system we show that these mRNAs not only associate but are also co-transported with mitochondria along axons. We tested the importance of different Cox7c mRNA regions and found that the coding sequence has a much greater contribution to localization than its 3'UTR. Specifically, the predicted N-terminal 15 amino acids mitochondrial targeting sequence (MTS) are important for localization. These results reveal that mRNAs encoding mitochondrial proteins are associated and transported with mitochondria in a manner that involve translation.

541 Cellular localisation of mRNA during angiogenesis

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Deciphering the complex biology of cell migration is key to understand human-related disorders. During angiogenesis, endothelial cells engage in coordinated migration events to form new blood vessels from parental counterparts. However, while subcellular localisation of mRNAs and localised translation are fundamental steps between gene transcription and protein activity, whether local regulation of gene expression controls the complex morphogenetic process of angiogenesis has never been addressed. Here, we set out to investigate the cytoplasmic distribution of mRNAs in migratory endothelial cells. We isolated RNA from endothelial cell protrusions from their cell bodies and used RNA-sequencing to identify asymmetrically distributed transcripts. These studies identified a set of transcripts enriched in endothelial cell protrusions over cell bodies, which included classically protrusion-enriched mRNAs and other intriguing transcripts encoding proteins implicated in cell migration To identify nucleotide sequences responsible for mRNA localisation, we present a set of *in vitro* and *in vivo* tools that permit live visualisation of transcript distribution in endothelial cells. Furthermore, we demonstrate how the localisation of a transcript translated at the leading front of endothelial cells is involved in angiogenic sprouting. In summary, the studies presented here will contribute to further understanding blood vessel formation and provide a novel avenue in the pursuit for angiogenesis-related therapies.

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542 Novel bioconjugation system for mRNA therapy

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Due to its chemical and biochemical properties, messenger RNA (mRNA) usually is degraded within a few minutes inside the cells, thus expression of a specific protein usually is a transient process. Moreover, the polyanionic mRNA molecule does not cross efficiently a cell membrane which renders the delivery of mRNA extremely difficult. Despite these challenges, scientific and technological advances of the recent years have made mRNA a promising candidate for a novel class of drugs including vaccinations, cancer immunotherapy and protein replacement therapy.

Click Chemistry is herein used to modify mRNA molecules to possibly enable the use of such modified mRNA molecules in the context of mRNA-based therapy. We demonstrate here the efficient enzymatic incorporation of different nucleotides, including: 7-ethynyl-ATP (EATP), 5-ethynyl-UTP (EUTP) and 3'-Azido-2',3'-ddATP for subsequent click conjugation for cell delivery and protein expression.

Furthermore mRNA coding for the enzyme α-galactosidase A (α-Gal A) was labelled via click chemistry and studied in Fabry disease cells, a disease caused by the lack of function of the enzyme, for a future promising treatment of such disease.

We consider this work as an important starting point for the development of various conjugation molecules that can add beneficial properties to the mRNA in the context of basic and biomedical research including pharmaceutical applications regarding e.g. delivery and stability of the mRNA.

543 Atlas of Subcellular RNA Localization Revealed by APEX‐seq

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We introduce APEX‐seq (Fazal*, Han* et al., bioRxiv 2018), a method for RNA sequencing based on direct proximity labeling of RNA using the peroxidase enzyme APEX2. APEX‐seq in nine distinct subcellular locales produced a nanometer resolution spatial map of the human transcriptome, revealing extensive and exquisite patterns of localization for diverse RNA classes and transcript isoforms. We uncover a radial organization of the nuclear transcriptome, which is gated at the inner surface of the nuclear pore for cytoplasmic export of processed transcripts. We identify two distinct pathways of messenger RNA localization to mitochondria, each associated with specific sets of transcripts for building complementary macromolecular machines within the organelle. APEX‐seq should be widely applicable to many systems, enabling comprehensive investigations of the spatial transcriptome.

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544 Functional study of *Plasmodium* tRNA import machinery

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Malaria continues to be a global burden for health, with 445,000 deaths and 216 million cases in 2016 (1). Our results bring an unprecedented observation: mosquitoes salivary gland sporozoites import exogenous host tRNAs. This tRNA trafficking is made possible by a unique surface protein, named tRip (transfer RNA import protein). The protein tRip mediates tRNA entrance in the parasite, and this mechanism is crucial for infection by a presently unknown mechanism (2). So far, we established that (i) *in vitro* tRip binds tRNAs with high affinity and recognizes the elbow of the tRNA molecule through its C-terminal domain. (ii) *In vivo* immunolocalization experiments found tRip in both the liver and the blood stages of *Plasmodium* in the mammalian host, as well as in the mosquito vector. (iii) tRip is located at the surface of the parasite, its tRNA binding domain being exposed to the outside. (iv) *In vitro*, exogenous tRNAs enter rapidly live sporozoites. Protein biosynthesis is reduced in a knock-out parasite, deleted for the TRIP gene (tRip-KO) and its infectivity is diminished at the blood stage as compared to the wild-type parasite. Moreover, the gene-encoding tRip is specific for *Apicomplexa* parasites, such as *Plasmodium*, *Toxoplasma*, and *Cryptosporidium*.

How are tRNAs imported into *Plasmodium*? What is their role in the infectious process of the parasite? We provide a functional and structural description of this parasite-specific import process by investigating recognition between tRip and host tRNAs, comparative proteomics between wild type and tRip-KO parasites, identification of tRip partners and structure of tRip, tRip/tRNA complex and the complex formed between tRip and its *Plasmodium* protein partners.

1. World Malaria Report 2017

2. Bour T., et al. (2016) Apicomplexa-specific tRip facilitates import of exogenous tRNAs into malaria parasites. *Proc Natl Acad Sci U.S.A.* **113**:4717-4722.

545 A change in cell fixation protocol provides a clue on transferred mRNA biology

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In eukaryotic cells, a small percentage of mRNA molecules can undergo transfer from one cell to another. This process of mRNA transfer occurs primarily via membrane nanotubes1. Nanotubes are long thin protrusions that are structurally distinct from filopodia. Nanotubes are produced by numerous cell types and connect cells that can be up to hundreds of microns apart. There are no known nanotube markers yet and nanotube visualization is achieved primarily by membrane staining or staining for the cytoskeletal fibers (typically F-actin). Since nanotubes are very thin and fragile, it is difficult to image them and their cargo. This is particularly true in single molecule fluorescent in situ hybridization (smFISH) for detection of mRNA transfer. smFISH requires the fixation of the cells, followed by multiple rounds of washing the samples prior to imaging, a process that damages the nanotubes. In order to improve the preservation of nanotubes in smFISH, we added glutaraldehyde (GA) to the fixation step. GA increased nanotube preservation and detection by ~4-fold, and long nanotubes (>10μm) were also better preserved. Surprisingly, transferred mRNA was not detected when GA was used. In contrast, endogenous mRNA was readily detected. We found that treatment with proteinase K and Urea after GA fixation has exposed ~30% of the transferred mRNA molecules for detection (compared to regular smFISH)2. We therefore suspect that transferred mRNA, unlike endogenous mRNA, is encapsulated in a unique protein "shell" which prevents the FISH probes from accessing the mRNA in samples that are cross-linked with GA. Since mRNAs were also not detected inside nanotubes in GA-fixed cells, we suspect that the mRNA is encapsulated prior to its transport along the nanotube. We are currently performing experiments aimed at identifying proteins that associate specifically with transferred mRNA. Such proteins may facilitate its transport or create the hypothesized protective "shell" around the transferred mRNA.

1 Haimovich et al. (2017) PNAS 114(46): E9873-E9882.

2 Haimovich and Gerst (2019) Methods in Molecular Biology (In press).

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546 The function of the DEAD-box ATPase Dbp5 in the regulation of mRNA export

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Transport of mRNA from the nucleus to the cytoplasm via nuclear pore complexes (NPCs) is an essential step in eukaryotic gene expression. Although factors involved in mRNA transport have been characterized, the lack of information on molecular mechanisms and dynamics of directional mRNA transport has hampered a comprehensive understanding of this critical process and its regulation. mRNA export depends on several NPC-associated proteins, including the DEAD-box ATPase Dbp5, which has been suggested to drive directional transport *in vivo*. Based on extensive *in vitro* work several groups have proposed models for Dbp5 function, but whether this can be transferred to Dbp5's role *in vivo* is unclear. Here, we demonstrate that acute depletion of budding yeast Dbp5 using an inducible degron system causes rapid nuclear accumulation of single labeled mRNAs. Furthermore, we show that while steady-state localization of many factors involved in mRNA export is unaffected in the absence of Dbp5, we observed dramatic changes in nuclear dynamicity. In particular, the essential export factor Nab2 ceases to shuttle between the nucleus and cytoplasm and forms a gel-like structure throughout the nucleus. This potential phase-separation phenotype can be recapitulated *in vitro*, with Nab2 forming liquid droplets in the presence of RNA, which can be dissolved by the addition of Dbp5. Intriguingly, we also observe nuclear Nab2 condensation in physiologically relevant stress conditions such as acute carbon source switch or glucose withdrawal, which are accompanied by global nuclear mRNA retention. Importantly, stress-induced mRNAs overcome nuclear retainment to allow for a redirection of the translational machinery to produce stress proteins to elicit a timely cellular stress response. We will present results using various Dbp5 and Nab2 mutants that characterize Dbp5-dependent gel formation of Nab2 and the preferential export of stress-induced mRNAs as a novel layer of gene expression regulation.

547 The mammalian nuclear basket and protein Tpr prevent nuclear exit of transcripts with retention elements but do not monitor the occurrence of splice sites

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The nuclear basket (NB), anchored at the nuclear side of the nuclear pore complex, is an evolutionally conserved structure of eight interconnected fibrils. Tpr is a main architectural element of the NB and has formerly been proposed to contribute to the surveillance of mRNA export in both yeast and humans, bypreventing leakage of un-spliced mRNAs to the cytoplasm via monitoring the presence of splice sites.

Here we have investigated in further detail how Tpr might contribute to such monitoring process. To this end, we created a series of inducible reporter cassettes that were then stably integrated into the same chromosomal locus of a parental cell. This approach allowed for direct comparability of the data from the different reporter cell lines, and for facilitating phenotype interpretation. Among these reporters were also such that allowed for expressing parts of the HIV genome and mutated variants thereof. The corresponding collection of reporter cell lines were then used for studying the subcellular distribution of un-spliced and spliced transcripts in the presence and absence of Tpr.

In most cases, we found that absence of Tpr does not cause any detectable leakage of their un-spliced versions. However, confirming earlier studies to some extent, certain types of HIV transcripts were more numerous in the cytoplasm of Tpr-deficient than control cells, suggesting that more were exported upon Tpr depletion. Remarkably, however, such phenotype did not depend on the presence of splice sites, branch point sequences, or poly-pyrimidine tracks, since cytoplasmic occurrence of the HIV transcripts was also observed when these elements had been eliminated. On the other hand, when changing other HIV sequence segments, a Tpr deficiency-related phenotype was no longer observable. Our results show that cytoplasmic occurrence of distinct HIV transcripts upon Tpr depletion does not reflect the loss of a splice site surveillance mechanism. Moreover, these data also reveal that Tpr is not part of a general retention mechanism for all intron-containing transcripts. Instead, they suggest that other RNA sequence elements can provoke nuclear retention of distinct types of RNAs and that Tpr, or the NB as a whole, plays a role in such a process.

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548 A region overlapping exon 1-exon 2 junction facilitates nuclear export of singly-spliced MMTV env mRNA

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Mechanistic details of the post-transcriptional regulation of alternative splicing remain largely unknown. Like cellular pre-mRNAs, retroviral primary transcripts are spliced in multiple ways and the alternatively spliced transcripts encode essential viral gene products. The singly- and multiply-spliced mRNAs encode an envelope and accessory viral proteins, respectively. The unspliced viral transcript encodes Gag polyprotein and serves as the viral genome that is transmitted to recipient cells. Retroviruses share common strategies of post-transcriptional gene regulation to produce Gag. It depends on structured RNA export elements in coding regions or in the 3'untranslated region of the unspliced mRNA that are recognized by a cellular nuclear export receptor, NXF1 (e.g. MPMV, MLV), or by a virus-encoded accessory protein mediating binding to another cellular nuclear receptor, CRM1 (e.g. HIV-1, MMTV). Post-transcriptional regulation of the env gene expression is less well understood. It is believed that the env mRNA, which contains the same structured export element as gag mRNA, is translocated from the nucleus to the cytoplasm via the same pathway as the mRNA encoding Gag. However, we surprisingly found that it was not the case for some retroviruses. We found that the env mRNA from one of the complex retroviruses, MMTV, which contains the same structured RNA export element as the mRNA encoding Gag (RmRE) and hence it should be directed to the CRM1-export pathway, is in fact exported to the cytoplasm via a CRM1-independent mechanism. The export is directed by a dominant cis-acting structured element (termed MPPE) generated by splicing of exon one to exon two. The MPPE is situated at the 5'end of env mRNA and overrides RmRE that is located at the 3'end of the same transcript. The MPPE function is transferable and can replace HIV-1 Rev-RRE-regulated expression of HIV-1 Gag.

549 Understanding the role of RNA binding for Trim2 and Trim3 function

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Trim2 and Trim3 are two closely related proteins that regulate axon regeneration and synaptic plasticity by yet unknown mechanisms. Trim2 deficiency or mutation causes Charcot-Marie-Tooth-Disease, an early-onset axonal neuropathy, while Trim3 has been associated with Schizophrenia.

Trim2 and Trim3 belong to a conserved family of developmental regulators that are characterized by their N-terminal TRIM motif their C-terminal NHL domain. While the TRIM motif confers ubiquitin ligase activity, the NHL domain has been shown to function as an RNA-binding domain and several members of the TRIM-NHL protein family bind mRNAs to control gene expression post-transcriptional. The role of RNA binding for Trim2 and Trim3 function, however, has not yet been investigated.

To systematically identify the RNA targets of Trim2 and Trim3 and to determine their impact on gene expression I combined RNA-Immunoprecipitation experiments with knock-out studies using induced spinal motor neurons in cell culture.

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550 Development and Analysis of Mouse Models to Study the Function of the Constitutive Transport Element (CTE) in the NXF1 Gene

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Intron retention, a form of alternative splicing once thought to be common mainly in plants, is rapidly becoming recognized as an integral process in mammalian gene regulation. The Nuclear Export Factor 1 (NXF1) gene is implicated in export of mRNA with and without retained introns. The NXF1 gene itself expresses two major different mRNA isoforms: one fully spliced and one that retains intron 10. The fully spliced isoform gives rise to a protein that functions as an mRNA export receptor. This Nxf1 isoform interacts with an element in intron 10 called the CTE, resulting in export of the mRNA that retains this intron, which can be translated into a small Nxf1 (sNxf1) isoform. We used CRISPR/Cas9 to generate deletions in the NXF1 CTE in both 293T cells and mice. In 293T cells, CTE deletions did not affect the relative expression of the different mRNA isoforms, but mRNA with the retained intron was not efficiently exported. In mice, we generated lines with 4 or 19 nt deletions in the CTE. The 19 nt deletion in the CTE includes part of the internal loop that interacts with the Nxf1 protein. Analysis of the 4 nt and 19 nt CTE deletions in a reporter system confirmed that the 19 nt deletion abolished CTE function, whereas the 4 nt deletion retained some function.

The mice with the 4 nt CTE deletion develop normally, show no obvious defects and have a normal life span. In contrast, the homozygous 19 nt deletion mice show some hyperactive behavior and unexplained "sudden" death in several of the mice. To study effects on gene expression, polyA+ RNA from hippocampus and cortex of mice (16-19 weeks and 35 weeks) was subjected to RNASeq using Illumina NGS. Analysis of the data demonstrated down regulation of several genes classified as Immediate Early Genes (IEGs), including ARC, FOS, and EGR1, in the homozygous deletion mice. IEGs are implicated in learning and memory. We are in the process of backcrossing these mice to generate a congenic C57BL/6 line carrying the CTE deletion, before further testing of behavior and learning.

551 Quantifying gene expression noise via single mRNA imaging in living cells

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Biological systems are intrinsically noisy. Gene expression variability, or "noise", originates from a combination of externally caused fluctuations and the internal stochasticity of all biochemical processes involved. It has been identified as a major source of phenotypic variability between genetically identical individuals. The amount of protein generated from expression of a gene depends on the rates of mRNA transcription, translation and decay. While single-molecule methods have revealed insights into the dynamics of mRNA transcription, it has not been possible to investigate the variability of mRNA translation or degradation.

We have partially overcome this limitation by designing the TREAT (3¢(Three) RNA End Accumulation during Turnover) reporter that can monitor the degradation of individual mRNAs in living cells via stabilization of degradation intermediates using viral pseudo knots. In addition, several recent publications have described novel imaging-based techniques that can directly monitor translation by observing nascent polypeptides as they are synthesized on individual mRNAs.

Here, we describe our progress in the development of a fluorescent biosensor that enables simultaneous observation of the translation (SunTag) and degradation (TREAT) of individual mRNAs in living cells. We apply it to directly measure how efficiently different reporter transcripts in several subcellular localizations are translated before their turnover. Based on these measurements, we will continue to investigate how the variability of translation and decay affects specific post-transcriptional gene regulatory pathways.

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552 Activity studies of poxvirus decapping enzyme D9.

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Messenger RNA degradation is one of the mechanisms that is used by cells to regulate their gene expression and it could be initiated by decapping, the process during which the protective cap structure from the 5' end of target mRNA is removed. The major eukaryotic RNA decay enzyme is Dcp2 and in complex with its activator Dcp1 it is responsible for decapping of full-length mRNAs. According to studies, some viral proteins (e.g. from poxviruses) also possess this hydrolytic activity but their role in viral infection is not completely understood. [1] Vaccinia virus decapping enzyme D9 recognizes m7G-cap and cleaves the pyrophosphate bond between α and β phosphates, releasing m7GDP and the 5′-monophosphorylated RNA body. That, in turn, leads to the mRNA degradation and shutdown of host protein synthesis.

To study the activity of D9 enzyme and identify some small molecules that could act as potential D9 inhibitors we propose a simple fluorescence-based assay with pyrene-labelled m7G nucleotide as an activity probe. We used this approach to monitor the fluorescence intensity changes upon enzymatic cleavage on a 96-well plate reader in the presence of compounds that can modulate protein activity of D9 decapping enzyme. Then, hits from the screening experiments we verified for their selectivity towards the D9 enzyme and evaluate their binding affinity and inhibitory properties using microscale thermophoresis. Obtained data may significantly advance our understanding of decapping process during viral infection.

[1] Parrish, S., and Moss, B. (2007). Characterization of a second vaccinia virus mRNA-decapping enzyme conserved in poxviruses. J. Virol. 81, 12973-12978.

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553 No-Go Decay substrates are uniquely cleaved upstream of the collided disome, resulting in 5′-OH ends phosphorylated prior to 5′-3′ decay

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The No-Go Decay (NGD) mRNA surveillance pathway degrades mRNAs containing stalled ribosomes. An endoribonuclease has been proposed to initiate cleavages upstream of the stall sequence. However, primary site of cleavage remains unknown. Indeed, direct evidence that two RNA fragments resulting from a precise and unique cleavage has never been obtained. We used mRNAs expressing a 3'-ribozyme to produce truncated transcripts *in vivo* that mimic naturally occurring truncated mRNAs, known to trigger NGD. We analysed ribosome associated NGD cleavage products at single-nucleotide resolution and show that a precise endonucleolytic cleavage event occurs within the mRNA exit tunnel of the ribosome, 8 nucleotides upstream of the first P-site residue. We also demonstrate that this NGD cleavage, which occurs within the third or upstream ribosomes, produces 5'-hydroxylated RNA fragments, also proposed to be further phosphorylated by the Rlg1/Trl1 kinase. The resulting 5'-phosphorylated RNA fragments are digested by the 5'-3' exoribonuclease Xrn1, but surprisingly, can also be trimmed by the 5'-3' exoribonuclease activity of Dxo1 in Xrn1 deficient cells[(1)]. In accordance with recent work[(2)], we verified that this cleavage is Hel2-dependent. However, we propose that the first two stalled ribosomes, called disome, are not competent for mRNA endonucleolytic cleavages of truncated mRNAs or mRNAs containing rare codons and that cleavages observed in disomes result from 5'-3' exoribonucleolytic trimming.

[1] Navickas, A., Chamois, S., et al. (2018). A unique No-Go Decay cleavage in mRNA exit-tunnel of ribosome produces 5′-OH ends phosphorylated by Rlg1. bioRxiv 465633; https://doi.org/10.1101/465633

[2] Ikeuchi, K. et al. Collided ribosomes form a unique structural interface to induce Hel2-driven quality control pathways. The EMBO journal 38, doi:10.15252/embj.2018100276 (2019).

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554 Open reading frame controls mRNA stability in human pathogen and protist *Giardia lamblia*

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All messenger RNAs will eventually be destroyed, but different transcripts will be degraded at different rates. Regulation of mRNA decay is thus essential for proper gene expression, and the 3' untranslated region (UTR) has traditionally been considered the main site controlling stability. However, recent work has demonstrated that the open reading frame (ORF) also controls mRNA stability, raising a new issue of the relative importance of ORF- and 3'UTR-mediated control of mRNA decay. To understand this issue, we turned to the protist and human pathogen *Giardia lamblia* because its 3' UTRs are exceptionally short: in *Giardia*, the average length of 3'UTRs is ~80 nt; in contrast, in humans, these are ~1000 nt long. We have used genome-wide approaches to measure mRNA stability and translation efficiency. Despite its unusually short UTRs, we found control of mRNA stability contributes more to gene regulation in *Giardia* than in traditional model organisms, like yeast. Moreover, we found that mRNAs with 3' UTRs shorter than 50 nucleotides have as much variation in stability as those with longer 3'UTRs. We thus propose that the coding sequence plays a major role in controlling mRNA stability in *Giardia*. Further work into the fundamentals of gene regulation in this deeply branching species will provide key insights into how post-transcriptional regulation has evolved across eukarya.

555 mRNA decapping by an ApaH-like phosphatase in trypanosomes

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The 5′ ends of eukaryotic mRNAs are modified with a 5′-monomethyl guanosine cap structure (m^7G) that is linked to the first transcribed nucleotide via a 5' to 5' triphosphate linkage and protects from rapid and uncontrolled decay. mRNA decay is typically initiated by the shortening of the poly(A) tail, followed by degradation of the mRNA in either 5′ to 3′ or 3′ to 5′ direction. In the 5′-to-3′ decay pathway, the (m^7G) cap is removed by the nudix domain protein Dcp2 along with a specialized multiprotein factory called the decapping complex. Recently, several further decapping enzymes were identified, but all have nudix domains and are thus similar to Dcp2. The decapping complex is highly conserved among eukaryotes, with the exception of trypanosomes.

We have recently identified an ApaH-like phosphatase (TbALPH1) as the major mRNA decapping enzyme of *Trypanosoma brucei*. TbALPH1 was initially identified within the stress granule and RNA-bound proteome and fulfils all *in vitro* and *in vivo* criteria of a decapping enzyme. ApaH-like phosphatases are widespread throughout the entire eukaryotic kingdom, but the trypanosome enzyme is the first one with an assigned function. Trypanosomes have several unusual features in their mRNA metabolism and one is the highly unusual, hypermethylated cap structure, called type 4. It is still not understood why mRNA caps of kinetoplastids are so heavily methylated but it could be the reason for the absence of a conventional decapping pathway. To further investigate the mechanism of mRNA decapping in trypanosomes, we have started a full biochemical characterization of TbALPH1. We will study substrate specificity, the functions of the different ALPH1 domains as well as the ALPH1 interacting proteins (the decapping complex). Initial results will be presented.

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556 NRDE2 negatively regulates exosome functions by inhibiting MTR4 recruitment and exosome interaction

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The exosome functions in the degradation of diverse RNA species, yet how it is negatively regulated remains largely unknown. Here, we show that NRDE2 forms a 1:1 complex with MTR4, a nuclear exosome cofactor critical for exosome recruitment, via a conserved MTR4-interacting domain (MID). Unexpectedly, NRDE2 mainly localizes in nuclear speckles, where it inhibits MTR4 recruitment and RNA degradation, and thereby ensures efficient mRNA nuclear export. Structural and biochemical data revealed that NRDE2 interacts with MTR4's key residues, locks MTR4 in a closed conformation, and inhibits MTR4 interaction with the exosome as well as proteins important for MTR4 recruitment, such as the cap-binding complex (CBC) and ZFC3H1. Functionally, MID deletion results in the loss of self-renewal of mouse embryonic stem cells. Together, our data pinpoint NRDE2 as a nuclear exosome negative regulator that ensures mRNA stability and nuclear export.

557 The dual role of mRNA decay factors in transcription and mRNA decay

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mRNA level is controlled by the balance between its synthesis and degradation rates. We have previously found that various yeast factors that degrade mRNAs in the cytoplasm (DFs) shuttle between the nucleus and the cytoplasm. In the nucleus they physically associate with chromatin, together with the transcription pre-initiation complex, and stimulate transcription (Haimovich et al., *Cell* **152**, 1000 [2013]). Significantly, the capacity of the decaysome to function in the synthesis of a certain mRNA in the nucleus depends on its ability to complete degrading this mRNA in the cytoplasm. I will present our recent NET-seq data uncovering a role of these factors in regulating promoter proximal RNA polymerase II (Pol II) pausing, a recently appreciated key step in transcription regulation. These factors also regulate Pol II pausing at the cleavage and polyadenylation sites. The dual role of DFs in the nucleus and the cytoplasm suggests that regulating their shuttling activity is important for determining mRNA level. We have found that import of a number of DFs is mediated by two novel Xrn1 nuclear localization sequences (NLSs). Interestingly, the decaying RNA binds and masks the major NLS and prevents its recognition by the its cognate importin. Import is therefore possible only after RNA degradation. We propose that this is a mechanism for linking mRNA degradation with import of DFs and subsequent transcription activation.

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558 Biophysical studies of human NUDT16 stability and substrate specificity towards cap analogs

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Human Nudt16 protein (hNudt16) is a member of NUDIX family of hydrolases, known as a potential "housecleaning" and decapping enzyme. It catalyzes the hydrolysis of the phosphodiester bond in substrates consisting of nucleoside diphosphate combined with another moiety X. In the decapping process, Nudt16 cleave cap structure from a subset of cytoplasmic mRNAs and small nucleolar RNAs in the 5'→3' degradation pathway, releasing m^7GDP or $m_3^{2,2,7}$GDP and 5'monophosphate RNA. Recent studies also reported that hNudt16 can process protein ADP-ribosylation.

The main aim of the present study was an attempt to gain further insight into substrate specificity of hNudt16 towards selected mono- and dinucleotide cap analogs. Circular dichroism (far- and near-UV) measurements of free hNudt16 and protein-cap analogs complexes allowed to obtain the characteristics of the secondary and tertiary structural changes of the protein upon ligand binding. Interestingly, recorded near-UV CD spectra showed changes in different regions of protein upon binding of various cap analogs. In addition, kinetic parameters of hNudt16 mediated cap hydrolysis were determined using fluorescence and chromatographic methods and the binding affinity of selected compounds to hNudt16 was studied on the basis of the isothermal titration calorimetry (ITC). Our biochemical and biophysical data indicated the main structural requirements for hNudt16 substrates that determine their efficient binding and hydrolysis. Moreover, the differential scanning fluorimetry (DSF) method was applied to examine the thermal stability of the wild-type and mutated hNudt16 depending on the concentration of divalent metal ions (Mg2+, Mn2+) and cap analogs.

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559 Identification of Tissue-specific RNA Exosome Cofactors as an Approach to Define Disease Mechanism

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The RNA exosome, a 10-subunit complex that mediates both RNA processing and degradation, plays a critical role in defining cellular expression profiles. This complex is ubiquitously expressed, essential, and critical for fundamental cellular functions, such as ribosomal RNA processing. Recent studies have linked mutations in genes encoding multiple subunits of the complex to tissue-specific human disease. For example, missense mutations in the human EXOSC3 gene, which encodes an RNA exosome subunit, cause Pontocerebellar Hypoplasia type 1b (PCH1b), a disease characterized by atrophy of the pons and cerebellum. The missense mutations encode single amino acid changes in conserved regions of the EXOSC3 protein. How these amino acid substitutions confer tissue-specific phenotypes is not known. One possible mechanism underlying the distinct disease phenotypes could be a decrease in the interaction of the RNA exosome complex with cofactors that confer specificity for RNA targets. However, most studies that identify and characterize RNA exosome cofactors have been carried out in budding yeast and thus, do not provide insight into whether tissue-specific cofactors could exist. Our studies use immunoprecipitation from neuronal cell culture (N2A) and relevant mouse tissues to define RNA exosome cofactors. Biochemical experiments that employ cultured N2A cells were used to identify RNA exosome-interacting proteins in both the nucleus and the cytoplasm. Preliminary results from this analysis reveal an association between the RNA exosome and a large complex of cytoplasmic tRNA ligase enzymes, which could link defects in tRNA maturation to disease pathology. We are extending these studies to explore the possibility of tissue-specific cofactors by immunoprecipitating EXOSC3 and analyzing co-purifying proteins from the mouse cerebellum (affected in disease) and cortex (unaffected). To complement these approaches to identify tissue-specific RNA exosome cofactors, we are also exploring whether amino acid changes that are linked to disease alter cofactor interactions. These studies, which are being performed in N2A cells, have identified cofactors that show altered interactions with the EXOSC3 variants present in disease. These studies will provide insight into both the functional consequences of amino acid substitutions in the RNA exosome that cause disease and the role of cofactors in conferring RNA target specificity.

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560 Substrate specificity of the TRAMP and exosome complexes *in vivo*

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The exosome complex plays major roles in RNA 3'->5' processing and surveillance. The TRAMP complexes, which include the RNA helicase Mtr4 together with an RNA binding protein (Air1 or Air2) and a poly(A) polymerase (Trf4 or Trf5), are major cofactors for the nuclear exosome.Mtr4 is an essential cofactorfor all characterized nuclear RNA degradation and processing activities of the exosome, whereas only surveillance activities are known to require the other TRAMP components. It remains unclear how these cofactors cooperate on different substrate classes. Structural analyses of Mtr4 previously identified an "Arch" domain, which acts independently of the helicase activity to stimulate the exosome.

Analyses by mass spectrometry indicated that Air2 interacts almost exclusively with Trf4. However, Air1 interacts with both Trf4 and Trf5, indicating that three different TRAMP complexes exist *in vivo*, with distinct roles. In vivo UV-crosslinking (CRAC) was used to compare RNA targets of the different TRAMP and exosome subunits. We found that different regions of the pre-rRNA are predominately targeted by the Trf4/Air1/Mtr4 (TRAMP4-1) and Trf5/Air1/Mtr4 (TRAMP5-1) complexes. For nuclear surveillance of RNAPII transcripts two putative mechanism were identified, involving either TRAMP5-1 or Trf4/Air2/Mtr4 (TRAMP4-2). TRAMP4-1 binds strongly at most mRNA 5' ends, close to the transcription start site (TSS). Both Rrp44 and Rrp6 are also found at these sites. This suggests roles in degradation of promoter-proximal RNAs generated by early termination of transcription. In contrast, TRAMP5-1 targets a subpopulation of mRNA, binding the 3' region close to polyadenylation sites. Rrp6 was also found in this location (although not Rrp44) and it is possible that TRAMP5-1 participates in surveillance of defective 3'-end formation on these mRNAs.

Notably, Mtr4 exhibited lower association with TSS proximal region when Trf4 is deleted but was not affected by Air2 deletion, suggesting that substrate specificity is provided by Trf4 and Trf5. It had previously been anticipated that the Zn-fingers of Air1 and Air2 would provide specificity, but they appear to be interchangeable at these sites.

We conclude that distinct TRAMP complexes assemble on different classes of transcripts, with Air1, Air2, Trf4 and Trf5 performing distinct roles in surveillance and regulatory pathways.

561 The function of DIS3L2 in the mechanism of nonsense-mediated mRNA decay

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In the flow of information from DNA to mRNA to proteins, mRNAs undergo a number of processing steps, since they are synthesized in the nucleus, until they are translated in the cytoplasm. Eukaryotic cells tightly control the fidelity of this process, via quality control pathways, among them, the nonsense-mediated mRNA decay (NMD). NMD recognizes and degrades mRNAs harboring premature translation-termination codons (PTCs), protecting the cell from potentially harmful truncated proteins. However, NMD can also regulate normal and fully functional mRNA levels, arising as a surveillance and a gene expression regulation pathway. A new branch of the NMD pathway is starting to be revealed, which is characterized by the involvement of the DIS3L2 3' to 5' exoribonuclease. This protein has special relevance, given its exosome-independent action and its uridylation-mediated decay. In addition, mutations on this ribonuclease induce deregulation of cell-cycle genes leading to a faster cell growth and decreased chromosome stability, while DIS3L2 downregulation enhances cancer stem cell properties. Our results have shown that some natural NMD-targets are highly stabilized in DIS3L2-depleted human cells. In this work, we will present the mechanistic basis for the involvement of DIS3L2 in NMD.

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562 The nuclear poly(A) RNA binding protein, Nab2, cooperates with the RNA exosome to alter the transcriptome for survival under stress

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Recent evidence indicates that nuclear poly(A) RNA binding proteins (PABPs) can function both in the protection and destabilization of RNA transcripts; however, the mechanisms remain poorly characterized. The evolutionarily conserved, nuclear PABP, Nab2/ZC3H14, contains a novel tandem CCCH zinc finger (ZnF) domain that specifically recognizes polyadenosine RNA and plays key roles in RNA export, processing, and poly(A) tail length control. Nab2 is essential for function in *S. cerevisiae* and *Drosophila* and mutations in *ZC3H14* cause intellectual disability. Importantly, a budding yeast *nab2* ZnF mutant, *nab2-C437S*, exhibits RNA hyperadenylation and impaired growth under cold stress, suggesting Nab2 could be critical for altering RNA expression to survive under cold stress and potentially other types of stress. To examine the possibility that Nab2 regulates the levels/processing of specific RNAs for survival under cold stress, we performed RNA-seq analysis on the *nab2-C437S* yeast mutant grown at cold temperature. Notably, we find that the levels of specific ribosomal protein gene paralog (*RPL*/*RPS*) mRNAs are reduced and the levels of snoRNAs and tRNAs are elevated in *nab2-C437S* cells, suggesting Nab2 may alter ribosomal function/translation for survival under cold stress. To further characterize the function of Nab2 under cold stress, we performed a high suppressor screen with the *nab2-C437S* mutant. Strikingly, we identified specific structural subunits, Rrp41 and Rrp42, of the RNA exosome complex - the conserved, barrel-like, multisubunit ribonuclease, and exosome cofactors, Nrd1 and Nop8, as potent suppressors of the cold-sensitive growth defect of *nab2-C437S*. Importantly, overexpression of Rrp41/42 exosome subunits in *nab2-C437S* cells increases the levels of ribosomal protein gene paralog mRNAs and rescues the processing of snoRNA and tRNA precursors. In support of the genetic link between Nab2 and the RNA exosome in higher eukaryotes, we find that overexpression of Rrp41/42 exosome subunits in *Drosophila nab2* mutant flies also rescues function. Combined, these data suggest a model where Nab2 cooperates with the RNA exosome, potentially as a cofactor, to alter the transcriptome for survival under cold stress. Nab2/ZC3H14 could therefore potentially act as a stress sensor that switches the 3'end processing and levels of select transcripts to survive stress.

563 Effect of His-tag sequence location in Decapping Scavenger enzymes on their structure and hydrolytic activity towards dinucleotide cap analogs.

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Decapping scavenger enzyme (DcpS) participates in 3'-5' mRNA degradation pathway, following deadenylation and exosome-mediated digestion. DcpS is a member of the HIT family of pyrophosphatases and uses a histidine triad motif (HIT) to carry out catalysis, releasing m^7GMP from short capped oligoribonucleotides or the dinucleotide cap structure. DcpS enzymes are homodimers with two independent active sites and two structural domains in each subunit: highly conserved C-terminal domain containing HIT motif and a variable N-terminal domain.

Most recombinant DcpS used in biochemical and biophysical studies were obtained as N-terminal His-tagged proteins. Here, we directly compared the hydrolytic activity of three forms of human and *C.elegans* DcpS enzymes (N-terminally and C-terminally His-tagged, and the native untagged), towards dinucleotide cap analogs (m^7GpppG, $m_3^{2,2,7}GpppG$, GpppG). Our data demonstrate that all three forms of DcpS have the same specificity towards tested dinucleotides, hydrolyzing triphosphate bridge between β and γ phosphate. However, we observed significant differences in the reaction efficiency when different forms of DcpS were used in enzymatic assays. To get insight into observed differences, in terms of structural data, we used circular dichroism spectroscopy, what allowed us to notice interesting changes in the secondary structure of studied proteins as functions of temperature.

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564 The PERK mRNA: an unexpected non-NMD-target

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Nonsense-mediated mRNA decay (NMD) was firstly described as a surveillance pathway that recognizes and rapidly degrades mRNAs carrying premature translation-termination codons (PTCs) resulted from mutations or errors in RNA processing. Many transcriptome-wide studies demonstrated that NMD also targets mRNAs transcribed from a large subset of wild-type genes, thus arising as a post-transcriptional regulatory mechanism of gene expression. Hence, NMD contributes to the regulation of many essential biological processes, including stress responses. Recent reports revealed that NMD is capable of regulating the Unfolded Protein Response (UPR) that is induced in conditions of endoplasmic reticulum (ER) stress. This is achieved, at least in part, by the degradation of the IRE1α mRNA, a natural NMD-target and one of the three primary factors of the UPR. The other two factors are ATF6 and PERK, and there are evidences suggesting that the PERK-branch can also be involved in the NMD-mediated regulation of the UPR. In this work, we intended to test if the PERK mRNA is a natural NMD-target and, if so, determine the role and relevance of NMD to the PERK-mediated response during the UPR.

By using 5'/3' rapid amplification of cDNA ends (RACE) we have confirmed the sequences and lengths of the annotated 5' and 3' untranslated regions (UTRs) of the PERK mRNA. This allowed us to confirm the presence of two possible NMD-inducing features: upstream open reading frames (uORFs) in the 5'UTR and a long 3'UTR (~100bp). To test if PERK mRNA is a direct NMD-target, we have assessed its mRNA levels and stability in conditions of impaired NMD by UPF1 (NMD central factor) siRNA-mediated knockdown in HeLa cells. Surprisingly, the UPF1 knockdown did not induce the upregulation of PERK mRNA and neither stabilize it, suggesting that PERK mRNA is not a natural NMD-target. Our data suggests that NMD does not regulate the UPR through degradation of the PERK mRNA as it does with the IRE1α mRNA. However, we cannot rule out the hypothesis of NMD acting in the PERK-branch, for instance, by degrading mRNAs that encode downstream targets of PERK.

565 Mineral nutrient-dependent translational regulation in *Arabidopsis thaliana*

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Plants are autotrophic and require essential minerals for growth. Concentrations of minerals in soils are often in inadequate ranges and to adapt such conditions, plants utilize a wide range of mechanisms to regulate root development and transport activities. We found that expression of mineral transporters are transnationally regulated in response to mineral conditions. *Arabidopsis thaliana BOR1* and *NIP5;1* encode transporters of boric acid (B), an essential element, and both are required for efficient uptake of boron from soil and transport to shoots. Both *BOR1* and *NIP5;1* expressions are regulated in boron dependent-manners and expressions are high under low-boron conditions, although mechanisms of boron-dependent regulations are different to each other. *NIP5;1* mRNA accumulates to a high level in roots under the condition of low boron in soils. This is regulated mainly through B-dependent mRNA degradation and this degradation is regulated through AUGUAA sequence in the 5'UTR of the gene. We found that ribosome stalls at AUGUAA in a boron dependent manner and this stall induces mRNA degradation (Tanaka et al Plant Cell 2016). Furthermore, we found that for the control of *NIP5;1* mRNA accumulation over a long period, transcriptional regulation plays an important roles, suggesting importance of coordination between transcriptional and translational control. In the case of *BOR1*, boron-dependent translational control also plays an important role as is the case of NIP5;1, but unlike the case of NIP5;1, longer uORFs are key for the regulation (Aibara et al 2018). To further explore the role of translational regulation at genome scale, plants exposed to low- or high-boron conditions were subjected to ribosome profiling and revealed a number of boron-dependent translational regulation phenomena.

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566 Nonsense-mediated mRNA decay regulates the exit from pluripotency

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Mouse embryonic stem cells (ESCs) capture the developmentally transient pluripotent state in vitro. If cultured under defined conditions they give rise to all three germ layers and the germ line. However, the mechanisms underlying their differentiation are poorly understood. Recent evidence, from our lab and others, suggests a role for RNA-binding proteins in this process. In particular, components of the nonsense-mediated mRNA decay (NMD) pathway have been identified in a haploid genetic screen for factors promoting the exit from pluripotency.

NMD is a quality control mechanism, which promotes the degradation of target mRNAs. We generated ESCs deficient for the NMD effectors Smg5, Smg6 and Smg7. ESCs deficient for these key NMD-factors are no longer able to differentiate properly, whereas self-renewal and ESC identity are not impaired.

In order to dissect the molecular mechanism through which NMD controls the exit from pluripotency we are performing genetic disruption combined with detailed biochemical and functional validation of potential downstream mechanisms. Moreover, to identify direct NMD targets we are assessing the NMD dependent regulation of transcriptome-wide mRNA half-life. Our data indicate that, relevant NMD targets in ESCs show an upregulated expression combined with an increase in their mRNA half-life. Finally, we identified the presence of a link between NMD and translation initiation, which we believe is directly controlling the exit from pluripotency.

567 Network of the cofactor complexes interacting with the human nuclear RNA exosome

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The RNA exosome, an evolutionarily conserved macromolecular machine, is a major 3'-5' ribonuclease in the cell, present both in the nucleus and the cytoplasm. It can either degrade or process its substrates, being involved in regulating RNA levels (RNA turnover), eliminating defective RNAs (RNA quality control), and in maturation of precursor RNAs (RNA processing). Several Mendelian diseases are associated with mutations in the human exosome complexsubunits.

Genetic, biochemical, and structural studies, carried out so far mostly on the yeast complexes, revealed that the catalytically inactive 9-subunit exosome core associates with compartment specific cofactors. In the nucleoplasm, catalytic activity is provided by the 10th subunit Rrp44 also called Dis3 – a processive 3'-5' exoribonuclease which also contains an endonuclease domain. In addition, the nuclear exosome interacts with the distributive ribonuclease Rrp6 tightly bound with its interaction partner Rrp47, the adaptor protein Mpp6, and with the helicase Mtr4, which is pivotal in formation of cofactor complexes targeting various RNA substrates to the exosome.

It remained unclear how the Mtr4 helicase works within the nuclear exosome holo-complexes to remodel substrate RNPs and to target them to the degradation machinery. Our recent cryo-EM structure of the yeast nuclear exosome complex captured on one of its major physiological substrates, a pre-60S ribosomal subunit, revealed the intricate network of the nuclear exosome cofactors and showed how the Mtr4 bridges the pre-60S particle to the exosome core (Schuller et al. 2018). Subsequent cryo-EM structures and biochemical studies of the 14-subunit human nuclear exosome complex showed a distinct DIS3 nuclease conformation, underscoring in the same time the evolutionary conservation of the MTR4 helicase recruitment and the RNA-channeling mechanisms (Gerlach et al. 2018).

Here we present an update on interactions of the human RNA exosome with its nuclear cofactor complexes.

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568 Oxidized cofactor NAD^+ promotes RNA 3′ end decay

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In eukaryotes, RNA processing and degradation are critical for the regulation of gene expression. The RNA decay machinery is diverse and differentially affected by the sequence of individual RNAs and protein factors associated with the RNA. We recently demonstrated mammalian RNAs can carry a 5'-end nicotinamide adenine dinucleotide (NAD^+) cap that promotes RNA degradation through the NAD^+ decapping (deNADding) activities of DXO or Nudt12 proteins. We now show that the role of NAD^+ in RNA decay extends beyond an NAD^+ cap. NAD^+, an essential cellular cofactor for numerous enzymes involved in cellular energy metabolism, serves a previously uncharacterized role as a cofactor that promotes RNA decay. In HEK 293T extract, the addition of NAD^+, promoted RNA decay, but surprisingly, the reduced form of NAD^+, NADH, did not. Importantly, dependence of RNA decay on NAD^+ levels was also observed in cells where RNAs transfected into cells with reduced levels of NAD^+ were more stable than control cells. These findings indicate that the redox state of a cell could affect RNA stability.

To address the mechanism by which NAD^+ could influence RNA stability, RNAs blocked at either the 5′ end with a nonhydrolyzable cap or blocked on the 3′ end by 3′end immobilization onto a column, were used. RNA blocked on the 5′ end were still susceptible to NAD^+-promoted RNA decay while RNA blocked on the 3′ end were less susceptible indicating NAD^+ promoted RNA 3′ end decay. We next focused on the predominant 3′ end RNA decay complex in cells, the RNA exosome. Importantly, the combined reduction of cellular NAD^+ levels and knockdown of the core exosome complex components, Rrp41, dramatically stabilized exosome target RNAs relative to each individual perturbation alone. Similar results were also obtained with in vitro decay assays using extract derived from the different cell backgrounds which will permit future detailed mechanistic analyses. Collectively, our data indicate cellular NAD+ levels can influence the function of the RNA exosome and in turn RNA stability and provides an important previously unforeseen link between cellular and RNA metabolism.

569 LC-MS/MS-based analysis of mRNA 5′ cap metabolism in cytoplasmic extracts of mammalian cells

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The cap structure located at the 5' end of eukaryotic mRNAs consists of N7-methylguanosine linked to the first nucleoside of the transcript by the 5',5'-triphosphate bridge. This unusual structure plays important roles at various stages of gene expression, including translation initiation, intracellular transport and pre mRNA maturation. Cap also protects mRNAs from premature degradation, thereby contributing to the regulation of its turnover. Only specialized decapping enzymes are capable of cap degradation. Although Dcp1-Dcp2 and DcpS have been identified as decapping enzymes acting in 5'-3' and 3'-5' degradation pathways, respectively, other enzymes such as hNUDT16 and Fhit have also been identified. However, the individual contributions of these enzymes to bulk mRNA decay and the fate of downstream cap metabolites such as m^7GMP, m^7GDP and others are still unclear.

In this work, we used model dinucleotide cap analogs that are potential mRNA metabolites released after 3'-to-5' decay to pinpoint cap degradation pathways in cytoplasmic extracts of HEK293 cells. To this end, we designed an LC-MS/MS method to analyze time-dependent degradation of cap dinucleotides and its potential metabolites in the extracts. To avoid interference with naturally occurring compounds and facilitate metabolite identification, [^{2}H]- and [^{18}O]-isotopologs of cap dinucleotides and 7-methylguanine mononucleotides were used to spike the extracts. As a result, new products of cap metabolism and enzymatic pathways leading to its degradation were identified. We also identified a set of chemical tools that enables selective blocking of different stages of cap metabolism. We hope that our study will contribute to better understanding of the mRNA degradation pathways and benefit rational design of cap analogs with biological or therapeutic importance.

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570 A hepatic post-transcriptional control of whole body metabolic homeostasis through CCR4-NOT deadenylase and FGF21

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Hepatokine, Fibroblast growth factor 21 (FGF21), plays a critical role in the regulation of whole body metabolism and is a therapeutic reagent that is being tested in clinical trials. However, development of FGF21-based therapies has been challenging due to mRNA and protein stability issues and significant side-effects including dysregulation in bone homeostasis. Alternative approaches to enhance FGF21 production would be of utmost clinical merit. To develop such therapies, it is essential to understand the mechanisms underlying the control of FGF21 metabolism.

Here, we have uncovered a previously unknown hepatic post-transcriptional network centered on the CCR4-NOT deadenylase, which plays a pivotal role in regulating to FGF21 expression and its effects on systemic metabolism. Under physiological conditions, nutrient uptake stimulates CCR4-NOT activity in the liver, which reduces FGF21 levels by destabilizing its mRNA. This finding is of significant medical relevance, since it unravels a novel mechanism of FGF21 suppression in response to nutrient uptake. Accordingly, we show that disruption of CCR4-NOT deadenylase activity in the liver, by targeting its catalytic subunit CNOT6L, leads to an increase in FGF21 levels, which is paralleled by a dramatic improvement of metabolic syndrome associated abnormalities. The hepatic CCR4-NOT/FGF21 axis not only impacts liver performance, but also systemically controls BAT, WAT, and pancreas functions. Most strikingly, our findings show that a modest increase in basal FGF21 levels is sufficient to ameliorate metabolic syndrome and results in more sustained effects without causing changes in bone density as compared with transient administration of FGF21.

Overall, our findings describe a new paradigm in regulation of whole body metabolism whereby a post-transcriptional network in the liver governs systemic metabolic regulation via FGF21. Furthermore, our results raise the exciting possibility that hepatic CNOT6L could serve as a future target for devising therapeutic strategies to modulate FGF21 levels.

571 Regulation of metal homeastasis mRNAs by the *Saccharomyces cerevisiae* Nonsense-mediated mRNA decay pathway

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The Nonsense-Mediated mRNA Decay (NMD) pathway is a highly-conserved mRNA decay pathway that degrades mRNAs that prematurely terminate translation. NMD regulates mRNAs containing premature termination codons as well as fully functional natural mRNAs. We are interested in NMD's effect on natural mRNAs involved in metal homeostasis, specifically copper homeostasis. Copper, being an essential micronutrient, plays a cofactor role in cellular processes, but can be toxic to the cell at high concentrations. The pathway has been found to regulate a number of mRNAs involved in copper homeostasis, including *COX17*, *COX19*, *COX23*, *MAC1*, *FRE2*, *CTR2*, *FRE2*, *CRS5*, and *PCA1*. These regulation of some of these mRNAs is sensitive to environmental conditions such as low or high levels of copper and iron. The degree and rate of regulation of the mRNAs in different conditions remains unknown. Furthermore, the NMD pathway has been shown to play important physiological roles as *S. cerevisiae* cells. A physiological role demonstrated by NMD mutants is tolerance to copper. We investigated the regulation of select mRNAs in varying environmental copper conditions. Additionally, when differential regulation of an mRNA was observed we examined the rate of change in. This kind of regulation would is important because it allows yeast cells to regulate expression of specific mRNAs in response to copper availability.

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572 Comparative RNA interactome capture as a tool to study RNP complex topology

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RNA-binding proteins (RBPs) are key factors in the post-transcriptional regulation of gene expression. They are involved in all steps of RNA processing and determine function and fate of all RNA molecules in the cell. RNA-protein crosslinking-based approaches have been very powerful in the identification and characterisation of RBPs. Efforts have been undertaken to catalogue the entirety of the RNA-binding proteome in different cell types by selective capture of polyadenylated RNAs and analysis of associated proteins by mass spectrometry. With the identification of novel RNA-binders - and in particular with the recurrent detection in RNA interactomes of proteins with well-described primary functions that are not RNA-related, such as metabolic enzymes - came the debate of how prevalent the observed interactions are in the cell. Using the fission yeast poly(A)+ RNA interactome as an example, we demonstrate that data normalised to protein abundances much better reflects the degree of RNA association, and can be indicative of the topology of RNA-binding multi-protein complexes - in short, that normalisation significantly increases the informative value of interactome capture data. We exploit the normalised S. pombe dataset to characterise recently annotated non-classical RNA-binding domains and observe that these can be subdivided into different classes based on their behaviour in the normalised interactome. We propose a refined nomenclature of non-classical RNA-binding domains that takes in vivo RNA-binding activity into account, and that groups them into the categories "classical-like", "elective", or "substoichiometric". We further show that that the normalised RNA interactome data captures aspects of the topology of large RNA-binding complexes such as the cleavage and polyadenylation factor or the RNA exosome. Using defined mutants of the nuclear exosome complex, we demonstrate that protein-RNA interactions that preferentially occur (and crosslink) when the complex is in one given conformation tend to be covariant upon distortion of the system. We propose that comparative RNA interactome capture experiments can be useful to delimit discrete topological states of large RNP complexes.

573 Quantitative proteomics revealed MTRES as a factor preventing stress-induced transcription deficiency in human mitochondria.

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Maintenance of mitochondrial gene expression is crucial for cellular homeostasis. Stress conditions may lead to a temporary reduction of mitochondrial genome copy number or/and affect mtDNA transcription, raising a risk of insufficient expression of mitochondrially encoded genes. Little is known how compensatory mechanisms operate to maintain proper mitochondrial transcripts levels upon disturbed transcription and which proteins are involved in. Here we applied a quantitative proteomic screen to search for the proteins that sustain expression of mtDNA under stress conditions. We found novel, poorly characterized protein, which we named MTRES, to be elevated in cells with perturbed mitochondrial gene expression. We show that MTRES functions as a protective factor to maintain proper mitochondrial RNAs level during transcription arrest. In vivo crosslinking and immunoprecipitation (CLIP) experiments indicated that MTRES binds RNA in vivo, which was confirmed by biochemical experiments using purified MTRES. Transcriptomic analysis and quantitative fluorescent microscopy showed that upregulation of MTRES prevents mitochondrial transcripts loss under perturbed mitochondrial gene expression. This function of MTRES involves binding of RNA by MTRES since mutated version incapable of RNA binding, designed based of structural and biochemical data, does not prevent mitochondrial RNA decrease. Functional experiments showed that MTRES acts by increasing mitochondrial transcription, without changing the stability of mitochondrial RNAs. We propose that MTRES may be an example of the protein that may have been acquired to the transcriptional machinery from the translation apparatus to protect the cell from mitochondrial RNA loss during stress.

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574 Development of inhibitors and chemical tools to study cNIIIB nucleotidase

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Human cytosolic 5' nucleotidase cNIIIB hydrolyzes several nucleoside monophosphates to corresponding nucleosides and orthophosphate. One of the preferred cNIIIB substrates is m7GMP, which is the major metabolite of mRNA 5' cap degradation. Thus, it has been proposed that cNIIIB participates in mRNA cap turnover and protects cells against undesired accumulation or salvage of m7GMP. We envisaged that properly designed inhibitors or chemical probes would help in the verification of the predicted biological functions of cNIIIB. To this end, a synthetic library of nucleoside monophosphates was screened as cNIIIB substrates and inhibitors to identify structural requirements for recognition. The inhibitors found in the initial screening were then used as leads to design second-generation compounds and determine detailed structure-activity relationship. The most potent inhibitors were investigated in more detail to verify their selectivity in the context of other m7GMP binding proteins, including eIF4E and DcpS. The compounds characterized by highest potency and selectivity were transformed into fluorescently labelled probes and affinity resins. Finally, the ability of the inhibitors to interfere with cap metabolism in mammalian cell extracts was verified to reveal compounds that are potentially suitable as tools for studying cNIIIB in living cells.

575 YTHDF2-HRSP12-RNase P/MRP coordinate to destabilize m^{6}A-containing mRNAs

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N^6-methyladenosine (m^{6}A) is the most prevalent internal modification in eukaryotic mRNAs and many studies show that m^{6}A is involved in a variety of important biological and physiological processes. Although there are many studies about m^{6}A-mediated gene regulation, the molecular mechanism underlying these processes has yet to be fully understood. Here we show that when m^{6}A-containing transcripts are bound by one of its reader proteins, YTH domain-containing family 2 (YTHDF2), an adaptor protein heat-responsive protein 12 (HRSP12) is recruited to bridge YTHDF2 and RNase P/MRP[1], which destabilizes the transcript through endoribonucleolytic cleavage. Transcriptome-wide analysis support this model by showing that target m^{6}A-containing transcripts have HRSP12 binding sites and RNase P/MRP-directed cleavage sites upstream and downstream of the YTHDF2-binding site, respectively. Furthermore, we show that this YTHDF2-mediated mRNA decay also occurs in a subset of m^{6}A-containing circular RNAs. Thus, our study expands the knowledge of m^{6}A-mediated gene regulation. For future work, we are planning to test the possibility of HRSP12 recognizing a specific structure or motif.

References

1. Park, O.H., Ha, H., Lee,Y., Boo, S.H., Kwon, D.H., Song, H.K., Kim, Y.K. Endoribonucleolytic cleavage of m(6)A-containing RNAs by RNase P/MRP complex. *Mol.Cell* **74**, (2019) https://doi.org/10.1016/j.molcel.2019.02.034

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576 Disease-linked Amino Acid Substitutions in the EXOSC2 Cap Subunit Alter RNA Exosome Interactions

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The RNA exosome is an evolutionarily conserved, 3'-5' exoribonuclease complex that is critical for both precise processing and degradation of different classes of RNAs. The RNA exosome is comprised of three cap subunits (EXOSC1-3), six core ring subunits (EXOSC4-9), and the active 3'-5' exonuclease (DIS3). Recently, mutations in genes encoding structural subunits of the RNA exosome have been linked to human disease. Mutations in the RNA exosome genes *EXOSC3*, *EXOSC8*, and *EXOSC9* cause types of pontocerebellar hypoplasia. However, mutations in the RNA exosome gene *EXOSC2* cause a distinct syndrome with various tissue-specific phenotypes including retinitis pigmentosa, premature ageing, hearing loss, and mild intellectual disability. In this study, we investigate how patient mutations that cause single amino acid substitutions in the EXOSC2 cap subunit of the RNA exosome affect interactions with other exosome subunits, exosome cofactors, and novel proteins. In a candidate-based approach, we examined interactions of EXOSC2 variants with exosome subunits and known exosome cofactors, including MTR4 and MPP6, in mouse N2a neuronal cells. In a complementary, unbiased approach to identify novel interactors, we performed a proteomic analysis of EXOSC2 variants in N2a cells. Our results reveal that EXOSC2 variants exhibit differential interactions with exosome cofactors and novel proteins, suggesting potential mechanisms that could underlie disease pathology.

577 Insight into the role of Nudt15 nudix hydrolase in RNA decay.

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Removal of the 5' mRNA cap is a prerequisite step in 5' to 3' mRNA degradation pathway. Up to date several mammalian Nudix enzymes have been implicated to hydrolase cap structure in vitro or/and in vivo, next to the initially discovered activity of Dcp2 protein, yielding m^7GDP (or m^7GMP) and corresponding decapped RNA. Nudt15 is one of the identified enzymes with decapping activity towards m^7GpppG- and GpppG-RNA substrates. Interestingly, it could also hydrolyze m^7GDP diphosphate into m^7GMP, what might suggest its role in subsequent steps of mRNA decay following decapping.

Here we will present the kinetic properties and specificity of Nudt15 for a set of differentially methylated di- and triphosphate mononucletides of guanosine, based on HPLC, differential scanning fluorimetry (DSF) and colorimetric measurements of reaction products. In the case of diphosphate analogs Nudt15 showed preference for monomethylated substrate in comparison to di-, tri- and unmethylated counterparts. Analysis of hydrolytic susceptibility of differentially capped ribooligonucleotides to Nudt15 will be also presented.

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578 Dynamic Profiling of Human RNA Decapping Protein hDcp2 Reveals the Regulation Of RNA Stability by a *cis*-Regulatory Motif

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Transcriptome-wide analysis of enzymatic RNA decay processes in human cells has been hindered by compensatory pathways and the absence of high throughput sequencing approaches that can differentiate mRNA decay from processing and transcription, as well as inhibition of RNA decay by transcriptional inhibitors. Based on the combination of 4-thiouridine metabolic labeling of RNAs and U-to-C conversion via oxidative nucleophilic-aromatic substitution, TimeLapse-seq can distinguish newly-made from pre-existing RNA pools in a single RNA-seq experiment and gives temporal resolution of dynamic changes in mRNAs, independent of potential side effects from transcription inhibitors employed in prior studies. Here we describe the application of this advanced method in the identification of hDcp2 targets, from which we unexpectedly find a previously unreported role of the MBE *cis*-regulatory motif in post-transcriptional regulation. Evidence will be presented in support of a mechanism that proceeds via protein-RNA interaction that promotes binding to mRNA decapping complex and localization to P-bodies.

579 rRNA degradation in Escherichia coli under the various nutritional conditions

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Bacterial cells of the liquid butch culture enter into the stationary phase with growth arrest when the available nutrients are exhausted, and can remain viable for several days under the starvation conditions. In the case of Escherichia coli, cells undergo physiological and morphological adaptation to the nutritional environment and perform rapid growth in the rich nutrients medium (log phase) or survive in the unfavorable condition of nutritional depletion (stationary phase).

Our previous data showed that in some nutrient condition, the amount of total RNAs in the cells of stationary phase is degraded quickly. Considering that the rRNA is the most abundant RNA in the cells, it is likely that the degradation rate of rRNA is varying according to the nutritional condition, and which may be the important strategy of physiological adaptation to the starvation stress.

To understand the change of the state degradation and turnover of RNAs in the bacterial cells, we have tried to investigate the degradation pattern of RNAs in the course of the life time of E. coli cells under the various nutrient conditions. The results showed that the ratio of mRNA and rRNA is varied according to growth stage and the nutritional environment, but the ratio of rRNA species (5S, 16S and 23S) were almost stable through cell cycle. Detail investigation of the quantification of various region of rRNAs showed that the relative amount of 3' region of 16S rRNA was unstable, suggesting that the degradation of rRNA is not uniformly occurred through the entire region of rRNA. The analysis of gene-knockout mutants of some RNases showed a similar tendency of the degradation pattern between rRNA and some mRNAs, indicating that the degradation of them share some mechanism of RNA turnover. Based on these results and transcriptome analysis, we will discuss about the bacterial adaptation to the starvation.

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580 Contribution of mRNA 3' UTRs in Substrate Recognition by the Nonsense-Mediated mRNA Decay Pathway

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The accurate transmission of genetic information during gene expression is essential for cell function and survival. For this reason, regulatory mechanisms exist to ensure fidelity is maintained throughout the process. One checkpoint at the level of the mRNA intermediate is the Nonsense-Mediated mRNA Decay (NMD) pathway, a translation-dependent process that identifies and rapidly degrades mRNAs harboring nonsense codons that direct premature translation termination. An essential attribute of this pathway is the ability of the NMD machinery to accurately discriminate between normal and 'aberrant' mRNAs.

The mechanism of how mRNA targets of the NMD pathway are identified remains unclear. Key observations, however, indicate that the core NMD factor, Upf1p, binds RNA in a length-dependent manner, and that the length of RNA sequence downstream of a termination codon is an important determinant in recognition of the termination event as premature. Consistent with this, endogenous mRNAs lacking a premature termination codon but harboring lengthy 3' untranslated regions (UTRs) downstream of a normal termination codon represent common targets of the pathway. Notwithstanding, while mRNAs with long 3' UTRs are preferential substrates, not all mRNAs with this attribute are targeted to NMD, suggesting that there are features of the RNA, in addition to the length downstream of the termination codon, important in discriminating between an NMD-sensitive and NMD-insensitive mRNA. As a means to better understand how NMD substrate selection occurs, we are characterizing long 3' UTRs from endogenous yeast transcripts to identify RNA features that elicit, or confer resistance to, recognition by the NMD machinery. Preliminary data using RNA immunoprecipitation of Upf1p confirms previous observations that there is a correlation between the 3' UTR length and the amount of Upf1p associated with NMD-sensitive mRNAs. Interestingly, reporter mRNAs harboring long 3' UTRs from NMD-insensitive genes showed a drastic reduction in their association with Upf1p, despite equivalent or longer 3' UTR lengths. This observation suggests features within these NMD-insensitive long 3' UTRs preclude association with the NMD machinery. Mechanistic studies aimed at identifying *cis*-acting sequences and/or *trans*-acting factors involved in modulating the susceptibility of endogenous transcripts to NMD will be presented.

581 The RNA helicase UPF1 remodels histone mRNPs to facilitate 3′-5′ decay of histone mRNA.

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Replication-dependent histone mRNAs lack a poly(A) tail and instead contain a 26 nucleotide stem-loop structure at their 3'-UTR. This stem loop structure, in association with a dedicated stem loop binding protein (SLBP) at the 5'-end and a 3'-5' exonuclease, 3'hExo at the 3'-end, forms a stable mRNP which acts as a roadblock to 3'-5' degradation of histone mRNAs. In addition to these proteins, the RNA helicase UPF1 was shown to be positioned in between the stop codon and stem loop structure in the 3'-UTR by mediating interactions with SLBP [1] and play a vital role in histone mRNA decay [2], [3]. We present here the biochemical reconstitution of an mRNP comprising of the above-mentioned proteins with an aim to understand UPF1 function and regulation in context of the histone mRNP. We have investigated the intricate network of protein-protein/RNA interactions within histone mRNP complex and have identified novel interacting partners that play an important role in mediating efficient histone mRNA decay. Our studies clearly define a role for the RNA helicase UPF1 in this decay pathway, which is to remodel the histone mRNP and unwind the stem-loop at a precise time-point, enabling limited degradation of the histone mRNA by the exonuclease 3'hExo.

[1] L. Brooks et al., "A multiprotein occupancy map of the mRNP on the 3′ end of histone mRNAs," RNA, vol. 21, no. 11, pp. 1943-1965, Nov. 2015.

[2] H. Kaygun and W. F. Marzluff, "Regulated degradation of replication-dependent histone mRNAs requires both ATR and Upf1," Nat. Struct. Mol. Biol., vol. 12, no. 9, pp. 794-800, Sep. 2005.

[3] S. A. Meaux, C. E. Holmquist, and W. F. Marzluff, "Role of oligouridylation in normal metabolism and regulated degradation of mammalian histone mRNAs," Philos. Trans. R. Soc. B Biol. Sci., vol. 373, no. 1762, p. 20180170, Dec. 2018.

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582 Strategies for studying the roles of NMD factors in human induced pluripotent stem cells

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Nonsense-mediated mRNA decay (NMD) is a eukaryotic RNA degradation pathway that modulates the abundance of 3-10% of all cellular mRNAs in mammalian cells. NMD targets for degradation faulty mRNAs with premature termination codons as well as many physiological mRNAs that encode full-length functional proteins. Consequently, NMD is conceived not only as a quality control mechanism but also as a post-transcriptional regulator of gene expression, and it has been implicated in a wide range of biological processes. To gain further insight into the roles of NMD factors during cellular differentiation, we aim at establishing two different approaches to conditionally deplete NMD factors in human induced pluripotent stem cells (hiPSCs). In one approach, we are establishing an auxin-inducible degron (AID) system, which is a ligand-inducible system that enables the rapid degradation of AID-tagged proteins in mammalian cells. The system relies on the heterologous expression of the plant F-box protein Tir1, which can interact with endogenous ubiquitin ligases SCF to form the SCF-Tir1 complex. In the presence of the ligand (auxin), the SCF-Tir1 complex can promote the proteasomal degradation of AID-tagged proteins (1). As a second approach, we are establishing a tetracycline-inducible system for knockdowns and rescues based on pKK-RNAi vectors (2). These vectors allow the simultaneous expression of i) an RNA interference (RNAi) cassette against the gene of interest and ii) an mRNA coding for an RNAi-resistant version of the target gene. We will present the genome editing strategy and preliminary results for both approaches.

(1) Nishimura K. et al. (2009) Nature Methods 6:12:917-922

(2) Szczesny R.J. et al. (2018) PLoS ONE 13(3): e0194887

583 Processive mode of action and substrate recognition are intertwined processes during the hydrolytic cycle of poly(A)-specific ribonuclease (PARN)

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Poly(A)-specific ribonuclease (PARN) is a processive poly(A) degrading exoribonuclease. It degrades poly(A) tails present on a subset of mRNAs and non-coding RNAs, including among others certain snoRNAs, miRNAs and precursor rRNAs. Human patients with genetic lesions in *PARN*suffer from a spectrum of syndromes called telomere biology disorders (TBD), which are associated with short telomeres. Here, we have investigated molecular mechanisms behind PARN's preference to degrade poly(A). We have used enzyme kinetics and divalent metal ions as mechanistic probes to show that PARN's poly(A) specificity is tightly linked to a translocation event during the hydrolytic cycle of PARN action. To understand the mechanism of PARN's processive mode of action we are developing a kinetic model that will allow us to study the probability of processive action for each round of the hydrolytic cycle. Our kinetic model will be general and applicable to the processive action of any processive enzymatic activity. In conclusion, our study has so far established a mechanistic link between PARN's processive mode of action, hydrolytic activity and preference for degrading poly(A).

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584 Post-transcriptional regulation of maternal mRNAs by NMD factor, Upf1

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Upf1 is an ATP-dependent RNA helicase known for its canonical role during nonsense-mediated mRNA decay (NMD). Upon splicing, it dynamically associates with the exon junction complex (EJC) and recruits downstream factors, including exoribonucleases, that degrade transcripts (1) harboring a premature termination codon (PTC) or (2) to downregulate endogenous levels of a certain gene. During *D. melanogaster* oogenesis, core EJC factors have been shown to regulate a key maternal transcript, *oskar*, but Upf1's role in this process is poorly understood. Here we characterize Upf1's localization pattern during oogenesis and propose a role for it in clearing *oskar* mRNA following bulk translation. Additionally, we find that the MAP kinase cascade that regulates *gurken* mRNA translation may also be affected when one of Upf1's targets, *gadd45*, is not properly degraded.

585 Structural studies of the yeast mitochondrial RNA degradosome

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Degradation pathways play a key role in RNA metabolism, from the regulation of gene expression to the efficient removal of defective RNA molecules. The main RNA-degrading enzymes are processive exoribonucleases that together with other proteins can organize into macromolecular complexes gaining new functions. In yeast the main executor of mitochondrial RNA degradation is the mtEXO complex composed of Dss1 3′-to-5′ exoribonuclease and Suv3 helicase that act in concert and efficiently remove defective RNAs and excised introns. Crystal structure of Dss1 from Candida glabrata reveals it is a unique member of the RNB superfamily of ribonucleases with specialized domains responsible for interactions with Suv3 helicase. The arrangement of both subunits deciphered in the crystal structure of the complex enables the helicase motor to feed the 3′ end of the RNA into the catalytic channel of Dss1 for effective degradation. This co-operation of both helicase and nuclease activities within the complex is particularly important for degradation of structured RNAs which cannot be handled by Dss1 on its own and for which the unwinding activity of Suv3 is required [1].

References

[1] Razew, M. (2018) Nature Communications 9:97

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586 Cell-to-cell variability in Nonsense-mediated mRNA decay

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Nonsense mediated mRNA decay (NMD) is an mRNA degradation pathway that eliminates transcripts containing premature termination codons (PTCs). The measurements of mRNA half-live have detected subpopulations of mRNA that escape NMD. However, it is not known whether NMD escape represents variability of mRNA degradation efficiency within cells or variability in the cell population. Here we demonstrate a single-cell approach with a bi-directional reporter construct, which expresses two β-globin genes with and without PTC in the same cell, to characterize the efficiencies of NMD and the variabilities in cells. Using this system, we found a broad range of NMD efficiencies in the cell population; some cells degrade 95% of the mRNAs, while other cells escape NMD almost completely. We also found that NMD efficiency does not correlate with the level of mRNA expression. Our findings argue that the cells have distinct NMD efficiencies within the cell population instead of a heterogeneous population of mRNA molecules, some of which escape NMD.

587 Withdrawn

588 Hidden networks: probing the non-canonical functions of Arabidopsis SMG7

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SMG7 is an evolutionary conserved phosphoserine-binding protein and its primary function is linked to nonsense mediated RNA decay (NMD). Nevertheless, SMG7 proteins underwent independent multiplications during the course of evolution indicating their propensity to adopt novel functions. Our functional studies in Arabidopsis showed that while SMG7 has retained its role in NMD, in addition it evolved another function in meiosis and germ line differentiation. Arabidopsis SMG7 mutants fail to exit meiosis and instead, attempt to perform third meiotic division. As consequence, these mutants form aberrant spores and largely infertile. The meiotic function of SMG7 appears to be distinct from its function in NMD. With the aim to decipher the non-canonical function of SMG7 in meiosis, we conducted a forward genetic suppressor screen in the background of *smg7-6* mutants that exhibit reduced fertility, to identify plants that revert to full fertility. In the suppressor screen of *smg7-6*, we identified a mutation in the EVH1-like domain in decapping 1 (DCP1) gene, a critical component of mRNA decapping complex. The DCP1 EVH1-like domain is highly conserved among eukaryotes and is predicted to have proline-rich sequences (PRS)-binding activity, suggesting it acts like a protein-protein interaction module that recruits specific proteins to link decapping with decay. Our phenotypic analysis showed that the *dcp1* mutation is among the strongest suppressors of the meiotic defect of *smg7*. This indicates that RNA turnover is a critical determinant of meiotic progression and anther development in plants. Further studies are underway to probe the mechanisms, which links SMG7 and DCP1 in meiosis.

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589 A macromolecular microRNA turnover complex from *Caenorhabditis elegans*

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MicroRNAs (miRNAs) are endogenous small non-coding RNAs, involved in regulation of gene expression by modulating post- transcriptional expression of large number of mRNAs. Regulation of these small regulators plays an important role in determining their functionality. Recent reports indicated that differential turnover plays an important role in determining their abundance, and thus functionality of miRNAs. The biogenesis pathway of miRNAs has been extensively studied, but very little is known about the basic miRNA turnover pathway. Notably in *Caenorhabditis elegans* it has been shown that miRNA turnover is a stepwise process, where the degradation of miRNA is modulated by their targets. The miRNA turnover was reported to be an active process in *Caenorhabditis elegans*; where XRN-2 has been shown as a 'miRNase' and later PAXT-1, a co-factor exerting a stabilizing effect on XRN-2. XRN-2 has been implicated in the processing and turnover of wide variety of RNA substrates. Therefore, in order to understand the mode of operation of XRN-2 in its actual molecular niche and decipher its regulatability, XRN-2 was purified from endogenous sources as a component of a macromolecular protein complex using a novel biochemical strategy. Although recombinant XRN-2 acts on variety of different RNA molecules, the complex is active on specific miRNAs and not on other groups of RNAs. The miRNA- binding receptor component of the complex was not only found to be important for worm development and physiology, but also provided *in vivo* substrate specificity to the complex which was further validated with its *in vitro* activity. Assays with the purified complex in an isolated system revealed its mode of action, where the newly identified components govern the function of XRN-2.

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590 Regnase-1 and Roquin - partners in an evolutionarily conserved RNA decay mechanism

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Regnase-1 and Roquin are mammalian RNA binding proteins (RBPs) essential for many aspects of immune regulation. Regnase-1, also known as Zc3h12a or MCPIP1, acts as an endoribonuclease responsible for the degradation of mRNAs involved in inflammatory responses. Many of its mRNA targets are also regulated by another RBP, Roquin. However, the functional relationship between Regnase-1 and Roquin is not yet fully understood. The two proteins have been originally suggested to cooperate in T cells, and this partnership requires the nuclease activity of Regnase-1, and the RNA binding ability of Roquin. More recently, however, studies in other cell types, showed that Roquin and Regnase-1 control common mRNAs, but they do so in different sub-cellular compartments and by different mechanisms.

We have recently reported that the *C. elegans* homolog of Regnase-1, which we called REGE-1, contains the same functional domains as the human homolog and also acts as a cytoplasmic endonuclease. The key mRNA target of REGE-1, *ets-4*, encodes a fat loss-promoting transcription factor. Using *in vivo* studies in worms, we showed that the REGE-1 mediated degradation of *ets-4* also requires the nematode counterpart of Roquin, called RLE-1. Remarkably, using human cells, we found that RNA elements, which direct the REGE-1 and RLE-1 mediated degradation of *ets-4* mRNA, also mediate mRNA silencing by mammalian proteins. Thus, we have uncovered an evolutionary conserved functional relationship between Regnase-1 and Roquin in RNA decay, potentially indicating the general importance of their collaboration for various cellular functions, which we can now address using a simpler experimental model.

591 Assessing in vivo consequences of disease-linked RNA exosome mutations using a budding yeast model.

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The RNA exosome is a conserved, riboexonuclease complex that processes/degrades numerous coding and non-coding RNA classes. The 10-subunit core exosome forms a ring-like structure composed of three S1/KH cap subunits (yeast Rrp4/40/Csl4-human EXOSC2/3/1), a lower ring of six PH-like subunits (yeast Rrp41/42/43/45/46/Mtr3-human EXOSC4/7/8/9/5/6), and a 3'-5' riboexonuclease subunit, Rrp44/DIS3, at the base. The nuclear RNA exosome contains an eleventh, cap-associated riboexonuclease subunit, Rrp6/EXOSC10. Recently, mutations in four exosome structural subunit genes, EXOSC2, EXOSC3, EXOSC8, and EXOSC9 have been linked to tissue-specific human diseases. Mutations in EXOSC2 cause a novel syndrome characterized by retinitis pigmentosa, hearing loss, premature aging, and mild intellectual disability. In contrast, mutations in EXOSC3 and EXOSC8 cause pontocerebellar hypoplasia type 1b and 1c, respectively - autosomal recessive diseases characterized by cerebellar hypoplasia and neuronal degeneration that lead to early mortality - and mutations in EXOSC9 cause cerebellar atrophy. To gain insight into the functional consequences of the mutations in EXOSC2/3/8/9 identified in patients, we generated the corresponding mutations in the S. cerevisiae genes RRP4/40/43/45 and examined their function in vivo. We find that rrp variants cause differential effects on cell growth and RNA exosome function, which could shed light on why impairments in different exosome subunits give rise to distinct disease phenotypes. We hypothesize that differences in disease phenotypes could reflect altered interactions with RNA exosome co-factors and/or misprocessing/accumulation of specific RNA exosome target RNAs. To explore the possibility of altered co-factor interactions, we have assessed genetic interactions between RNA exosome mutations in budding yeast and co-factor deletions (MPP6, RRP47, RRP6) and used a non-biased high copy suppressor screen to identify functionally important interactions. To explore the possibility of altered RNA processing or degradation, we have used qPCR to assess levels of specific RNA exosome targets. In addition, we have used RNA-sequencing to assess global transcript levels comparing our budding yeast RNA exosome mutant models. Ultimately, these results can provide insight into both the function of the RNA exosome and the mechanisms that underlie the puzzling disease phenotypes associated with each identified mutation.

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592 Exploring how the caspase-mediated cleavage of HuR alters its regulation of apoptosis

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The RNA-binding protein HuR is well-established as a post-transcriptional regulator for a variety of functions, including cell survival and apoptotic cell death. We previously showed that severe stress causes HuR to be exported to the cytoplasm while associated with the apoptosome activator pp32/PHAPI, and accumulates there through a loss of binding with its import factor, Transportin 2. Cytoplasmic HuR is then cleaved at Asp226 by caspases-3 and -7 through a PKR/FADD-dependent pathway, to generate two cleavage products (HuR-CPs).

These cleavage products have distinct characteristics from full-length HuR, which may help explain how HuR can regulate the opposing processes of survival and death. For example, under normal conditions, full-length HuR associates with and promotes the expression of caspase-9 and Prothymosinα(ProT) mRNAs, pro- and anti-apoptotic factors respectively. When apoptosis is engaged however, HuR enhances the protein expression of caspase-9, but not ProT. This selective effect is due to the generation of the HuR-CPs, which associate with and stabilize caspase-9 mRNA.

We have expanded on these results by now exploring which other mRNAs differentially associate with HuR and its CPs, to understand more fully the role of the cleavage products. As we have also shown that the HuR-CPs can bind to proteins to different extents when compared to full-length HuR, we have also begun investigating which protein partners may contribute to the pro-apoptotic effects of cleaving HuR.

Collectively, our results point towards mechanisms whereby HuR cleavage promotes apoptosis through protein partners and the regulation of particular mRNA targets.

593 The Achilles heel of LINEs. A newly discovered regulatory mechanism of human mobile genetic elements.

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There is a growing experimental evidence of non-templated nucleotide additions to RNA 3' ends. Among these uridylation by terminal uridyltransferases has come into focus as an important player in regulation of RNA stabilities and functionalities. The scope of RNAs regulated by uridylation is broad and includes cytoplasmic coding RNAs, non-coding structured RNAs and retrotransposon RNAs. The latter are RNA intermediates in a life cycle of mobile genetic elements, retrotransposons, that can propagate in the human genome by a copy-and-paste mechanism called retrotransposition. Besides shaping human genome in an evolutionary time scale also retrotransposonal RNAs impact cellular homeostasis with confirmed roles in autoimmunity and senescence. This report summarizes a newly discovered uridylation-mediated LINE-1 regulatory mechanism and discusses its potential role in LINE-1 insertional activity and impact of LINE-1s onto cellular homeostasis.

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594 The role of MCPIP2 in the human neuronal model cell line - SH-SY5Y.

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The MCPIP family of proteins contains four members. Similarly to the founder member of this family, MCPIP1 (also called Regnase-1), the other proteins, including MCPIP2, contain a NYN/PIN-like ribonuclease domain and a CCCH-type zinc finger. Our data indicate that all MCPIP proteins are able to promote the decay of a common subset of transcripts including *IL-6*, *IER3* and *MCPIP1*. The observed functional redundancy is surprising taking into account the very divergent phenotypes of mice lacking MCPIP1 and MCPIP4 coding genes. The former develop a systemic inflammation and die soon after birth whereas the latter develop without problems. One possible explanation of these discrepancies may be the tissue specific expression of these proteins. The highest level of *MCPIP1* mRNA is detected in bone marrow and *MCPIP4* transcript is predominantly expressed in appendix, lymph nodes and spleen. *MCPIP3* seems to be present in most tissues at quite a similar level while *MCPIP2* is expressed mainly in brain. Moreover, the expression of the MCPIP-family proteins seem to be regulated differently.

Recently we found that MCPIP2 is able to bind and destabilize *IL-6* mRNA. Using luciferase reporter constructs we have shown that MCPIP2-induced decay of IL-6 depends on presence of a specific, highly conserved stem-loop structure located in the 3'UTR of this transcripts. The ability of target destabilization by MCPIP2 depends on the presence of a functional NYN/PIN-domain. Expression analysis of *MCPIP2* mRNA in different human tissues reveals its high abundancy in the brain. From the analysed cell lines of neural origin the highest level of *MCPIP2* transcript is found in the SH-SY5Y cell line. Interestingly, the expression of MCPIP2 at mRNA level can be further increased in these cells upon differentiation towards neuron-like phenotype. Lentivirus based knock-down of MCPIP2 in SH-SY5Y cells correlates with increased levels of its target mRNAs. Microscopic analysis of SH-SY5Y and HeLa cells shows that MCPIP2 is a cytoplasmic protein excluded from the cell nucleus that forms granule-like structures characteristic for other known proteins involved in mRNA decay control.

595 Down-regulation of different ribonuclease affect gene expression of multiple biological pathways and indicate functional diversity of ribonuclease

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All organisms contain many kinds of ribonuclease (RNase). RNases play key roles in RNA metabolism and RNA maturation in cells. Over the years, this has overshadowed the true versatility and importance of RNase in gene expression regulation and other biological functions. Our studies indicate that exogenous RNase A affects cell morphology and migration, RNase L is involved in the process of DNA damage repair in radiation damaged cells, Angiogenin (ANG) plays an important physiological role in cell stress response by cleaves tRNAs into tRNA halves. siRNA interference down-regulates the expression of RNAse L, RNaseT2, ANG, AGO2, Dicer and Drosha in A549 cells and the differential gene expression profiles were obtained by high-throughput sequencing. Bioinformatics analysis of high-throughput sequencing data revealed that different types of RNase affect multiple biological pathways including cellular metabolic pathways. These results imply that RNase plays an important physiological role in cells, except for the known function.

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596 The effect of the Perlman syndrome DIS3L2 exoribonuclease in the regulation of gene expression

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The 3'-terminal uridylation is catalyzed by the terminal uridyltransferases (TUTases). It is a widespread RNA modification mediating processing and/or degradation of various RNAs in most eukaryotes. Aberrant uridylated transcripts are specifically recognized by the Perlman syndrome exoribonuclease DIS3L2. In 2016 others and we demonstrated, that TUT-DIS3L2 (TDS) is a conserved RNA surveillance pathway in the cytoplasm [1,2,3,4]. TDS targets mostly aberrant noncoding RNAs. In addition, our studies uncovered uridylated transcripts corresponding to 5' termini of protein-coding genes (5' mRNA fragments, 5'mRFs). These fragments appear to originate from aberrant transcription initiation because the uridylation position overlaps with the position of stalled RNA Polymerase II.

In our follow up study, we investigate the process leading to the 5'mRFs formation, their uridylation dynamics and putative function in the cell. We are able to reconstitute their production from a heterologous reporter system. Our quantitative sequencing analyses show that 5'mRFs are formed in the nucleus. However, only after the export to the cytoplasm, they are targeted by the TDS. To test whether 5'mRF formation affects gene expression, we performed differential transcriptome-wide analysis of long and small RNAs from control and DIS3L2 K.O. cell lines. Next, we used the RNA-based immunoprecipitation to identify factors involved in the 5'mRF biogenesis and turnover. In summary, we will present a comprehensive analysis of the biogenesis and quality control of aberrantly terminated PolI transcripts in human cells.

1. Ustianenko D., et al. (2016), EMBO J
2. Pirouz M., et al. (2016), Cell Rep
3. Łabno A., et al. (2016), NAR
4. Reimão-Pinto MM.,et al. (2016), EMBO J

597 ARF1 SBS - STAU1 complex structure uncovers target recognition by staufen1.

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RNA elements in mRNAs recognized by RNA-binding proteins (RBPs) are vastly important in posttranscriptional regulation of gene expression. RNA structure in 3'UTRs of mRNAs represents an additional information to primary sequence elements. Structures in 3'UTRs can be bound with double-stranded RBPs (dsRBPs) which in turn apply their function based on recognition of dsRNA target through a structure and sequence combination. One of dsRBP involved in mRNA transport and localization is a Staufen1 (STAU1). It provides translational control and mRNA decay by a STAU1- mediated mRNA decay (SMD) pathway. One of the targets is the STAU1 binding site (SBS) within human ADP-ribosylation factor1 (ARF1) 3'UTR. Binding of STAU1 to the SBS provides a regulation ARF1 cytoplasmic mRNA levels by the SMD pathway. Nethertheless, exact way of recognition of specific mRNA targets by STAU1 is still unrevealed.

The ARF1 SBS - STAU1 complex structure helps to understand target recognition by STAU1. We found that STAU1 dsRNA binding domain (dsRBD) 4 interacts with two pyrimidines and one purine from the minor groove side via helix α1, β1-β2 loop anchors the dsRBD at the end of the dsRNA. Simultaneously lysines in helix α2 bind to the phosphodiester backbone from the major groove side. The same binding mode with specific recognition of one guanine base is observed for STAU1 dsRBD3. Minor groove recognition of ARF1 SBS disrupted by mutations reduce SMD in vivo but have minor effect on in vitro binding. Thus our data demonstrate how various functions in gene expression pathways can be mediated by STAU1 recognition of dsRNA.

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598 Human cytomegalovirus harnesses multiple RNA-processing machineries for efficient viral replication

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RNA represents a pivotal component of the host-pathogen interactions. Human cytomegalovirus (HCMV) infection causes extensive alterations in host RNA metabolism, but the functional relationship between the virus and cellular RNA processing remains largely unknown. HCMV harbors multiple viral genes that modulate cellular mRNA processing, with some host cell genes capable of positively or negatively regulating viral infection. To identify genes essential for HCMV lytic production, we performed an RNA interference-screening experiment using genes involved in cellular RNA processing. Through loss-of-function screening, we show that HCMV requires multiple RNA-processing machineries for efficient viral lytic production. In particular, cellular RNA-binding protein Roquin, whose expression is actively stimulated by HCMV, plays essential roles in inhibiting innate immune responses. Transcriptome profiling of HCMV-infected cells reveals Roquin-dependent global downregulation of proinflammatory cytokines and antiviral genes. Furthermore, using CLIP-seq, we identify *interferon regulatory factor 1* (*IRF1*), a master transcriptional activator of immune responses, as a Roquin target gene. Roquin reduces IRF1 expression by directly binding to the 5'-UTR of its mRNA, thereby enabling suppression of a variety of antiviral genes. This study demonstrates how HCMV exploits host RNA-binding protein to prevent cellular antiviral response and offers mechanistic insight into the potential development of CMV therapeutics.

599 Non-coding RNAs directly interact with key pluripotent transcription factors in human embryonic stem cells

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In recent years non-coding RNAs, specifically long non-coding RNAs (lncRNAs) are emerging as important players in transcriptional and post-transcriptional processes. Moreover, it has been demonstrated that lncRNAs have many different roles in pluripotency and differentiation of human embryonic stem cells (hESCs). Employing an RNA interactome capture experiment, conducted in nuclear extracts from hESCs, we noticed a significant enrichment of proteins involved in transcriptional regulation and chromatin organization, many not well known to bind (coding or non-coding) RNAs. Among these proteins, we observed 40 transcription factors (TFs), including key pluripotent factors. To validate these findings and identify the RNA targets, we performed eCLIP (enhanced crosslinking and immunoprecipitation) for two key pluripotency TFs OCT4 (octamer-binding transcription factor 4) and STAT3 (signal transducer and activator of transcription 3). Consistent with their known role as TFs, we found that both STAT3 and OCT4 interact with only a small subset of RNAs, showing a highly specific binding pattern. Among the RNA targets, we identified the telomerase RNA component (TERC), which was found to bind OCT4 and the lncRNA NORAD (non-coding RNA activated by DNA damage) that was specifically associated with STAT3. Overall, our results support the notion that non-coding RNAs can mediate transcriptional regulation via directly binding to transcription factors. We propose that such interactions involving key pluripotent TFs may play critical roles in maintaining pluripotency in hESCs and in differentiation.

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600 Regulatory cascades underlying kidney development; A WT1 perspective

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Abstract:

Regulation of gene expression occurs at the transcriptional as well as the post- transcriptional level, which determines RNA turnover and protein output, in health and disease. RNA binding proteins (RBPs) are essential components of this regulatory network, orchestrating and fine-tuning cellular processes during differentiation and development.

Wilms' tumour 1 (WT1) is one such unique, transcription factor and a tumour suppressor protein, essential for the development and homeostasis of multiple tissues derived from the intermediate and lateral plate mesoderm. Its transcription factor ability regulates, the essential process of Epithelial-Mesenchymal Transition (EMT) and the reverse MET. WT1 has been hypothesized to be an RBP, hence, we generated an unbiased Global RNA-protein Interactome Map to identify endogenous WT1 RNA targets. This led to the identification of a role for WT1 in regulating stability of developmental mRNAs through secondary structure interactions at the 3' UTR.

Furthermore, we have now identified WT1 to be involved in the microRNA let7 pathway, thus correlating the genetic evidence and molecular mechanism underlying the aetiology of Wilms Tumour (WT). In humans, germ line WT1 mutations lead to the eponymous pediatric cancer, genitourinary anomalies and in some rare cases, heart disease. WT is a cancer where nephrogenesis is impaired. In an attempt to understand the role of regulatory events in the context of nephron progenitor cell lineage, CRISPR/Cas9 mediated genome editing of WT1 isoforms, followed by functional assays have been ongoing. This has now led to the elucidation of possible WT1 isoform specific regulatory cascade in the context of kidney development with potential implications in disease.

601 Stress-induced changes to the RNA binding proteome in yeast

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Unicellular organisms, such as *Saccharomyces cerevisiae*, respond to changes in nutrient availability and environmental stress by rapidly reprogramming their gene expression. In part, this response is mediated by RNA-binding proteins (RBPs), which direct global changes in RNA transcription, translation, and turnover. The RBPome has previously been investigated using techniques involving UV crosslinking and oligo(dT) selection, which take advantage of the mRNA poly(A) tail to affinity purify proteins crosslinked to mRNAs. However, most cellular transcripts are not polyayadenylated and proteins binding to these RNAs will escape detection. We therefore developed TRAPP (total RNA-associated proteome purification) to identify the total RNA binding proteome (Shchepachev et al., bioRxiv doi.org/10.1101/436253). In TRAPP, RNP complexes are UV-crosslinked in vivo, denatured, and isolated through their RNA component using silica beads. Subsequently, crosslinked proteins are detected and quantified using SILAC-MS/MS. The related iTRAPP technique identifies precise sites of protein-RNA interaction.

We applied TRAPP to yeast cells exposed to various cell stresses, including heat shock, glucose withdrawal, osmotic shock, and rapamycin treatment. In the minutes following each stress, we observed robust changes in RNA binding for dozens of proteins, without altered protein abundance. Surprisingly, each stress generated a relatively distinct response profile. For example, several RBPs targeting cell wall-related mRNAs were specifically altered only following heat shock. Ribosome biogenesis factors showed globally reduced RNA binding after heat shock or inhibition of the TOR pathway, but only a handful were altered following glucose starvation.

By contrast, translation initiation factors appeared to be a common target across multiple stresses. In particular, the translation initiation factors eIF4A and eIF4B showed especially reduced RNA binding, suggesting these proteins could mediate translation shutoff during stress. Analysis of eIF4A/B RNA targets after cell stress revealed greatly decreased binding to the 5′ regions of almost all mRNAs, consistent with a general shutdown in cap-dependent translation. For eIF4A, binding to mRNA was lost within 30sec of glucose withdrawal. Analysis of multiple signaling pathway mutants implicated the PKA pathway in loss of eIF4A/B binding to RNA during stress. We are currently investigating the mechanism by which PKA and other signaling pathways regulate eIF4A/B in response to stress.

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602 RNA secondary structures bound in vivo by Staufen 2 across mammalian brain development

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Interactions between RNA and associated *trans*-acting factors, notably RNA binding proteins, are important for post-transcriptional regulation. The structure of RNA molecules plays an important role in this interplay. The Staufen protein family binds double-stranded RNA and is highly conserved, with important roles in mRNA localisation, stability and translation. It is known that Staufen proteins contain multiple domains that bind to RNA duplexes, held together primarily with complementary base-pairing. However, their mechanism of action and the properties of their interactions with *in vivo* RNA structures remain poorly understood. To investigate these in a physiological context, we studied RNA secondary structures that are bound by the neuron-specific Staufen 2 in rat cerebral cortex at multiple developmental stages.

We previously developed the hiCLIP technique (RNA hybrid individual-nucleotide resolution UV cross-linking and immunoprecipitation) to identify *in vivo* RNA duplexes bound by Staufen proteins. Now for the first time, with iterative experimental and computational improvements, we have succeeded in performing hiCLIP in primary tissue. We identify an atlas of Staufen 2 bound duplexes in rat cerebral cortex. The majority of these structures are in *cis*, with thousands of unique structures in mRNA transcripts. These are predominantly located within the long 3' untranslated regions (UTRs) present in neurons and can bring together regions that are kilobases apart. Their arrangements are more complex than those previously identified in HEK293 cells, with multiple long-range duplexes suggesting alternative or intercalated structures formed within these long 3' UTRs. Furthermore, by examining multiple developmental stages we show quantitatively how usage of these structures is dynamic across brain development. Finally, we integrate our findings with newly generated RNA-seq data and publicly available orthogonal data, including localisation and stability studies, to gain insight into the possible functions of these Staufen-bound structures.

Altogether, the RNA secondary structures bound by Staufen 2 that we detect here may reflect hitherto underappreciated complex 3' UTR structural configurations and appear to be physiologically relevant, changing across brain development.

603 Metaserver for the docking of RNA-protein complexes

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RNA-protein (RNP) interactions play pivotal roles in various biological processes, such as protein synthesis, regulation of gene expression, RNA splicing, transport, storage, and stabilization. To understand the functional and mechanistic details of these processes, it is essential to have information about the three-dimensional structures of these complexes. The inherent flexibility of RNA molecules and the transient nature of these complexes makes it technically difficult to determine these structures experimentally[1]. The computational docking of RNP structures is an alternative, and consists of two steps: i) conformational sampling, i.e., searching for possible conformations and mutual orientations of the docking components to the generate decoys, and ii) scoring of decoys: a mathematical function used to assess decoys to distinguish the ones with different degrees of similarity to the unknown "true" structure[2]. The existing docking methods can be broadly classified as i) rigid docking algorithms that do not account explicitly for conformational changes and ii) flexible docking algorithms which attempt to account for the conformational changes. A major challenge in RNP docking is molecular flexibility[3] and computational complexity associated with flexible docking. Generating conformations similar to the bound conformation from the starting structures, and discriminating them from others is a challenging task[4].

Here, we propose a meta-predictor that combines various existing methods for docking and scoring to obtain biologically, chemically and physically relevant predictions. Such meta-predictions were successfully applied previously for modeling protein structures[5] and protein-protein docking[6]. RNP docking is performed using different methods, and the top scored docking poses from each of these is rescored by different scoring functions. If the scoring methods reach a consensus, then decoys obtained from different methods are clustered together. However, in the absence of a consensus scoring, top models proposed by different methods are suggested as alternative solutions.

References: 1 Ponce-Salvatierra, A. et al. Biosci. Rep. 39, (2019); 2 Vajda, S. et al. Proteins 81, 1874-1884 (2013) 3 Mukherjee, S. et al. Sci. Rep. 8, 10546 (2018); 4 Nithin, C. et al. Genes 9, (2018); 5 Kurowski, M. A. et al. Nucleic Acids Res. 31, 3305-3307 (2003); 6 Anashkina, A. A. et al. Bioinformatics (2017)

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604 A global binding map of hnRNP A2/B1

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Correct splicing of exons with weak splice sites depends on a tight balance between positive and negative splicing regulatory elements (SREs). A large fraction of disease-causing mutations disrupt mRNA splicing by decreasing splice site strength or disrupting/creating SREs. We used eCLIP (enhanced crosslinking and immunoprecipitation) and targeted RNA immunoprecipitation (tRIP) combined with next generation sequencing to create an in vivo binding map for the splicing regulatory protein hnRNP A2/B1. This map can be used to identify important SREs that function through hnRNP A2/B1 binding, and which can potentially serve as binding sites for splice switching oligonucleotides (SSO) to modulate splicing.

HeLa cells with inducible expression of T7-tagged hnRNP A2/B1 were UV irradiated generating irreversible crosslinks between RNA and RNA binding proteins allowing stringent purification of the bound RNA. The eCLIP and tRIP libraries were subject to next-generation sequencing. We correlated the hnRNP A1/B2 binding map with hnRNP A2/B1-regulated exons identified by hnRNP A2/B1 knockdown and RNA-seq to identify regulated exons and the responsible SREs. Furthermore, we employed Surface Plasmon Resonance imaging (SPRi) to validate the hnRNP A2/B1 binding sites discovered by eCLIP and tRIP, as well as elucidating the RNA-protein interactions by investigating the kinetics of protein association and dissociation with RNA oligonucleotides of interest.

The heterogeneous nuclear ribonucleoprotein particle (hnRNP) A2/B1 has been widely associated with human disease, including several cancers and amyotrophic lateral sclerosis (ALS). Our project may therefore generate new knowledge, which can be important for development of new treatments for these severe diseases.

605 U7 snRNP, FUS and hnRNP UL1 interact with each other out of the S phase of the cell cycle in human cells

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U7 snRNP and FUS are positive regulators that activate replication-dependent histone gene expression in the S phase of the cell cycle (1). In contrast, in G1 and G2 phase FUS interacts with heterogeneous ribonucleoprotein (hnRNP) UL1 (1). hnRNP UL1 is known to repress histone gene expression by interaction with U7 snRNP in cell cycle-arrested condition (2). Therefore, we suggested that FUS can mediate U7 snRNP/hnRNP UL1 complex assembly outside of S phase in order to inhibit histone synthesis that could be harmful to the cell.

By immunoprecipitation (IP) and proximity in situ ligation assay (PLA) techniques, we confirmed that FUS and hnRNP UL1 interact differently in different phases of the cell cycle. Moreover, we figured out, that posttranslational modifications of proteins are also diversified during the cell cycle, thus they might determine the binding affinity. Finally, we determined which domain of FUS and hnRNP UL1 are required for mutual interactions as well as for interaction with U7 snRNA.

1. Raczynska et al., 2015, Nucleic Acids Res. 43(20):9711-28.
2. Ideue et al., 2012, PNAS 109(15):5693-8.

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606 iCRAC combined with NMR structures reveal the motif bound by Npl3 in vivo and an unexpected separation of the involvement of each RRM in different functions of Npl3

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Serine-arginine rich proteins (SR proteins) belong to an RNA binding protein family that is involved in multiple steps of RNA metabolism including the regulation of alternative splicing events. Npl3 is the only SR-like protein in budding yeast shown to promote the splicing of pre-mRNAs. In addition, Npl3 is involved in other functions such as linking splicing to chromatin remodeling and nuclear export of mRNA and pre-60S ribosomal subunit. Npl3 is composed of two consecutive RRMs towards the N-terminus followed by a C-terminal RS/RGG domain. The first RRM is canonical and the second is a pseudo-RRM. Although Npl3 binding to RNA is important for its functions, its mode of RNA recognition remains elusive.

The pseudo-RRM of Npl3 was previously reported to bind GU rich RNA sequences. In this study, we used a combined CRAC and iCLIP approach in yeast and determined the consensus sequence bound by Npl3 in vivo. Combined with NMR structures of the RRMs bound to RNA, these data show that Npl3 RRM2 recognizes a 5´-GNGG-3´ motif, whereas RRM1 binds preferentially upstream of this sequence to a CC dinucleotide. Interestingly, RRM2 binds to UGG if no guanine is present upstream of the motif. However, in the presence of a GUGG sequence, RRM2 does not contact the uridine anymore and instead recognizes the upstream guanine, which is strongly conserved in the iCRAC consensus sequence.

Using the structures, we engineered mutations that decrease the binding of Npl3 RRM1 or RRM2 to RNA and investigated their effect on Npl3 function in yeast. Strikingly, each RRM of Npl3 seems to be involved in a different function of the protein. The binding of RRM2 to RNA is important for the splicing function of Npl3, while RRM1 is important for splicing as well as chromatin remodeling. Finally, these data also suggest that Npl3 involvement in splicing could come at least in part from its direct specific interaction with the U2 snRNA.

607 Characterization of the novel RNA endonuclease, EndoU

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The RNA binding protein, EndoU, is a novel but poorly characterized RNA endonuclease with a role in B cell apoptosis. However, the biochemical properties and physiological impacts of this enzyme are yet to be discovered in most tissues where EndoU is expressed. Expression of EndoU is particularly high in developing thymocytes. Here, we characterize the biochemical function and the affected RNA populations in a model thymocyte cell line. An established immunoprecipitation/cleavage assay allows for the determination of the optimal reaction conditions and sequence binding specificity. Our results show that immunoprecipitated EndoU favors an environment closely matching intracellular conditions and is activated by calcium. Since the calcium concentrations increase transiently as immature thymocytes develop at each stage, EndoU is likely functional during this maturation process. We have determined that EndoU is capable of cleaving a relatively broad range of RNA substrates in vitro, yet in cells with high calcium, only a few specific products are created. Further analysis of these products will aid in identifying the direct RNA targets in thymocytes and illuminate the role of EndoU on thymocyte maturation. While the direct targets are currently not known, we have measured the effects of EndoU on the transcriptional landscape in maturing thymocytes and observe differential expression of genes involved in apoptosis. Through our research, we have biochemically characterized this novel RNA endonuclease that is expressed in high turnover cells and determined that it is able to cleave a broad range of RNA targets to affect genes regulating apoptosis.

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608 The solution structure of Dead End bound to AU-rich RNA reveals an unprecedented mode of tandem RRM-RNA recognition required for mRNA repression

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The RNA binding protein Dead End (DND1) is essential for normal germline development, through its role in the clearance of specific AU-rich mRNAs. The molecular mechanisms required for its target selection are unknown. DND1 contains two RNA recognition motifs (RRMs) followed by a double-stranded RNA binding domain (dsRBD). Here, we present the solution structure of DND1's tandem RRMs bound to an AU-rich RNA recognition element. Both RRMs are involved in RNA binding, with RRM1 acting as a main binding platform, including unusual helical and beta-hairpin extensions to the canonical RRM fold. RRM2 acts cooperatively with RRM1, capping the RNA, in an unprecedented mode of RRM-RNA interaction, together leading to a unique RRM:RRM arrangement. The structure reveals how an NYAYUNN element is recognized in agreement with recent genome-wide studies. We show that mutation of the RNA binding interface of the RRMs weakens affinity *in vitro* and *in cellulo* while DND1's dsRBD does not contribute to the binding of a set of abundant RNA targets. Subsequently, we demonstrate that repression of a reporter gene by DND1 does depend on its dsRBD in addition to its RNA binding interface. Our results point to a model where RNA recognition by DND1 is mediated by an uncanonical mode of binding by the tandem RRMs and a role for the dsRBD in the recruitment of repressing factors.

609 Activation of hypoxia-inducible factor signaling modulates the RNA protein interactome in Caenorhabditis elegans

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RNA binding proteins (RBPs) bind to all known RNA species and thereby playing an important role by regulation of expression, stability and localization of their targets. Even more importantly, mutations in genes encoding RBPs can lead to cancer and developmental disorders. In the last decade, the list of known and putative RBPs has been increasing in size and complexity across species. Thanks to the development of techniques that allow crosslinking of RNA to interacting proteins followed by both RNA pulldown and mass spectrometry (RNA interactome capture). Little is still known about the molecular function of many RBPs and their global dynamics in different conditions. A recent study from our group found differences in RBP-binding to mRNA upon exposure to hypoxia. Key genes involved in sensing hypoxia are the hypoxia-inducible transcription factors (HIF). Stabilization of HIF can also be gained by a loss of function of the VHL gene. In this study, we chose C. elegans as a model organism to address this complex biological question how hypoxia-inducible factor signaling modulates the RNA protein interactome. Performing RNA interactome capture in wild type and vhl-1 mutant worms we identified 1340 RBPs 311 out of which had not been described before. In addition, we found 44 RBPs to be overrepresented in vhl-1 mutant and 54 RBPs in wild-type worms. A comparison of the proteome in both strains showed that all but one of these are not differentially regulated on the level of protein abundance pointing towards differences in RNA-binding capacity. Our results will add to the understanding of the RBPome in the nematode and its modulation by HIF-signaling.

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610 Deciphering the function of FUS-dependent sdRNAs in human cells.

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FUS is a multifunctional protein involved in many pathways of RNA metabolism in human cells, including transcription, splicing, alternative splicing, RNA transport, miRNA processing and replication-dependent histone gene expression. Interestingly, we have recently found that FUS also binds small nucleolar RNAs (snoRNAs) in human cells and induces their processing into shorter fragment, called sdRNAs (snoRNA-derived sdRNAs). Such sdRNAs could be involved in regulation of gene expression.

Indeed, using in silico approach we predicted putative targets for our sdRNAs. Some of them can hybridize to the 3'UTRs of target mRNAs, suggesting their role in posttranscriptional regulation of transcript stability and/or protein synthesis. Moreover, we have found sdRNAs that can interact with noncoding transcripts. In analyzed cases the RNA duplex is formed between the sdRNA and the unique region that distinguishes noncoding transcript from protein coding mRNA transcribed from the same gene. Biological significance of regulation of gene expression by FUS-dependent sdRNAs will be discussed.

611 Protein RsmC shows RNA annealing and chaperone activity during ribosome biogenesis

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Ribosome is the ribonucleoprotein (RNP) particle that is essential for protein biosynthesis in all kingdoms of life. Formation of proper secondary structures and rRNA folding is critical to maintaining accuracy during the ribosome assembly. Several ribosomal proteins and ribosome assembly factors are known to have RNA chaperone activity. Here we report the RNA chaperone activity of rRNA modification enzyme ribosomal RNA small subunit methyltransferase C (RsmC). RsmC modifies guanine (G) to m2G at position 1207 of 16S rRNA (E. coli nucleotide numbering) located at helix 34 (h34). Our stopped-flow fluorescence measurements illustrated a 40-fold increase in the annealing rates of h34 RNA strands. The circular dichroism (CD) thermal melting experiment showed that the lagging strand (3′-h34) that carries the methylation site (G1207) forms a stable hairpin secondary structure. Single molecule FRET (smFRET) experiments confirmed the ability of protein RsmC to destabilize the hairpin structure of 3′-h34. In addition, 16S h34 RNA duplex is also stabilized in the presence of protein RsmC. These auxiliary functions of protein RsmC play a vital role in the biosynthesis of functional ribosomes.

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612 Identification of RNA-RBP networks

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The scope and RNA interaction partners of prokaryotic RNA-binding proteins (RBPs) are poorly understood. We focus on the roles of RBPs in post-transcriptional control in the gram-negative food-borne bacterial pathogen *Salmonella enterica* serovar Typhimurium that is able to invade eukaryotic cells. New approaches are needed for the identification of RNA-RBP interactions for full understanding of non-coding RNA-based control mechanisms. We use *in vivo* UV-crosslinking to covalently fix the RNA and RBP interaction partners at native conditions and apply phenol-based extraction techniques for selection of x-linked RNA-RBP complexes. Our goal is to increase the resolution of purification techniques for the identification of new RNA chaperones for a deeper understanding of their interaction network.

613 Cap structure modifications influence stability of IFIT/mRNA complexes and determine specificity of IFIT1 and IFIT5.

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Different mechanisms are responsible for distinguishing between self and non-self RNAs. Many epitranscriptomic modifications include methylations of bases and riboses near the 5' end of mRNA. m^7GpppNm modification of the cap structure is known as cap 1 while the unmethylated counterpart m^7GpppN is cap 0. It has been shown that the stress-inducible proteins IFIT1 and IFIT5 have an ability to bind the very terminal 5' end of mRNA depending on its structure modifications. Published reports prove that IFIT1 binding of cap 0 RNA results in inhibition of translation.

We will present the first kinetic data on the interaction between IFIT1 or IFIT5 and a spectrum of differently capped or uncapped mRNAs. The difficulty in such investigations arises from limited quantity of different in vitro transcribed and capped mRNA that can be produced in laboratory conditions. We developed accurate methods that require only very small quantities of material and allow to study this biomolecular interaction, based on fluorescence titration assay and bio-layer interferometry. Our data indicates that each protein favors a specific subset of mRNAs. Obtained data also shows that modification of the cap structure is especially related with the stability of a formed IFIT1/mRNA complex. Finally, we collected kinetic data for a newly identified ligands of IFIT5. Overall, our study shows how engineering of a cap structure can protect mRNA from translational inhibition via IFIT1 binding.

Acknowledgments:

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614 Structural insights into MLE-UNR-roX2 complex assembly during early steps of *Drosophila* dosage compensation

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The male-specific lethal (MSL) complex in *Drosophila* targets the male X chromosome to achieve 2-fold hypertranscription of X-linked genes. High affinity sites on the roX gene act as initial assembly points from where the complex spreads throughout the entire X chromosome. A crucial step in this remarkable chromosome specificity is the early assembly of the MSL complex and involves remodelling and incorporation of the long non-coding (lnc) RNA roX2 into the complex by the RNA helicase MLE. This process is assisted by another protein, Upstream-of-N-Ras (UNR). However, the structural basis of roX2 lncRNA recognition by MLE and UNR remains unknown. We employ an integrated structural biology approach to study the single proteins and their RNA interactions as well as the MLE-UNR-roX2 ternary complex by combining NMR, crystallography, small-angle scattering, and cryo-EM.

Our data on MLE demonstrates that the first double-stranded RNA binding domain (dsRBD) is a non-canonical dsRBD important for RNA binding in general but dispensable for roX2 binding *in vivo*. It is also essential for localization to the X territory but not via its RNA binding property. Thus, other protein factors are important for MSL assembly and localization, where UNR has been suggested.

Our high-resolution structures of UNR surprisingly reveal the presence of more cold shock domains than the usually predicted five. We identify four more non-canonical cold shock domains, which do not bind RNA but are critical for structural integrity of full-length UNR by forming inter-domain contacts. This in turn has implications on RNA binding as shown by *in vitro* translation assays and cell-based experiments.

Furthermore, cryo-EM data on roX2 lncRNA confirm predicted stem loop structures and the formation of stable MLE-UNR-roX2 complexes.

615 The RNA-Protein Interactome of Differentiated Kidney Tubular Epithelial Cells

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From biogenesis to degradation, RNA molecules interact with and are in many ways affected by RNA-binding proteins (RBPs). Among other effects, these interactions modulate the stability and translation of RNA profoundly leading to consecutive changes of the proteome. Here we set out to study the regulation of RBPs in clinically relevant experimental conditions. We analyze hypoxia-induced changes of the RNA-binding proteome (RBPome) in murine ciliated epithelial cells of the inner medullary collecting duct.

To this end, we employ RNA interactome capture (RIC), an oligo-d(T) bead based pulldown method followed by masspectrometric measurement to determine the RBPs bound to polyadenylated RNA species. Since hypoxia is one of the key players in both kidney physiology as well as acute and chronic kidney disease, we use cells cultured under hypoxic conditions. We then quantify changes of the RBPome compared to cells cultured under standard conditions.

Comparison of the two conditions revealed hypoxia-induced changes in RBP-binding to polyadenylated RNAs. Additionally, apart from confirming over thousand RBPs that have been described previously in other models, we identified 25 novel RBPs possibly specific to differentiated kidney epithelial cells.

Our findings demonstrate that the interaction between proteins and transcripts is altered by environmental stimuli. These data broaden the understanding of signaling in epithelial cells of the kidney prompted by hypoxia. For a better understanding and to guide the way towards a collaborative in-depth analysis of the role of RNA-protein interactions in kidney tubular epithelial cells we present an interactive online repository providing the RBPome and proteome of this cell type.

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616 NMR solution structure of a *Legionella* ProQ-homolog and its interaction with RNA

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Regulation of the gene expression by small non-coding RNAs (sRNAs) is a common mechanism in bacteria. sRNAs base pair with sequences in the 5'- or 3'- UTR of their target mRNAs and thereby regulate translation initiation or mRNA stability. This mechanism often requires specialized RNA-binding proteins (RBPs), which stabilize the sRNA and facilitate the sRNA-mRNA interaction. An extensively studied RBP is the RNA chaperone Hfq that interacts with hundreds of different sRNAs. Recently, the ProQ/FinO protein family was identified as a new class of sRNA chaperones. Only a few members of this family have been characterized so far and the structural basis for RNA-binding and recognition has not yet been established. The gram negative bacterium *Legionella pneumophila* harbors a ProQ protein that is specifically involved in the regulation of natural competence. Interestingly, a second homolog (Lpp1663) can be found in *L. pneumophila*. The homologous protein is of unknown function, but the presence of the ProQ/FinO domain makes a role in sRNA based regulation very likely. To gain insight into the RNA binding mechanism of the family of ProQ/FinO proteins, we solved the NMR solution structure of the *Legionella* ProQ homolog Lpp1663. Structurally, the protein resembles a classical ProQ/FinO fold. Further, we examined the RNA binding ability of Lpp1663 and could identify the potential RNA binding site of the protein. This is the basis for further studies on the protein-RNA complex in order to understand the RNA-recognition and binding mechanism of ProQ/FinO proteins.

617 NMR analysis reveals a structural and binding mechanism for SRSF3 and hnRNP A1 with hsa-pri-mir-30-1 RNA

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MicroRNAs (miRNAs) are small, non-coding RNAs that function at a post-transcriptional level, inhibiting the expression of specific target genes. Single nucleotide polymorphisms (SNPs) that occur in pri-miRNAs, pre-miRNAs, and mature miRNAs have been shown to have an effect on processing of specific target genes, through both Drosha and Dicer processing and RNA-binding protein (RBP) interactions. The hsa-pri-mir-30c-1 RNA has a single G-to-A SNP that results in an increase of the mature miRNA, miR-30c, an effect that is observed in gastric and breast cancer patients. This genetic variant was found to cause a frameshift in base pairing of the RNA, thus changing the overall secondary structure. Critically, this rearrangement in secondary structure results in the exposure of a CNNC motif located at the 3' end of the RNA, which is recognized by the SR protein family member, SRSF3, and promotes miRNA biogenesis[1].

So far, structural mechanisms underlying how SNPs affect miRNA processing have been poorly studied. Here, we use NMR analysis to compare molecular and structural features of the wildtype and G/A variants of pri-mir-30c regarding the secondary and tertiary structure of the pri-mir-30c-1 RNA and the interaction with the RNA recognition motif (RRM) of SRSF3. Our results suggest that the G/A mutation only locally disrupts base pairing near the apical loop, thus making it more flexible. We characterize the previously reported interaction of wildtype and G/A variant pri-mir-30c-1 with SRSF3 and identified a binding motif for the RNA-binding protein hnRNP A1 in the apical loop of the RNA transcript. Results of these studies are presented and a potential mechanism for processing of pri-mir-30c-1 RNA involving hnRNP A1 is discussed.

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618 The nuclear mRNA-binding protein Tho1 regulates TREX occupancy and nuclear mRNP assembly

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Nuclear mRNP biogenesis is an integral part of gene expression and encompasses mRNA processing as well as mRNP assembly by the binding of nuclear mRNA binding proteins to the mRNA. TREX is a nonameric complex that couples transcription elongation to nuclear mRNA export. It is composed of the THO complex, the SR proteins Gbp2 and Hrb1, the RNA helicase Sub2 and the mRNA export adaptor Yra1. The protein Tho1 is a nuclear mRNA binding protein in *S. cerevisiae*. It was identified as a high copy suppressor of THO complex mutants. Tho1 binds to RNA and is recruited to transcribed genes in a THO- and RNA-dependent manner. CIP29, the human homolog of Tho1, binds to the TREX complex. Thus, Tho1 is thought to be a loosely associated TREX subunit, but its precise role in mRNP assembly has remained elusive.

Here, we show that Tho1 regulates TREX recruitment in a negative manner. In contrast, Tho1 positively influences the occupancy of another protein complex involved in transcription elongation, the PAF complex. However, PAF decreases the occupancy of Tho1. In addition, phosphorylation of the transcription elongation factor Spt5 is necessary for Tho1 and PAF occupancy but decreases TREX occupancy. Importantly, Tho1 is needed for correct formation of nuclear mRNPs. Taken together, we analyze the function of Tho1 in the regulation of occupancy of TREX, PAF and Spt5 at the transcribed gene as well as the assembly of nuclear mRNPs.

619 RNA-binding activity of Npl3 is required for mRNP assembly and nuclear mRNP export

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An integral step of gene expression is the formation of an mRNP by assembly of nuclear RNA-binding proteins onto the mRNA and the subsequent export of the formed mRNP out of the nucleus. The function of the proteins involved in these processes have been largely analyzed by deletion or depletion of the whole protein or at least protein domains, which probably abrogates several functions of each protein at once. In order to determine specifically the RNA-binding function of proteins involved in nuclear mRNP assembly, we first determined the amino acids involved in RNA binding by RNPXL. We identified about 100 amino acids cross-linked to RNA in vivo in Npl3, Nab2, Tho1, Mex67-Mtr2, and the TREX complex. Second, we can now specifically elucidate the function of the RNA-binding activity of these proteins by mutation of the identified amino acids.

Npl3 is an SR-like protein with functions in transcription elongation, poly(A) tail formation, mRNP assembly, and nuclear mRNA export. The middle part of Npl3 consists of two RRM domains connected by an eight amino acid long flexible loop. In order to analyze the function of the RNA binding activity of Npl3, we changed amino acids that cross-linked to RNA. We generated two Npl3 mutants, one in the loop region, named npl3-loop, and one within RRM1, named npl3-RRM1, and elucidated the functional consequences of these mutations. Interestingly, npl3-loop leads to a nuclear mRNA export defect, while npl3-RRM1 does not. Furthermore, both mutants show distinct and specific changes in the composition of nuclear mRNPs. Thus, abrogation of mRNA-binding in different regions of Npl3 has different functional outcomes.

Taken together, we identify the in vivo RNA binding sites of nuclear mRNA binding proteins involved in mRNP assembly and nuclear mRNA export. In addition, we show that abrogation of RNA binding in different regions of the protein Npl3 has specific and surprisingly different functional consequences. Thus, our approach unraveled novel and unexpected insights into the process of nuclear mRNP assembly.

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620 Ferritin Iron Response Element (IRE)-mRNA Binding to Eukaryotic Translation Initiation Factor (eIF)4F

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Iron deficiencies and overload represent major public health problems throughout the world. Cellular iron homeostasis is accomplished by the coordinated and balanced expression of proteins involved in uptake, export, storage and utilization. Iron regulate the translation of ferritin IRE-mRNA by interaction with an iron regulatory protein (IRPs) and eIF4F. The Protein repressors (IRPs) bind 5'UTR IRE RNA to inhibit protein synthesis or 3'end IRE containing RNA degradation. Iron increases rates of protein synthesis encoded in iron responsive element (IRE)-mRNAs. The noncoding structure IRE-mRNA, about 30-nucleotide, folds into a stem loop to control synthesis of proteins in iron trafficking, cell cycling, and nervous system function. We have recently shown that iron binding to IRE-mRNA destabilized repressor IRP equilibria and enhances activator eIF4F equilibria. To further explore the differences in ferritin IRE-mRNA stability for eIF4F, we compared the thermodynamic parameters for eIF4F and IRP with IRE-mRNA. Fluorescence measurements showed the change in intensity of eIF4F upon addition of ferritin IRE RNA. The binding constants (K_a) and binding capacity (n) of eIF4F for ferritin IRE-mRNA were determined to be 11.1 x 10^7 M^{-1} and 1.0, respectively. In order to understand the relative importance of equilibrium and stability, we further report the temperature dependence binding measurements for the interaction of ferritin IRE-mRNA with eIF4F in the absence and presence of iron. Equilibrium dissociation constant increased with an increase in temperature. Temperature dependence binding data reveals that ferritin IRE-mRNA binding to eIF4F was enthalpy-driven and entropy favorable. Addition of iron increased in enthalpic and decreased in entropic contribution for ferritin IRE-mRNA/eIF4F complex. The decrease in entropy involved in the formation of the IRE-mRNA/eIF4F complex suggested weakened hydrophobic interactions and increased hydrogen bonding for complex formation and an overall conformational change, and more stable platform for effective ferritin IRE-driven translation.

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621 Dynamic Recognition at Interfaces of Protein/RNA Complexes: What Can Computations Tell Us?

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The ribonucleic acid (RNA) molecules are involved in countless key processes in living organisms, including gene expression, cellular defense and catalysis of chemical processes. In vivo, RNA molecules always interact with proteins, since they are synthesized to the point of their degradation. Understanding the principles of protein/RNA interaction is therefore a matter of biologically imminent importance as it allows us to fully grasp the nature of nucleic acids and to appreciate the irreplaceable role they play in living organisms. At the same time, structural studies of protein/RNA complexes by the three leading experimental methods for structure determination (e.g. X-ray crystallography, NMR spectroscopy, Cryo-EM) are inherently more complicated than determining the structure of the individual monomers.

In my research, I use molecular dynamics (MD) simulations to study the protein/RNA complexes. Many biomolecular complexes are inherently dynamical, which makes MD an important tool to complement the experimental techniques of structural biology which typically provide only static ensemble-averaged pictures of the molecular complexes. MD can also provide detailed information on structural hydration which is an important element contributing to binding affinity in non-covalently bound complexes.

I have successfully applied MD to study dynamic recognition at protein/RNA interfaces of Fox-1, CUG-BP2, HuR, and HIV-1 reverse transcriptase (RT) proteins. I showed that dynamic recognition is an important and so far under-appreciated element of RNA recognition by proteins. It constitutes unique evolutionary response to situations in which specific protein interactions with RNA are vital, but at the same time the cell needs to rapidly switch between numerous RNA sequences. In case of HIV-1 RT, I have showed that dynamic recognition and high-energy states of the substrate determine both coordination of its nuclease and polymerase activities and its substrate specificity. Both of these processes are critical for successful proliferation of the virus in infected cells. I predict that dynamic recognition by proteins will be increasingly recognized as important structural and functional element in biology of RNA and DNA molecules. Studies of such systems will require diverse methodologies, ranging from structural biology and computational methods to advanced biochemical approaches.

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622 Unconventional protein-RNA interactions in human embryonic stem cells

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Transcriptional regulation and specifically Transcription Factors (TFs) are known to play a pivot role in pluripotency and differentiation. However, much less is known about the role of post-transcriptional regulation in these processes. In recent years, accumulating evidence supports the involvement of RNA-binding proteins (RBPs) and non-coding RNAs in embryonic stem cell regulation and in reprogramming. Specifically, it has been shown that long non-coding RNAs (lncRNAs) are involved in activation and repression of pluripotency-related genes via epigenetic and transcriptional regulation. To identify novel RBPs and protein-RNA interactions involved in pluripotency we conducted whole cell and nuclear RNA Interactome Capture (RIC) experiments in human embryonic stem cells (hESCs). We identified over 800 high confident RBPs in hESCs, among them many novel RBPs with yet unknown function in pluripotency. Surprisingly, among the RBPs detected in the RIC experiments, we observed a significant number of proteins annotated as DNA-binding proteins, including several TFs which comprise the human pluripotency network. To confirm these findings and identify the RNAs that are directly targeted by these TFs, we performed eCLIP (enhanced crosslinking and immunoprecipitation) followed by sequencing for key pluripotency TFs. Computational analysis of the CLIP data revealed a small subset of non-coding RNAs with significantly enriched peaks relative to the input control, indicating novel TF target sites on RNA. Specifically, the eCLIP results signify a direct association between STAT3 (signal transducer and activator of transcription 3) and the lncRNA NORAD (non-coding RNA activated by DNA damage) in human pluripotent cells. Based on our findings, we propose that non-coding RNAs may contribute to stemness by directly interacting with TFs, possibly acting as co-factors to modulate and fine-tune the transcriptional program of their target genes.

623 YB-1, an abundant core mRNA-binding protein, has the capacity to form an RNA nucleoprotein filament

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The structural rearrangements accompanying mRNA during translation in mammalian cells remain poorly understood. Here, we discovered that YB-1 (YBX1), a major partner of mRNAs in the cytoplasm, forms a linear nucleoprotein filament with mRNA, when part of the YB-1 unstructured C-terminus has been truncated. YB-1 possesses a cold-shock domain (CSD), a remnant of bacterial cold shock proteins that have the ability to stimulate translation under the low temperatures through an RNA chaperone activity. The structure of the nucleoprotein filament indicates that the CSD of YB-1 preserved its chaperone activity also in eukaryotes and shows that mRNA is channeled between consecutive CSDs. The energy benefit needed for the formation of stable nucleoprotein filament relies on an electrostatic zipper mediated by positively charged amino acid residues in the YB-1 C-terminus. Thus, YB-1 displays a structural plasticity to unfold structured mRNAs into extended linear filaments. We anticipate that our findings will shed the light on the scanning of mRNAs by ribosomes during the initiation and elongation steps of mRNA translation.

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624 RNA Binding Activity of Signal Transduction Proteins

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Recently, studies have underscored the importance of RNAs in the regulation of protein-protein interactions. By allowing the assembly of nuclear protein complexes, long non coding RNAs act as scaffolds and thus promote protein-protein interactions in order to regulate the chromatin state. However, the potential role of RNAs in the direct regulation of protein-protein interactions of key cytoplasmic signal transduction pathways remains largely unknown. We hypothesize that RNAs act as scaffolds to directly regulate protein-protein interactions in the cytoplasmic signal transduction pathways. Using a combination of CLIP (crosslinking and immunoprecipitation) and silica matrix based affinity capture (2C complex capture) approaches that can uncover direct interactions between proteins and RNAs in vivo, we demonstrated an unprecedented direct interaction of key MAPK (mitogen-activated protein kinases) signalling proteins with RNA. Subsequent studies using proximity ligation assay (PLA) demonstrated an RNA-dependent modulation of protein-protein interactions in the MAPK pathway, suggesting that an RNA component is able to stabilize these critical protein-protein interactions. We are currently mapping the RNA binding domain in these MAPK proteins to generate RNA binding mutants that will allow us to study the role of the interaction with RNA in signal transduction. Our study could thus provide novel insights in the regulation of the MAPK pathway whose dysfunction is often correlated with several cancers.

625 Is there room for RNA during early meiosis?

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Defects in homologous chromosome segregation during meiosis are a major cause of aneuploidy and infertility. The accurate segregation of homologous chromosomes during meiosis is partly ensured by proper pairing between homologs, which is then stabilized by the assembly of the synaptonemal complex along the entire chromosome axis. Although defects in synaptonemal complex assembly lead to severe errors in meiosis, our understanding of how the synaptonemal complex is assembled correctly between homologs remains limited. Some sporadic evidence in the literature suggests that RNA may be involved in chromosome pairing and may co-localize with the synaptonemal complex. Here, we use *Caenorhabditis elegans* as a model to tackle the intriguing question of whether RNA is an integral player during synaptonemal complex assembly between homologous chromosomes in early meiosis. Our results may thus uncover a novel role for RNA in the production of functional gametes that will give rise to healthy offspring.

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626 Application of RNA-binding proteins in mRNA capture method to analyze the transcriptome

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RNA sequencing (RNA-Seq) is an approach to profile transcriptomes with deep-sequencing technologies. RNA-Seq allows for the detection and analysis of a variety of RNA species within a sample, including mRNA, long and small non-coding RNA, as well as pathogenic RNA. It gives insight into many cellular processes and provides information about gene expression level, gene fusions, alternative splice variants, mutations, transcript isoforms in terms of their 5′ and 3′ ends, etc.

In order to performe RNA-Seq-based coding transcriptome analysis, mRNA need to be efficiently separated from other RNA species, especially highly abundant ribosomal RNA which account for majority (80-90%) of total RNA in the cell. This is typically accomplished by selective hybridization or priming-based methods like rRNA depletion or oligo-dT mRNA capture. Alternatively, selective rRNA and tRNA degradation can be achieved by using the Terminator 5′-phosphate-dependent EXonuclease (TEX), which recognizes RNA molecules with 5′-monophosphate. Efficiency of all those methods is species dependent and suffers from off-target effects, for example, internal poly(A) priming. The oligo-dT mRNA capture is the most commonly used enrichment method, however, it depends on the length of the poly-A tail and is not an option in samples of prokaryotic origin.

With the emergence of RNA-Seq and its various applications, there is a need for developing new, alternative mRNA capture methods. We test several RNA binding proteins with unique RNA binding properties based on the 5′ moiety selection for mRNA sample enrichment prior to RNA-Seq library preparation. We believe that our approach could be particularly useful in lower eukaryotes or bacterial samples. It could also be employed for the detection of the 5′ modification on targeted transcripts.

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627 Investigating the role of L18 in pre-5S rRNA maturation: structure and function.

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In *E. coli*, the precursor (pre) of 5S rRNA is cleaved by a combination of RNase E and T to yield the mature 5S rRNA. In many low G+C gram-positive bacteria such as *G. stearo*, this cleavage is instead performed by RNase M5. Unlike RNase E and T, RNase M5 requires a small, ribosomal protein, L18, for activity. The role of L18 in this reaction is still unknown. To understand the catalytic differences in rRNA processing between *E. coli* and *G. stearo*, the three-way *G. stearo* system of RNase M5, pre-5S rRNA and L18 was studied structurally and functionally. Here, we present data suggesting that binding of L18 to the pre-5S rRNA reshapes the rRNA, to allow recognition by the catalytic domain of RNase M5. In the absence of L18, a second domain of RNase M5 can still recognise the pre-5S rRNA, suggesting that this domain could function as an anchor on the rRNA, prior to binding of the catalytic domain. The structure of the small anchor domain was solved to 1.5 Å. It resembles a death domain (DD) previously identified to be solely a protein-protein interaction scaffold. The RNA-binding properties of this domain in RNase M5 thus represents a novel function of this DD-like fold. The catalytic domain, solved to 1.3 Å, has a toprim domain fold, but lacks a catalytic tyrosine utilised in catalysis by other proteins containing this domain. The catalytic RNase M5 toprim domain therefore likely cleaves its substrate in a distinctively different way than other toprim domain family members such as topoisomerases and gyrases. The full-length RNase M5 protein was modelled from SAXS, X-ray crystallography, and NMR data combined. HDX-MS showed that each RNase M5 domain bind the RNA. The L18 cofactor protein nteracts extensively with the pre-5S rRNA in solution, explaining how this could function as an RNA-folding chaperone, allowing RNase M5 to recognise, bind and cleave its pre-5S rRNA substrate.

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628 P23 acts as functional RBP in the macrophage inflammation response

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Macrophages are critical cellular components of the innate immune system. Their phagocytic activity, migration and signaling molecule expression can be induced by pathogen components, such as lipopolysaccharides (LPS) of gram-negative bacteria. The LPS dependent activation of Toll-like receptor 4 (TLR4) activates downstream pathways, which comprise mitogen activated kinases and NFκB to stimulate cytokine synthesis and secretion. Importantly, post-transcriptional regulation of TLR4 downstream signaling molecule expression by RNA binding proteins (RBPs) contributes to the tight regulation of the macrophage immune response[1].

To comprehensively identify RBPs that modulate the LPS-induced macrophage activation, we employed RNA interactome capture in untreated and LPS-induced RAW 264.7 cells[2]. We identified 402 proteins as the macrophage RNA interactome, of which 91 were previously not considered as RBPs. RNA interactome comparison classified 32 as unique RAW 264.7 cell RBPs. Nineteen of these are not linked to nucleic acid related biochemical activities including P23, a HSP90 co-chaperone that was also demonstrated to exhibit cytosolic prostaglandin E2 synthase activity. P23 was validated to interact differentially with poly(A)$^+$ RNA from naïve and LPS-induced macrophages[2].

Interestingly, RNAi mediated reduction of P23 expression resulted in increased phagocytic activity. To identify P23 interacting RNAs and uncover potential regulatory RBP functions, we immunoprecipitated P23 from cytoplasmic extracts of untreated or LPS-induced cross-linked RAW 264.7 cells. RNAseq revealed 52 differentially associated mRNAs. Annotation of their corresponding proteins classified a group with cytoskeleton-related functions, from which we selected kinesin family member 15 (Kif15) for further analysis. Kif15 is implicated in the regulation of cytoskeletal reorganization and cell mobility[3].

Currently, we analyze whether m^{6}A mRNA modification contributes to the LPS induced differential P23-Kif15 mRNA interaction and investigate the function of the newly identified P23-Kif15 mRNP in the regulation of LPS-induced macrophage response.

[1]Liepelt, A, et al. (2014) *RNA* 20(6), 899-911
[2]Liepelt, A, et al. (2016) *Mol Cell Proteomics* 15(8), 2699-2714
[3]Feng, J, et al. (2016) *J Cell Sci* 129(12), 2438-2447

629 Multidomain convergence of Argonaute during RISC assembly correlates with the formation of internal water clusters

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In humans, four Argonaute proteins (AGOs) load miRNAs to assemble the RNA-induced silencing complex (RISC) while interacting with TNRC6 proteins that further recruit mRNA degradation complexes for post-transcriptional gene silencing. Previous studies reported that loading of miRNAs increases the affinity of AGO for TNRC6 proteins, but the molecular mechanism remains elusive. Here, we report the 1.9 Å crystal structure of human Argonaute4 (AGO4) in complex with guide RNA. Our structural comparison with the previously determined apo structure of *Neurospora crassa* QDE2, an Argonaute homolog, revealed that the PIWI domain is composed of two subdomains, which are fastened with the MID domain upon binding of guide RNA. A mutation at their subdomain interface disrupted TNRC6 binding, suggesting that the conformational change is indispensable for binding to TNRC6 protein. In addition, the current high-resolution structure enabled us to notice that loops and domain linkers wrap 13 and 4 water molecules, forming two clusters inside the AGO4-RISC (We named them LAKEs, Loop-Associated Key Estuaries). Notably, the corresponding water molecules are also found in AGO1 and AGO2 but not in silkworm PIWI protein. Consistent with the observation, the surrounding residues that form hydrogen bonds with the water clusters are conserved throughout eukaryotic AGOs but not PIWIs. The significance of LAKE is evidenced by our *in vitro* assay that AGO2 lost or lowered slicer activity when their corresponding residue was mutated. Lastly, molecular dynamics simulations show that the water molecules always occupy specific positions at the domain interfaces but are exchangeable with bulk solvent. Altogether, these results suggest that water molecules are essential for maintaining the functional RISC structure after the guide-driven conformational changes, and presumably important also for RISC disassembly when releasing guide RNA.

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630 Global identification of RNA-binding proteins in *Arabidopsis* using mRNA interactome capture.

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RNA-binding proteins (RBPs) are involved in virtually all aspects of the lifecycle of RNAs including processing, expression, cellular location, chemical modifications and decay. However, relatively little is known about RNA-protein interactions as their scope and heterogeneity has impeded their study on a system-wide basis. Here, we have adapted 'mRNA interactome capture', a method recently developed for animal cells, to identify the set of proteins bound to mRNA in Arabidopsis etiolated seedlings. In brief, this approach involves UV-crosslinking of RNA and proteins, followed by cell lysis, capture of mRNA-protein complexes with oligo(dT) beads, release of proteins by RNase treatment and finally mass spectrometry for protein identification. The approach has identified more than 700 proteins in total, 300 with high confidence (FDR below 1%), which is defined as the Arabidopsis mRNA interactome. Gene ontology (GO) analysis links approximately 75% of these proteins with RNA biology and includes well known RBPs such as RNA recognition motif and K homology domain-containing proteins, validating many bioinformatically predicted RBPs. However, many proteins had no GO annotations associated with plant RNA biology, including WHIRLY, LIM, ALBA, and YTH domain-containing proteins, the latter of which have recently been shown to act as readers of m6A RNA methylation in plants. We captured major signalling proteins, cytoskeleton-associated proteins, membrane transporters and a host of other proteins not previously associated with RNA-binding, implying the existence of many unknown RNA-protein interactions within a plant cell. Our study provides novel insights into RNA biology and is a foundation resource for future studies investigating the function of RBPs in plants.

631 Role of m^{6}A in modulating hnRNP A2/B1-mediated RNA metabolism to promote breast cancer progression

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Recent reports have implicated the most common mRNA modification, N6‐methyladenosine (m^{6}A), in breast cancer initiation and progression. m^{6}A residues are typically deposited onto nascent pre-mRNA molecules and play an important role in mediating gene expression, primarily through regulating alternative splicing and RNA turnover. Interestingly, the RNA binding ability of the pre-mRNA processing protein hnRNP A2/B1 is influenced by m^{6}A residues, though the precise mechanism of this interaction remains to be elucidated. Similar to the role of m^{6}As, hnRNPs primarily function to regulate pre-mRNA stability and splicing. Notably, hnRNP A2/B1 has also been implicated in the invasion and migration of breast cancer cells. To fully investigate how m^{6}A modifications modulate A2/B1 binding within the context of breast cancer, we employed a modified eCLIP strategy called meCLIP ('methyl-eCLIP', i.e. 'meCLIP') to identify m^{6}As at single-nucleotide resolution. Briefly, the meCLIP method utilizes UV crosslinking to covalently link anti-m^{6}A antibody to m^{6}A modified transcripts and then immunoprecipitates the antibody:RNA complex. Reverse transcription over the anti-m^{6}A crosslink site results in detectable C-to-T mutations that are then used as input for a custom algorithm that identifies sites of elevated conversion frequency occurring within the m^{6}A consensus motif. Using this technique, we successfully identified 10,870 m^{6}A residues in MCF-7 breast cancer cells (to our knowledge, this is the first report of single-nucleotide resolution m^{6}A sites in this cell type). Correlating these residues with our previously identified hnRNP A2/B1 binding sites revealed a profile where A2/B1 was markedly reduced at the m^{6}A site compared to surrounding regions. These results support a model where m^{6}A modifications directly inhibit A2/B1 binding and led us to hypothesize that modulation of m^{6}A levels in breast cancer will cause a significant change in hnRNP A2/B1 binding and subsequent dysregulation in RNA metabolism of cancer-associated genes. By continuing to profile m^{6}A:A2/B1 interactions using the approach outlined above, we will gain valuable insight into the dynamic regulatory potential of the 'epitranscriptome' of breast cancer and potentially provide promising new routes of therapy for targeting m^{6}A-mediated cancer progression pathways.

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632 Structural characterization of Mei-P26 protein - a central regulator of RNA biosynthesis during stem cell fate decision

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Post-translational regulation of gene expression plays an essential role in the early development of many organisms. By controlling different aspects of RNA metabolism and forming highly complex feedback loops, RNA-binding proteins (RBPs) represent major regulators of the germline stem cells' (GSCs) self-renewal and differentiation.

Here, we use fly development as a model system to shed light on the role of a yet poorly characterized central stem cell regulator, namely Meiotic P26 (Mei-P26). The Mei-P26 protein is a TRIM-NHL family member which interacts with key differentiation factors, like Bam (Bag-of-marbles) and Bgcn (Benign gonial cel neoplasm), and regulates germline stem cell differentiation at different levels. In detail, (i) it is an integrated component of the nanosRNA repressing complex, (ii) it represses the Nanos-Pumilio maintenance pathway, and (iii) it binds to Argonaut 1 to repress the microRNA pathway. Interestingly, loss of mei-P26 also impairs the Bone Morphogenetic Protein pathway, suggesting that Mei-P26 is crucial for both, stem cell self-renewal and differentiation.

During our studies, we have obtained high resolution structural information on the C-terminal domain of Mei-P26, which adopts a known propeller-like shape. We have confirmed that it binds RNA in a sequence specific manner by recognizing unique motifs in single stranded RNA. Furthermore, we have identified and defined the consensus RNA recognition motif for Mei-P26 using a multi-disciplinary approach, combining in silico modeling and experimental validation. Finally, we compare our findings with other known NHL domains (e.g. DmBrat and DrLin41) to gain deep insights into the specificity of this domain family. Our results provide information about novel RNA-protein interactions and strongly contribute to a better understanding of the conserved molecular mechanisms that guide stem cell differentiation in the germline.

633 RBPs TRAPPed – insights and challenges in characterizing the RNA associated proteome

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RNA binding proteins (RBPs) modulate all aspects of RNA biology, thus affecting the fundamental cellular processes such as gene expression and stress response. We have devised a toolkit to study the RBPome under steady state conditions and its evolution in challenging environment. The RNA-bound proteome is revealed with TRAPP (total RNA-associated proteome purification). At its core TRAPP is a simple one step protein affinity purification under denaturing conditions, utilizing SILAC mass spectrometry to identify enriched proteins over background. The affinity tag in TRAPP is the RNA itself, crosslinked to proteins *in vivo* with UVC irradiation, which efficiently binds to silica beads. TRAPP is potentially applicable to any organism and has no inherent sequence bias. We have successfully applied TRAPP in *S. cerevisiae* and *E. coli*. In both systems, we identified hundreds of unexpected RBPs, in addition to established RNA biology proteins.

A complementary technique, PAR-TRAPP uses the photoactivated ribonucleotide analogue 4-thiouracil (4tU) for metabolic labelling of RNA species, followed by RNA-protein crosslinking with UVA light. Comparing TRAPP and PAR-TRAPP data in yeast reveals that UVC irradiation more readily crosslinks abundant cellular proteins to RNA in a dose-dependent manner. We speculate that UVC crosslinking reveals transient RNA-protein contacts; the significance of these largely remains to be determined. We successfully utilized PAR-TRAPP to monitor specific changes to the RBPome in yeast challenged with sorbic acid, as well as several other stresses (see abstract by Bresson et al.). Furthermore, our preliminary data indicates that short pulses of 4tU labeling in kinetic TRAPP (kTRAPP) allows characterization of the evolution of the RBPome from nascent transcripts to the final products of RNA maturation.

The final tool in the set is iTRAPP, which offers individual amino acid resolution based on purification of RNA-crosslinked peptides using TiO_2 columns. Peptides, and the exact amino acid, crosslinked to RNA can be identified using mass spectrometry together with the Xi bioinformatic pipeline. The iTRAPP data identified likely targets for specific mutagenesis to abolish RNA binding. Notably, protein phosphorylation sites were enriched in close proximity to RNA-protein interfaces mapped by iTRAPP. Phosphorylation is predicted to reduce RNA interactions, suggesting regulatory mechanisms.

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634 RNA binding landscape and function of a molecular slave oscillator in circadian timekeeping and abiotic stress response

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A key function for RNA-binding proteins in orchestrating plant development and environmental responses is well established. However, the lack of a genome-wide view on their in vivo binding targets and binding landscapes has for a longtime represented a major gap in understanding the mode of action of plant RNA-binding proteins. We have for the first time adapted individual nucleotide resolution crosslinking immunoprecipitation (iCLIP) for genome-wide determining the binding repertoire of the circadian clock-regulated Arabidopsis thaliana glycine-rich RNA-binding protein AtGRP7. iCLIP identified around 800 transcripts with significantly enriched crosslink sites in plants expressing AtGRP7-GFP and absent in plants expressing an RNA-binding-dead AtGRP7 variant or GFP alone. To independently validate the targets, we performed RNA immunoprecipitation (RIP)-sequencing of AtGRP7-GFP plants subjected to formaldehyde fixation. Half of the iCLIP targets were also identified by RIP-seq, thus representing a set of high-confidence binders. To determine the fate of the bound targets in the cell, we cross-referenced the targets against transcriptome changes in AtGRP7 loss-of-function mutants or overexpressing plants. This revealed a predominantly negative effect of AtGRP7 on its direct targets. Additionally, we observed changes in circadian expression patterns of numerous target genes, in line with a proposed function of the protein as a molecular slave oscillator in the circadian timing system. Furthermore, several targets show changes in alternative splicing in response to altered AtGRP7 levels that frequently lead to PTC-containing transcript isoforms. In particular, AtGRP7 mediates splicing changes in response to abiotic stresses. iCLIP targets of the paralog AtGRP8 point to overlapping but not entirely redundant functions of these proteins. Taken together, establishing iCLIP for plants represents a major advancement in plant RNA biology and paves the way to investigate the dynamics of posttranscriptional networks in response to exogenous and endogenous cues.

635 An improved RIP-seq technology to capture both direct and indirect protein-RNA interaction sites

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Protein-RNA interactions are fundamental to core biological processes ranging from epigenetic regulation, transcriptional regulation and post-transcriptional regulation. The complicate interaction network context predicts a wealthy amount of indirect RNA binding via protein-protein interactions inside of a living cell. RNA immunoprecipitation combined with deep sequencing (RIP-seq) is a potential technology to capture such indirect RNA binding activity of a protein via its RNA binding protein partner. In this study, we developed an improved RIP protocol (iRIP-seq) that captures the full landscape of protein-RNA interaction site with a high resolution and specificity while requiring much smaller number of cells and maintaining high library generation efficiency. In iRIP-seq protocol, we applied ultraviolet (UV) crosslinking, RNA input library and template switching in the protocol to preserve the sites of protein-RNA interactions, improve signal-to-noise, and increase the cDNA library efficiency, respectively. Ribonuclease digestion was applied to restrict the length of the protein-bound RNA fragments, further improving the specificity in the discovery of authentic binding sites. We used PTBP1 as an example to prove the concept of the method, allowing us to detect not only the binding sites of PTBP1, but also those of hnRNPH1 and SRSF1, in HeLa cell. The physical interaction between these two proteins was proven by co-immunoprecipitation assay. These results suggest that iRIP enables global mapping of footprint regions of both direct and indirect protein-RNA interactions, at a resolution comparable to those of the recently improved CLIP-seq technologies.

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636 Regulation of the p53 expression profile by hnRNP K under stress conditions

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P53 protein is one of the transcription factors responsible for cell cycle regulation and prevention of cancer development. It has been shown that more than 50% of human tumors are connected with mutations in the *TP53* gene which result in p53 dysfunction. Despite expanding knowledge about the regulation of p53 expression only few proteins interacting with the 5'-terminus of p53 mRNA have been shown so far to act as a p53 regulators at the translational level.

Here, we applied RNA affinity chromatography combined with mass spectrometry analysis to identify proteins that have the potential for binding to the 5'-terminus of p53 mRNA and thus are able to regulate the p53 expression profile. We used two variants of the 5'-terminal region of p53 mRNA: P1-Δ40p53 and P0-Δ40p53 which is a consequence of the existing two major p53 mRNAs. Approximately 20-25% of the proteins which were identified by MS were linked to the translation machinery. The next group, approximately 1-3% of the identified proteins, was represented by proteins which have already been experimentally proved to interact with the 5' UTR of p53 mRNA. The most abundant group, 35-48% of the identified proteins, was called by us the candidate group. In this group, we included proteins with high potential for binding to RNA such as hnRNPs, RNA helicases, splicing and transcriptional factors. One of the top candidates was heterogeneous nuclear ribonucleoprotein K. Since it has been shown that hnRNP K is involved in many processes including translation, transcription and RNA processing we decided to verify whether hnRNP K might act as a p53 regulator. We demonstrated that changes in the level of hnRNP K protein strongly affected the p53 expression profile under genotoxic, nucleolar and endoplasmic reticulum stress conditions. Our results suggest that HRNP K is not only a mutual partner of p53 in the transcriptional activation of target genes under stress condition but it may act as a regulator of p53 expression at the transcriptional and translational levels which is particularly striking in the presence of stress agents.

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637 Identification of new *cis*-regulatory elements based on structural conservation

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Post-transcriptional gene regulation controls the amount of protein produced from a particular mRNA by altering both the rate of decay and the rate of translation. Such regulation is primarily achieved by the interaction of *trans*-acting factors, mainly RNA-binding proteins (RBPs) and non-coding RNAs, with *cis*-regulatory elements in the untranslated regions (UTRs) of mRNAs. These interactions are guided either by sequence- or structure-based recognition. Similar to sequence conservation, the evolutionary conservation of a UTR's structure thus reflects its functional importance. We used such structural conservation to identify previously unknown *cis*-regulatory elements in human 3'UTRs. With the RNA folding program Dynalign, we scanned all UTRs of humans and mice for conserved structures. Characterization of a subset of putative, conserved structures revealed two conserved stem-loops in the 3'UTR of *UCP3*. *UCP3* encodes a mitochondrial membrane protein, mainly expressed in skeletal muscle. It is involved in fatty acid metabolism, obesity and insulin resistance. The two conserved stem-loops constitute a tandem binding site for the RBP Roquin, enabling efficient, cooperative mRNA destabilization. Detailed functional characterization of the conserved tandem site enabled us to redefine the binding preferences of Roquin. Using this newly defined consensus, we identified novel Roquin targets genome-wide. Many of the newly identified targets are unrelated to Roquin's established role in inflammation and immune responses, highlighting additional unstudied cellular functions of this important repressor. Moreover, the expression and regulation of several Roquin targets is cell type specific. Consequently, they are difficult to detect using methods that depend on mRNA abundance, but easily detectable using our unbiased strategy.

Braun J, Fischer S, Xu ZZ, Sun H, Ghoneim DH, Gimbel AT, Plessmann U, Urlaub H, Mathews DH, Weigand JE (2018) Identification of new high affinity targets for Roquin based on structural conservation. *Nucleic Acids Res*. 46:12109-12125.

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638 Structural insights into binding of N7-modified cap analogs by human eIF4E isoforms.

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The recognition of 5' mRNA cap by eIF4E is a key point of the translation initiation regulation. In eIF4E cap binding slot the N7-methyl guanine base of cap is sandwiched by the indol rings of conserved tryptophans (Trp56 and Trp102) via cation-π stacking interaction. What is interesting, the biophysical and crystallographic studies showed that eIF4E1a can efficiently bind the cap analogs with benzyl group instead of methyl in N7 position of guanine ring. The big benzyl group packs into hydrophobic pocket behind the Trp moieties involved in stacking interaction. What is more, the N7 benzyl cap analogs are tested as potential anticancer therapeutics in oncogenic cells with increased eIF4E1a expression where are employed to inhibit translation by blocking the interaction between canonical translation factor, eIF4E1a and 5' mRNA cap.

In our research we extend the investigation of specificity for human eIF4E isoforms (eIF4E1a, eIF4E1b, eIF4E2, eIF4E3) toward different variants of N7- modified mononucleotide cap analogs (m^7GTP, bn^7GTP, et^7GTP, 1-naf^7GTP and 2-naf^7GTP). Whereas eIF4E1a, the canonical eIF4E factor, binds m^7GTP and bn^7GTP with the similar association constants (K_{as}), which are higher from that observed for other cap analogs from 2 to 10-fold, eIF4E1b shows an exceptionally higher specificity toward bn^7GTP and 2-naf^7GTP. Moreover eIF4E2 binds m^7GTP about 3-fold weaker than other investigated N7-modifided cap analogs. The performed analysis of secondary and tertiary structure using CD spectroscopy also showed different conformational changes of eIF4E's tryptophan residues as a results of binding N7-modified cap analogs among eIF4E isoforms.

639 Stabilization of dystrophin mRNA as a novel therapy for treating DMD.

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The mutations causing Duchenne muscular dystrophy (DMD) lay within the dystrophin gene which encodes for a protein that is essential for muscle structure and function. Approximately 13% of the alterations in the dystrophin gene are nonsense mutations. Nonsense mutations lead not only to premature translational termination and truncated polypeptide products, but also promote mRNA destabilization and degradation by a process called nonsense mediated decay (NMD). In many patients, nonsense mutations stop the synthesis of the dystrophin protein and also lead to the degradation of the RNA. Read-through therapies for DMD nonsense mutations, such as PTC124 (Ataluren), show great promise. However, read-through therapies rely on the abundance of mutant dystrophin mRNA, which in most cases is degraded by the NMD process, thus diminishing the success of read-through therapy. We propose to use NMD inhibitors to increase dystrophin mRNA levels and enhance read-through therapy.

We have analyzed patient-derived skin fibroblasts from 10 DMD patients and determined that there is varied expression of dystrophin mRNA. Treatment of these cells with NMD inhibitors stabilized the dystrophin mRNA. A known target of NMD, SRSF1, had increased protein expression in the cells treated with NMD inhibitors. Since fibroblasts do no express dystrophin protein, we were not able to determine dystrophin protein levels in these cells. We are now in the process of differentiating the patient-derived fibroblasts to muscle cells to determine dystrophin protein levels after treatment with NMD inhibitors either alone or in combination with Ataluren.

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640 miRNAs in arthritis pathogenesis and therapy

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MicroRNAs (miRNAs) play critical roles in various biological processes by targeting specific mRNAs. We have reported that cartilage-specific miR-140 plays a critical role both in skeletal formation and arthritis pathogenesis. However, miR-140 exists into an intronic region of E3 ubiquitin ligase, Wwp2, and its knockout mice generated from Wwp2 gene trap ES line showed similar skeletal phenotype, it is unclear which molecules critically contribute to this phenotype. By generating single deletion and double deletion mice for miR-140 and Wwp2 by CRISPR/Cas9, we could examine the exact function of both molecules and found that only miR-140 contribute to the skeletal phenotype, but both molecules act synergistically in cartilage homeostasis and arthritis pathogenesis.

To identify miRNA's functional targets, we created a cell-based screening system using a luciferase reporter library composed of 4,891 full-length cDNAs, each of which was integrated into the 3′-untranslated region (3′-UTR) of a luciferase gene. Using this reporter library system, we conducted screening for targets of miR-34a (a tumor suppressor miRNA) and miR-140.

Our strategy is useful for elucidation of miRNA functions and their therapeutic application.

641 Towards understanding the role of aberrant splicing in prostate cancer disease progression

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In prostate cancer (PCa) a major clinical challenge is posed by the occurrence of constitutively active androgen receptor (AR) splice variants, as AR-V7, that lack the ligand binding domain and are therefore resistant to AR signaling inhibitors, such as abiraterone or enzalutamide. Emerging data suggests that alternative RNA splicing leads to increased transcriptome diversity and plays a pivotal role in cancer development and progression. This non-genetic variability promotes isoform switching in oncogenes and tumor suppressors potentially enabling new mechanisms of therapy resistance. To date, the most prominent example of this is vemurafenib treatment resistance in BRAF(V600E)-mutant melanomas through the emergence of alternatively spliced isoforms of BRAF. Currently, the precise molecular mechanisms by which these splice variants are generated remain unclear. Further investigation is needed to close this critical gap in knowledge and to pave the way for new therapeutic insights. While the role of canonical splicing in cancer has been studied extensively, the present understanding of the interplay between minor splicing and cancer is still lacking. Minor introns recognized by the minor spliceosome constitute only 0,35 % of all human introns, yet they are highly conserved and serve as critical molecular switches providing rapid control of gene expression. Intriguingly, oncogenes or tumor suppressors, such as BRAF, Erk1/2 and PTEN, are highly enriched in minor introns, suggesting that tumors reliant on these genes may be particularly susceptible to manipulations of the minor spliceosome. In unpublished preliminary work, we show that minor splicing is significantly higher in castration resistant and neuroendocrine PCa tumor cells as compared to the hormone sensitive PCa cell line LNCaP. However, certain conditions, such as androgen depletion, increase minor splicing in LNCaP cells to nearly the same level as seen in castration resistant PCa tumor cells. A knockdown of a minor spliceosome component decreases the viability and proliferation of PCa cells, and this correlated with enhanced minor intron splicing of the respective PCa cell type/subtype. we further demonstrated that genes that play major roles in PCa transdifferentiation, such as AR, EZH2, SYP and SMARCA4, are deregulated upon minor spliceosome KO.

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642 The onco-ribosome: A frontier of ribosome heterogeneity

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The ribosome is an RNA-protein complex essential for translation in all life. The structural and catalytic core of the ribosome is its four rRNAs: 18S, 28S, 5S, and 5.8S. While ribosomal protein gene mutations have been identified as oncogenic drivers of human cancers (*RPS5*, *RPS15*, *RPL10* and *RPL22*), the mutational and variation landscape of rRNA has remained largely unexplored.

Investigating rRNA sequence variation in colorectal carcinoma (CRC), we discovered an epigenetic variation in the 18S rRNA present in **44% of patients** (a rate higher than *KRAS* mutation). The variation occurs at a base which is perfectly conserved in eukaryotes, and which normally undergoes hyper-modification to *1-methyl-3-α-amino-α-carboxyl-propyl pseudouridine* (macpΨ). The macpΨ modification occurs only at 18S U.1248, and is located at the core of the ribosomal P-site, where molecular dynamics implicate it in direct tRNA interaction. We validated macpΨ-deficient rRNA in 4 of 12 CRC cell lines and show that it incorporates into mature ribosomes. CRISPR-Cas9 knockout of the *TSR3* gene, which encodes the enzyme responsible for macpΨ modification, allowed us to investigate the molecular function of this ancient modification and explore the distinct functionality of a so called, "Onco-Ribosome".

To gain mechanistic insights on the oncogenic and tumour suppressive domains of the ribosome, we projected ribosomal protein and rRNA mutations from >280,000 cancer genomes onto the human ribosome structure. We found mutational hotspots in 3-dimensional clusters encompassing the known oncogenic driver mutations, as well as several novel inter-protein mutational clusters. Notably, the ribosomal P-site showed significant enrichment for both ribosomal protein (RPS15 and RPL10) and rRNA mutations, suggesting perturbation of P-site functionality may be a common feature in oncogenesis.

Understanding cancer-variant ribosomes has paradigm-shifting potential in both its consequence to aberrant translation in cancer and as a novel chemotherapeutic target. Thus, this innovative stream of research has truly translational potential.

643 Alternative mRNA polyadenylation modulates influenza A virus induced innate immune response

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Many viruses modulate mRNA and protein expression in infected cells, most extreme cases being transcriptional and translational host shut-off. We used a combination of -omics approaches to study the relationship between mRNA and protein expression levels in virally infected cells, focusing on various strains of influenza A virus (IAV). Interestingly, we detected a large number of post-transcriptional changes of host's mRNA occurring in infected conditions.

Here we show that alternative polyadenylation (APA), characterized by differential usage of polyadenylation sites, affects a broad spectrum of host mRNA in a host-shutoff independent manner. Using a variety of Influenza A strains in vitro, we characterized the effect of viral proteins on APA of the host's mRNA. We functionally mapped the effect to a single amino acid residue of a viral protein. Conversely, using affinity purification followed by mass spectrometry and a comprehensive knock-down screen, we identified the host interactor that is required and sufficient to mediate APA.

Using a combination of functional proteomics, transcriptomics, and quantitative mapping of polyadenylation sites, we characterized APA and corresponding downstream effects. Notably, we show that ability to induce APA correlates with cytokine expression inhibition in vitro and in vivo, which is pivotal for virulence in an in vivo mouse infection model.

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644 Splicing defect of the profilin gene alters actin dynamics in a *S. pombe* SMN mutant

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Spinal muscular atrophy (SMA) is a devastating motor neuron disorder caused by mutations in the multi-functional *survival motor neuron* (SMN) gene. To characterize biological pathways connected to SMN, we performed an Epistatic MiniArray Profile (E-MAP) screen with an hypomorphic tdSMN fission yeast mutant exhibiting differential snRNPs assembly and splicing defects. We found that deletion of the acp1 gene (CAPZA1 in human) alleviates the growth defect of tdSMN cells. Acp1 together with acp2 forms the heterodimeric actin-capping protein, which binds to the barbed end of the actin filaments.

To characterize the molecular bases of the protective effect of acp1 deletion in the tdSMN mutant, we first characterized the Filamentous/Globular-actin ratio and found that tdSMN cells contain excessively polymerized actin. The tdSMN mutant is able to grow on plates containing 0.3uM Latrunculin A, demonstrating that it is more resistant to actin depolymerization signals compared to wild-type and suppressor cells. We also analyzed splicing of genes coding for actin-binding regulators and found a strong splicing defect in the profilin gene, which leads to a decrease in the level of profilin both in the tdSMN mutant and tdSMNΔacp1 suppressor cells.

Taken together, these studies allow to propose a model in which defective splicing downregulates profilin in the SMN mutant and this alters actin turnover. In the suppressor cells, the capping protein is not functional and this redistributes more actin monomer to the different actin networks. Our data show a clear correlation between an impaired function of SMN in snRNP assembly and dysregulation of actin dynamics.

645 CRISPR/Cas9 knockin modeling of a short tandem repeat disease

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The human genome contains an array of short tandem repeats, that are susceptible to expansions that underlie multiple neurological and neuromuscular diseases. In the disease setting, a diverse range of repeat numbers can be detected due to somatic mosaicism, and the numbers observed in readily obtainable sample types may not reflect the size of the repeats in other affected tissues. Attempts at modeling repeat expansion diseases in mice have relied on heterologous promoters and gene contexts which disconnects the tissue specificity, developmental timing and spatial expression of a repeat expansion from its endogenous context. This disassociation can mask discoveries that would be important to the disease biology.

Here, we employ a combination of rolling circle amplification (RCA) and CRISPR/Cas9 genome editing using the *DMPK* CTG expansion (CTG^{exp}) disease myotonic dystrophy type 1 (DM1) as a model. Zygote injections of *Dmpk* CTG^{exp} homology directed repair templates resulted in knockin mice carrying a range of expansion mutations due to repeat contractions *in vivo*. DM1-relevant disease manifestations, including nuclear RNA foci and RNA mis-splicing, were detectable in tissues and cells, including choroid plexus epithelial cells responsible for cerebral spinal fluid production. These results demonstrate that microsatellite expansions can be effectively introduced into their endogenous gene loci using RCA and CRISPR-Cas9 resulting in knockin mice that serve as multisystemic experimental and therapeutic platforms for short tandem repeat diseases.

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646 Mechanism of Alcohol mediated tissue injury in Chronic Pancreatitis.

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Background: Chronic pancreatitis (CP) is an inflammatory condition leading to progressive and irreversible damage to both exocrine and endocrine components of the pancreas. Alcohol increases the risk of CP in a dose-dependent manner, and it has been shown that continued exposure increases chances of progression to CP. But the exact mechanism by which alcohol mediates the tissue injury is not completely understood. So, we hypothesized that investigation of transcriptome and miRnome profile of pancreatic serum and tissue samples might provide some more insights about the mechanism of alcohol mediated tissue injury.

Objective: To identify a putative mechanism of alcohol mediated tissue injury in Chronic Pancreatitis.

Methods: We did a serum miRNA profiling to identify differentially expressed miRNAs in CP. It was followed by extensive literature review and exploration of different databases to identify miRNAs which are supposedly secreted from diseased pancreatic tissue via exosomes. Simultaneously we also looked for datasets having transcriptomic profile of CP pancreas to find out differentially expressed genes in Pancreatic tissue. In order to extract biologically relevant information from the miRNome and transcriptome data, we did a combined miRNA-gene analysis using hypergeometric tests to identify the miRNAs enriched with target genes in inverse direction of expression. We also explored the role of differentially expressed Transcription factors.

Results: We identified 150 miRNAs and 652 genes to be differentially expressed in chronic pancreatitis. We linked the miRNAs to their target genes based on their expression profile and discovered 14 miRNAs to be enriched with 69 unique targets in inverse direction of expression. We also found 4 Transcription factors i.e RUNX2, RUNX3, TGFB1 and ZEB1, supposedly regulating 14 selected miRNAs as well as some of DEGs. These TFs were also found to be differentially expressed in our selected dataset. This led to the creation of miR-Target gene-Transcription factor network and a mechanistic model of alcohol mediated tissue injury was proposed.

Conclusion: Alcohol induces the differential expression of genes and miRNAs which act as pro and/or anti-inflammatory molecules leading to the activation of pancreatic steallate cells, ultimately culminating in fibrosis of Pancreas and development of Chronic Pancreatitis.

647 Retinitis pigmentosa linked mutation of Prpf8

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Prpf8 is a highly conserved pre-mRNA splicing factor and a crucial component of the U5 snRNP. Mutations in Prpf8 were found in patients with retinitis pigmentosa (RP), a human disease caused by a loss of photoreceptors in the retina and degenerative changes in the retinal pigment epithelium (RPE). Previously, we showed that majority of RP-linked mutations impair incorporation of Prpf8 into splicing complexes and inhibit splicing. However, this does not apply to the protein carrying the Y2334N mutation, which was properly incorporated into splicing complexes and splicing defects were gene specific (Malinova et al., JCB, 2017).

Here, we utilized CRISPR/Cas9 system to tag endogenous Prpf8 with GFP and to introduce Y2334N mutation in the near-diploid RPE-1 cell line. We prepared homozygotic and heterozygotic cell lines expressing GFP tagged Prpf8 or the Y2334N mutant. The Y2334 mutant is localized to splicing speckles similarly to wild type. We further confirmed by immunoprecipitation that the Y2334N mutant is properly incorporated into snRNPs. In a mouse model, Prpf3 mutation reduced phagocytic abilities, which might impair photoreceptor function in retina. We therefore probe whether the mutation in Prpf8 also impairs phagocytosis of human RPE cells.

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648 Splice-site changing oligonucleotides targeting the serotonin 2C may reduce spasticity after spinal cord injury

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Spinal Cord Injury (SCI) affects approximately 300,000 Americans resulting in devastating neurological and physical limitations. SCI in the chronic phase is complicated by muscle spasms, which are to a large degree caused by hyperactivation of the serotonin receptor 2C (5HT2C) caudal to the injury site. Currently, there is no rational treatment available for these spasms. Through a combination of alternative splicing and editing of exon Vb, the 5HT2c pre-mRNA generates at least 25 isoforms with different regulatory properties: one intracellular truncated receptor 5HT2C_tr, one non-edited full-length receptor 5HT2C_FL_INI, and 23 full-length edited receptors 5HT2C_FL_ed. The full-length receptors are active in signaling, while the truncated receptor has a dominant negative inactivating function.

To intervene with the 5HT2c isoform ratios, we developed a series of oligonucleotides that either increase or decrease the 5HT2c_tr/5HT2c_FL_INI ratio, as well as an antiserum that is specific for the 5HT2C_tr protein. One of these oligos (oligo #21, 2'-O-methyl-phosphothioate, 18-mer) is localized in an intron downstream of the regulated splice site. Using minigenes derived from rat DNA, we found in transfection assays that Oligo#21 promotes skipping of all edited exon Vb, with an apparent efficacy of 10 nM in vitro.

To test its efficacy in vivo, we delivered oligo #21 into the spinal cords of injured rats through intrathecal injection. Oligo #21 accumulates in motoneurons after intrathecal delivery. We observe exon Vb skipping after delivering 20 µg of oligo #21 to each rat that is more pronounced when using 50 µg. Analysis of the EMG (electromyograph) after oligo treatment showed a change in duration, but not amplitude of the spasms.

Our data confirm that a deregulation of the 5HT2C pre-mRNA contributes to spasms occurring after spinal cord injury and show the principle that splicing-changing oligonucleotides could be used to treat spasticity, which is a major comorbidity of SCI. Furthermore, receptor-specific oligonucleotides can be used to dissect the role of structural highly related serotonin receptors in the brain.

649 Molecular dissection of the cancer microRNA miR-888 cluster associated with aggressive prostate disease.

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Our laboratory is studying how conserved non-coding RNAs arranged within genomic clusters function as complex networks to co-regulate overlapping cancer pathways. Specifically, we are characterizing how the microRNA (miRNA) miR-888 cluster coordinates tumor suppressor TIMP2/3/4 and SMAD4 signaling in the context of prostate cancer. We identified the miR-888 cluster consisting of seven miRNA genes (mir-890, -891a, -891b, -892a, -892b, -892c) in a profiling screen as elevated in specimens and urine-derived-exosomes from patients with advanced prostate cancer as well as in metastatic, drug-resistant human prostate cancer cell lines. Our in vitro assays showed that these miRNAs control proliferation, migration, and invasion activities in human PC3 hormone-refractory and LNCaP hormone-sensitive cells. Consistent with an oncogenic role in prostate cancer, miR-888 and miR-891a accelerated prostate tumor growth in mice and induced neuroendocrine transdifferentiation in cell culture. Interestingly, this cluster resides on human chromosome Xq27.3, which maps to a genetic locus linked to hereditary PCa. Cluster members are reported to be elevated in other types of human malignancies that include breast, renal, colon, and endometrial cancers. Therefore, we likely uncovered a novel signaling cancer network with immense therapeutic potential. We hypothesize that the relationship between the miR-888 cluster miRNAs and their messenger RNA (mRNA) targets are reciprocal and these mRNAs may act in a competing endogenous RNA (ceRNA) network to regulate expression of other mRNAs in trans via microRNA response elements. We are currently testing this theory using proteomics, published HITS-clip data, and CRISPR gene edited cell lines deleted for certain miR-888 cluster members. Moving forward, we will validate the utility of miR-888 cluster anti-mir reagents to block disease progression in animal models and determine to what extent exosomal miR-888 cluster cargo controls tumorigenesis in mice. This work could lead to effective biomarkers and therapeutic targets for aggressive prostate disease.

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650 Effect of ALS-linked FUS mutations on U7snRNP, processing of histone mRNA's and genome stability

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Abstract - Mutations in the FUS (Fused in Sarcoma) gene are repeatedly identified in the familial form of ALS (Amyotrophic Lateral Sclerosis). We have recently shown that U7 snRNP, a key factor responsible for correct 3'end processing of replication-dependent core canonical histone pre-mRNAs, interacts with FUS (1). U7 small nuclear RNA is a part of this RNP complex and is generally observed in the nucleus of the cell, but our recent FISH and Immunofluorescence data reveals interaction and miscolocalization of U7snRNA with ALS-FUS mutants into the cytoplasm of the cell. Further our observation shows that the 3'end processing is reduced to significant levels in the presence of ALS-FUS mutations thus giving rise to extended mRNA transcripts of replication-dependent core canonical histone genes. We also performed ChIP experiment on proliferating cells and observed that RNA polymerase 2 binding is reduced for ALS-FUS mutants as compared to FUS WT thus reducing transcription for cells with ALS-FUS mutations. Altogether our results indicate that ALS-FUS mutations are capable to cause cytoplasmic colocalization of U7snRNA, leading further to reduced mRNA levels of U7snRNA along with an increased amount of extended histone mRNA transcripts. This along with reduced transcription can further lead to disturbed repression or activation of histone gene expression resulting in genome instability and may be the explanation behind the molecular mechanisms in the progression of ALS.

1. Raczynska et al., 2015, Nucleic Acids Res. 43(20):9711-28.

651 Poly(A)-specific ribonuclease (*parn*) knockout zebrafish variants showed variable telomere lengths.

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Poly(A)-specific ribonuclease (PARN) is a deadenylating enzyme that degrades poly(A) tails of a subset of mRNAs and ncRNAs in eukaryotes. Recently, we (Dhanraj et al 2015) and others (Stuart et al 2015, Tummala et al 2015, Moon et al 2015) have found that mutations in the human gene encoding PARN cause telomere biology disorders (TBDs). At present the molecular details causing TBDs due to PARN deficiency are not well understood.

To investigate the physiological role of PARN during the whole life span of an animal and the link between PARN deficiency and TBDs we have established a *parn* knockout zebrafish model, using a CRISPR/Cas9 genome editing strategy. We have so far generated three *parn* compound heterozygotes with the following genotypes: Fish 1 (p.Gln119fs/p.Ser118fs; p.(Ser562_Leu563del)); Fish 2 (p.Lys310fs/p.Glu311fs); and Fish 3 (p.Glu311del/p.Glu311fs). The compound heterozygous mutations in fish 1 and 2 will abolish Parn activity/expression whereas Parn activity/expression will be reduced in fish 3.

The telomere lengths of the compound heterozygous fish have been investigated and we have found that the fish 3 has short telomeres (8 kb) while the fish 1 and fish 2 have telomeres of normal length as wild type (12 kb). We have crossed each of the compound heterozygotes with wild type zebrafish and generated heterozygous off-springs (F2) and subsequently in crossed the F2 fish and generated both heterozygous and compound heterozygous off-springs (F3). Both F2 and F3 fish variants showed variable telomere lengths ranging from 8-14 kb suggesting incomplete penetrance of the disease phenotype. We are currently characterizing phenotypes of the obtained off-springs to study the penetrance of the disease. Interestingly, some of the obtained off-springs show growth/developmental defects. To our knowledge, these are the first stable PARN knockout animals that have been developed so far.

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652 Improving fusion gene diagnosis in cancer using targeted RNA sequencing

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Chromosomal translocations join together two previously distinct parts of the genome. This genomic shuffling can alter expression levels of canonical genes or fuse separate genes to encode a single chimeric transcript of novel function. These resulting fusion genes account for ~20% of human cancer morbidity and are often tumour-specific. Since several drugs have been developed to specifically target fusion gene products, their precise identification is essential for accurate diagnosis and treatment. However, current clinical diagnostic techniques are limited in resolution and throughput.

To address these drawbacks, we developed Blood FuSeq and Solid FuSeq - two diagnostic tests utilizing targeted RNA sequencing to identify fusion genes in haematological malignancies and solid tumours, respectively. Each panel captures the full range of oncogenic fusion RNA transcripts, improving diagnostic performance by specifically enriching the sequencing coverage of all targeted regions. Utilising well-characterised cell lines and spike-in sequencing standards, we demonstrated the ability of the assays to accurately detect and quantify known fusion genes.

Expanding our analysis to clinical patient samples, we successfully diagnosed fusion genes from numerous cancer subtypes covering a broad range of sample sources and RNA qualities, from shelf archived formalin-fixed tissues to cryopreserved snap-frozen liquid and solid specimens. Applying the FuSeq assays improved the overall fusion gene diagnostic rate and demonstrated high concordance for samples with previous diagnoses, identifying many fusion genes for which approved therapeutic treatments exist.

In addition to identifying >50 different fusion genes across these samples, the resulting targeted RNAseq data revealed informative variations in exon usage and isoform diversity - all factors that can affect treatment efficacy. Additionally, the FuSeq assays simultaneously measured target gene expression levels and reported on immune receptor profiling.

Overall, Blood and Solid FuSeq provide an advanced sequencing-based approach to fusion gene diagnosis. Following further development into accredited clinical diagnostic tests, these assays will deliver detailed and medically relevant information on a wide range of fusion genes, representing a significant advancement for fusion gene molecular diagnostics in cancer. Further, the resulting expression level and exon-usage information for these cancer-associated genes can indicate additional treatment pathways, all within a single diagnostic test.

653 Transcriptomic studies provide insights into the tumor suppressive role of miR-146a-5p in non-small cell lung cancer (NSCLC) cells

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Non-small cell lung cancer (NSCLC) is a complex disease in need of new methods of therapeutic intervention. Recent interest has focused on using microRNAs (miRNAs) as a novel treatment method for various cancers. miRNAs negatively regulate gene expression post-transcriptionally, and have become attractive candidates for cancer treatment because they often simultaneously target multiple genes of similar biological function. One such miRNA is miR-146a-5p, which has been described as a tumor suppressive miRNA in NSCLC cell lines and tissues. In this study, we performed RNA-Sequencing (RNA-Seq) analysis following transfection of synthetic miR-146a-5p in an NSCLC cell line, A549, and validated our data with Gene Ontology and qRT-PCR analysis of known miR-146a-5p target genes. Our transcriptomic data revealed that miR-146a-5p exerts its tumor suppressive function beyond previously reported targeting of EGFR and NF-κB signaling. miR-146a-5p additionally targeted arachidonic acid metabolism genes, as well as the RNA-binding protein HuR, and decreased expression of many HuR-stabilized pro-cancer mRNAs, including TGF-β, HIF-1α, and various cyclins. miR-146a-5p transfection also reduced expression and cellular release of the chemokine CCL2, and this effect was mediated through both its 3' UTR and promoter region. Taken together, this work reveals that miR-146a-5p functions as a tumor suppressor in NSCLC by controlling various metabolic and signaling pathways through direct and indirect mechanisms.

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654 Repeat-associated non-AUG translation at CAG repeats in the ATXN3 gene context

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Spinocerebellar ataxia type 3 (SCA3) is a progressive neurodegenerative disorder caused by a CAG repeat expansion in the *ATXN3* gene encoding the ataxin-3 protein. Until recently, SCA3 was thought to arise solely from a protein gain-of-function mechanism. However, the emerging body of evidence indicates that the RNA-mediated mechanism might also contribute to neurodegeneration in SCA3. Importantly, recent discovery of the repeat–associated non-ATG (RAN) translation may further increase the pathogenic complexity of SCA3. In the present study, to gain insight into toxicity of expanded CAG repeats in SCA3, we investigated RAN translation in various cellular models of SCA3 expressing translated or non-translated CAG tracts. The presence of at least two of three possible RAN-translated proteins (polyglutamine and polyalanine) was observed in cells expressing expanded repeat region with short 5' and 3' flanking sequences. Microscopic analysis revealed that RAN proteins colocalize and alter nuclear envelope architecture. Importantly, RAN translation of polyglutamine protein was strongly influenced by the length of 5' flanking sequence. Moreover, cellular stress enhanced RAN translation in cellular models of SCA3. Based on our results it appears that RAN translation at the CAG repeats in SCA3 is occurring similarly as for expanded repeats present in non-coding sequences, and that a sequence context of the repeats may play an important role in triggering this unconventional translation.

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655 *RBFOX2 acts as a tumor suppressor in metastatic pancreatic cancer*

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RBFOX2 plays a key role in tissue-specific alternative splicing regulation. Aberrant expression of RBFOX2 in many cancers causes altered splicing of specific targets. Recent studies suggest that RBFOX2 is important in establishing an invasive phenotype through its regulation of mesenchymal-specific splicing events during EMT. In an attempt to investigate the role of RBFOX2 and its splicing targets in the development of metastatic pancreatic cancer we have established a model system using human primary pancreatic tumor samples (primary tumors) and human metastatic ascites cells (ascites) obtained from pancreatic cancer patients. The ascites was used to generate either solid tumors in mice (PDX tumors) or metastatic cell lines (PDX-derived cell lines) by xenografting. Using our model system, we found that RBFOX2 is down-regulated in the metastatic tumors compared to primary tumors. Overexpression of RBFOX2 in patient-derived metastatic cell lines inhibited survival, migration and invasion in vitro and metastasis into the lungs in vivo, while knockout of RBFOX2 promoted the invasive and tumorigenic abilities of primary pancreatic cancer cells. Taken together, these findings suggest that RBFOX2 acts as a tumor suppressor in metastatic pancreatic cancer. Deep RNA sequencing of these cell lines revealed both known and novel RBFOX2 regulated alternative splicing targets. Bioinformatics analysis of these splicing targets showed enrichment of targets in the Rho-Rac and Hippo signaling pathways known to have a role in migration and invasion. Modified antisense oligonucleotides, as well as splice site mutagenesis by CRISPR/CAS9 are used to manipulate the splicing of these targets in vitro and in vivo to demonstrate their function role in tumor progression. Understanding the biological importance of RBFOX2 and its involvement in pancreatic metastatic tumors has the potential to aid early diagnosis and shed light on novel approaches for treatment.

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656 Evidence for disrupted snRNP biogenesis links FUS-ALS to SMA

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The RNA-binding protein Fused in Sarcoma (FUS) is a ubiquitously expressed member of the hnRNP family and has been implicated in multiple steps of gene expression. Mutations in the *FUS* gene have been linked to the motor neuron disease Amyotrophic Lateral Sclerosis (ALS) and typically disrupt the nuclear localisation signal of FUS, leading to cytoplasmic mislocalisation and eventually aggregation.

To identify its binding sites in the physiological as well as pathological context, we used a reductionist CLIP approach including nuclear and cytoplasmic FUS constructs.

We consistently found the spliceosomal U1 snRNA among the most enriched transcripts in our CLIP data with a well-defined binding signature on stem loop 3. Our biochemical and functional data suggests that FUS employs its two RNA-binding domains to physically bridge the U1 snRNP to its pre-mRNA substrate during splice site definition. The atomic details of this interaction are currently under investigation.

In the disease context, we observed that cytoplasmic FUS cross-links to an additional site on the U1 snRNA overlapping the Sm site. We show that this aberrant cytoplasmic interaction interferes with U1 snRNP biogenesis and present evidence for a general snRNP assembly defect. Intriguingly, snRNP biogenesis defects were already reported for Spinal Muscular Atrophy and implicate a shared pathomechanism between FUS-linked ALS and SMA.

657 Specific inhibition of splicing factor activity by decoy RNA oligonucleotides

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Alternative splicing, a fundamental step in gene expression, is deregulated in many diseases. Splicing factors (SFs), which regulate this process, are up- or down regulated or mutated in several diseases including cancer. To date, there are no inhibitors that directly inhibit the activity of SFs. We designed decoy oligonucleotides, composed of several repeats of a RNA motif, which is recognized by a single SF. Here we show that decoy oligonucleotides targeting splicing factors RBFOX1/2, SRSF1 and PTBP1, can specifically bind to their respective SFs and inhibit their splicing and biological activities both in vitro and in vivo. These decoy oligonucleotides present a novel approach to specifically downregulate SF activity and have the potential to treat diseases where SFs are up-regulated, such as cancer.

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658 Molecular pathogenesis of Diamond-Blackfan anemia and drug screening for the disease using zebrafish as a model animal

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Mutations in genes involved in ribosome biogenesis have been identified in patients with specific disease conditions, called ribosomopathies. Diamond-Blackfan anemia (DBA) is one of such disorders, characterized by diminished numbers of erythroid progenitors and associated physical deformities.

We developed a zebrafish model of DBA by knocking down the zebrafish ortholog (*rps19*) of the human *RPS19*, which is the most frequently mutated gene in DBA patients. The knockdown embryos displayed a drastic reduction of red blood cells, whereas differentiation of other myeloid and endothelial cells seemed to be normal. The anemia phenotype was almost completely rescued by injection of wild-type *rps19* mRNAs, but not by mRNAs with patient-type mutations. The DBA model also showed developmental abnormalities in the head and tail regions due to increased apoptosis. Therefore, this zebrafish model nicely recapitulates the clinical conditions of DBA patients.

To isolate drug candidates for DBA, we carried out a compound library screening using this zebrafish DBA model. The *rps19* knockdown embryos were treated with 1,280 validated compounds and the red blood cell production was assessed by hemoglobin staining. We successfully identified 4 compounds that have a potential for improving the anemia phenotype in the DBA model, suggesting that zebrafish will be a powerful *in vivo* tool for drug discovery.

659 Plasma miR-320a as a liquid biopsy suppresses non-small cell lung cancer progression through AKT3 and its associated pathways

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Non-small cell lung cancer (NSCLC) comprises ~80-85% of the total lung cancer cases, the major cancer burden. Late presentation of the disease worsens the survivability of the patients due to tumor tissue unavailability for biopsy; which is of paramount importance for tumor presence and its information. Notably, tumor-associated circulating microRNAs (miRNAs), stable under harsh conditions and a minimally invasive approach, might serve as a liquid biopsy to improve current diagnostic strategies for NSCLC patients. In circulation, their measured differential expression can enumerate tumor related information like early detection, tumor grade, staging, histology and metastasis. In our study, we observed low plasma levels of miR-320a through NGS and validated in eighty NSCLC plasma samples through qRT-PCR compared with eighty healthy controls. Low circulating miR-320a showed a negative correlation with patient clinico-pathological features. Functional assays showed, *in vitro* up-regulated miR-320a levels affected the hallmarks of cancer viz., decrease cell viability, proliferation, colony formation, migration and invasion capabilities, cell cycle arrest and increased apoptosis; whereas its down-regulation showed the opposite effect. These results were supported by western blot and immunohistochemistry of EMT proteins. Furthermore, western blot analysis showed that AKT3, predicted miR-320a direct target, is negatively regulated by miR-320a levels. Even, immunohistochemistry analysis showed high AKT3 protein expression in NSCLC patient tissue; where circulating miR-320a expressions were low. In addition, AKT3 associated PI3K/AKT/mTOR, MAPK, JAK2-STAT3 pathways downstream target protein levels were also negatively affected by miR-320a. Taken together, our data suggest plasma miR-320a is a tumor-suppressive miRNA in NSCLC and negatively regulates AKT3 and its associated pathways. Thus, circulating miR-320a could be explored as liquid biopsy for improved diagnosis of NSCLC patients.

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660 A Potential Role of Extended Simple Sequence Repeats in Competing Endogenous RNA Crosstalk

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MicroRNA (miRNA)-mediated crosstalk between coding and non-coding RNAs of various types is known as the competing endogenous RNA (ceRNA) concept. Here, we propose that there is a specific variant of the ceRNA language that takes advantage of simple sequence repeat (SSR) wording. We applied bioinformatics tools to identify human transcripts that may be regarded as **repeat-associated ceRNAs (raceRNAs)**. Multiple protein-coding transcripts, transcribed pseudogenes, long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) showing this potential were identified, and numerous miRNAs were predicted to bind to SSRs. We propose that simple repeats expanded in various hereditary neurological diseases may act as sponges for miRNAs containing complementary repeats that would affect raceRNA crosstalk. Based on the representation of specific SSRs in transcripts, expression data for SSR-binding miRNAs and expression profiling data from patients, we determined that raceRNA crosstalk is most likely to be perturbed in the case of myotonic dystrophy type 1 (DM1) and type 2 (DM2). Key points: (1) Multiple non-coding and protein-coding transcripts harbor simple sequence repeats (SSRs), (2) Some SSRs in human transcripts participate in miRNA-mediated cross-regulation, (3) Repeat-associated ceRNAs crosstalk is most likely altered in two myotonic dystrophies (DM1 and DM2), associated with extended SSRs.

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661 Splice modulators license C9orf72 ALS repeat RNA into nuclear export and RAN translation

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An intronic G4C2 repeat expansion in the C9orf72 gene is the major known cause for Amyotrophic Lateral Sclerosis (ALS). The disease mechanism is still not fully understood, but a pathological gain of function of nuclear repeat RNA foci as well as translation into toxic dipeptide repeat (DPR) polyproteins have been proposed. We screened 100,000 small molecules in C9orf72 patient iPS derived neurons for modulation of RNA foci and identified analogs of known spliceosomal modulators targeting SF3B1. These compounds trigger elimination of RNA foci post-transcriptionally, independent of C9orf72 pre-mRNA splicing and gene context. As a result, the G4C2 repeat RNA is bound by SRSF1, exported into the cytoplasm and licensed into RAN translation. This enhances DPR cell toxicity, suggesting a potential protective role of RNA foci. In turn, trapping SRSF1 in the cytoplasm by small molecule inhibition of SRPK resulted in build of nuclear RNA foci and a reduction in RAN DPR cell toxicity. Thereby these data reveal a noncanonical role of the spliceosome in C9orf72 repeat RNA metabolism and provide orthogonal pharmacological tools to study C9orf72 ALS pathobiology.

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662 The impact of HAX-1 protein on regulation of transcripts involved in pro-inflammatory response of cancer cells

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HAX1 (HCLS1-associated protein X 1) protein is involved in cell migration, apoptosis, regulation of calcium ions homeostasis and probably plays a role in innate immune response (Kostmann disease). HAX1 was identified also as an RNA binding protein with known interaction with vimentin (2003) and DNA polymerase beta (2007) transcripts. Those interactions suggest its post-transcriptional regulatory function. HAX1 is a protein engaged in cancerogenesis but still its role stays unknown.

RNase MCPIP1 (Monocyte chemoattractant protein-induced protein 1) has a well-established role in inflammation and indirectly, in development of cancer. The inflammation is tightly associated with the cancerogenesis and growing cancer promotes the expression of pro-inflammatory factors. MCPIP1 controls an immune response by destabilization of mRNAs encoding immune related proteins including IL-6 and IL-12p40 via their 3' untranslated regions (UTR). MCPIP1 auto-regulates its own mRNA level through interaction with similar stem-loop in the transcript. So far, the molecular basis of MCPIP1 interaction with RNA remains unclear, since no sequence specificity has been found.

To verify the hypothesis that HAX-1 can interplay with MCPIP1 protein we tested this potential interaction using co-immunoprecipitation method. We found out that both proteins co-precipitate. Additionally, to confirm that result, BIFC (BImolecular Fluorescence Complementation) experiments were performed. To show potential role of this interaction, we studied levels of both proteins in clear cell renal carcinoma (ccRCC) clinical samples. Amounts of HAX1 and MCPIP decreased in ccRCC tissues comparing to normal tissue.

Results of this project are expected to bring the new knowledge about mechanisms how and where HAX-1 and MCPIP1 proteins cooperate in the cells to maintain the internal homeostasis between cells and inflammation process.

Acknowledgments

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663 Disruption of IntS13 Interaction with Integrator Cleavage Module Contributes to Ciliopathy Disease

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The Integrator complex (INT) consists of at least 14 highly conserved subunits, and is associated with RNA polymerase II (RNAPII). Key functions ascribed to INT are the co-transcriptional cleavage of UsnRNA and eRNA as well as regulating the activity of paused RNAPII at many genes critical for development. Cleavage of nascent RNA by INT is reliant on three of its subunits, IntS4/9/11, forming a 'cleavage module' with IntS11 housing the actual endonuclease activity. Not surprisingly, perturbation of INT function through mutation can cause developmental disorders in humans effecting multiple tissue types. Prominent among these disorders are ciliopathies, which are caused by disruption in the biogenesis of the primary cilium that is expressed in nearly all cells. We previously demonstrated that RNAi-mediated knockdown of INT subunits is sufficient to disrupt ciliogenesis but the underlying molecular basis of this phenotype is unknown.

Here, we describe two unrelated families with children that have a specific ciliopathy disease that have two distinct and homozygous recessive mutations within the C-terminus of IntS13 - a previously uncharacterized Integrator subunit. Either mutation leads to decreased levels of INTS13 protein in patient cells, and cilia defects are recapitulated in Xenopus embryos treated with antisense morpholino oligos to IntS13. Using a modified yeast two-hybrid assay, we determined that IntS13 utilizes its C-terminus to interact with the IntS4/9/11 heterotrimer and that any mutation within IntS4, 9, or 11 that disrupt the heterotrimer also disrupt association with IntS13. Importantly, the C-terminus is necessary and sufficient to mediate this interaction and the patient mutations completely abolish the binding. Co-immunoprecipitation and mass spectrometry data in human and Drosophila cells show that these patient mutations weaken or disrupt INTS13's association with the majority of INT subunits and RNAPII but retain interaction with IntS14 and the cohesin complex. We are currently using NGS approaches in patient cells and IntS13 knockdown cells to pinpoint the exact spectrum of ciliogenesis relevant genes whose transcription are disrupted when INT interaction deviates due to mutation.

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664 Translational re-programming by the unfolded protein response drives resistance to anti-folates

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Accumulation of unfolded proteins in the endoplasmic reticulum triggers the unfolded protein response (UPR), an adaptive signal transduction pathway aimed at reinstating cellular homeostasis, or, if that fails, at triggering of apoptosis. The UPR plays a key role in a variety of disorders (including diabetes, neurodegenerative disorders, and inflammatory processes) and has been implicated in cancer progression and resistance to chemotherapy. However, the mechanisms and pathways by which the UPR contributes to chemoresistance are only poorly understood.

We have employed a multi-omics approach to monitor changes to gene expression after induction of the UPR with two different compounds, probing in parallel the transcriptome, the proteome, and changes to translation. Stringent filtering reveals the induction of 267 genes (the UPR regulon), many of which have not previously been implicated in stress response pathways. We experimentally demonstrate that UPR-mediated translational re-programming (by phosphorylation of eukaryotic translation initiation factor 2 subunit alpha, eIF2α) causes an up-regulation of enzymes involved in a pathway that diverts intermediate metabolites from glycolysis to fuel mitochondrial one-carbon metabolism. This metabolic rewiring of the cells results in resistance to treatment with the widely-used folate anti-metabolites Methotrexate and Permetrexed.

665 Identifying RNA binding proteins relevant for melanoma progression

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RNA binding proteins (RBPs) are essential players in RNA metabolism and are gaining great attention in the cancer field because of their potential to regulate essentially every hallmark of tumour development. The number of RBPs with established roles in tumorigenesis is, however, small and their molecular mechanisms of action are poorly understood. To identify novel RBPs implicated in cancer and metastasis, we have performed unbiased mRNA interactome capture (RIC) analysis using melanoma cell lines with increasing aggressiveness. We have identified ~600 RBPs that comprise the high-confidence melanoma RBPome. Cluster analysis revealed seven RBP groups with consistent changes in RNA binding capacity across cell lines. Interestingly, for many RBPs we could not detect a concomitant change at the level of protein expression, suggesting that RNA binding activity is regulated during melanoma progression. We have functionally validated 35 RBPs using depletion followed by five cellular assays that monitor the tumorigenic potential of cells. The results indicate that RIC indeed reveals RBPs with roles in tumor development. As a proof-of-principle, we are investigating a previously uncharacterized RBP. The results of our in-depth analysis will be shown.

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666 miR-708-5p Suppresses Lung Cancer Cell Growth and Resistance Through Targeting of the Arachidonic Acid Signaling Pathway

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Our objective is to identify, define, and restore dysregulated post-transcriptional mechanisms in the arachidonic acid pathway in cancer progression and tumor microenvironment (TME) composition. Many cancers maintain an inflammatory microenvironment to promote their growth, which promotes other hallmarks of cancer, such as proliferation, invasion, angiogenesis, and immune evasion. Lung cancer is of particular importance, as it is the second most common cancer and the deadliest. Moreover, $13 billion is spent on lung cancer treatments in the United States annually, yet 5-year survival rates have not improved and remain exceedingly low. Currently, many patients initially respond to treatment, only to develop resistance. Hence, resolving how inflammation is dysregulated and contributes to resistance in cancer may provide fresh opportunities for therapeutic development to more comprehensively treat tumors.

One inflammatory pathway commonly dysregulated in cancer is the metabolism of arachidonic acid by Cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO) into potent lipid-signaling molecules called eicosanoids. Eicosanoids promote proliferation, invasion, resistance, and angiogenesis through autocrine and paracrine signaling to cancer cells and the tumor stroma. Additionally, eicosanoids have been shown to have a role in resistance and immune evasion. While the arachidonic acid pathway is commonly upregulated in cancer, the mechanisms governing this deregulation are poorly understood. One profound regulator of expression is microRNA (miRNA), which act post-transcriptionally to suppress target mRNAs through transcript degradation or translational stalling. miRNAs are commonly misexpressed in cancer and can perform oncogenic or tumor suppressive functions.

One miRNA in particular, miR-708-5p, is commonly underexpressed in cancer and has been shown to repress oncogenic signaling pathways. Interestingly, our data indicate miR-708-5p suppresses both COX-2 and 5-LO expression by targeting their 3' UTRs in lung cancer cells, decreasing their mRNA and protein levels. This direct targeting of COX-2 and 5-LO decreased oncogenic eicosanoid production, resulting in decreased proliferation of lung cancer cells *in vitro*. Moreover, we have observed additive effects of miR-708-5p when used in combination with chemotherapies and other tumor suppressive miRNAs. Our work is expanding to further study miR-708-5p's ability to alter lung cancer cell phenotype and TME composition.

667 Exploiting *Drosophila* to Examine RNA Exosome-linked Disease

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The RNA exosome is an evolutionary-conserved 3'-5' riboexonuclease complex critically important for both precise processing and complete degradation of a variety of cellular RNAs. Given, the crucial role of the RNA exosome in post-transcriptional regulation of RNA, it is not surprising that the complex is essential in systems examined thus far. The recent discovery that mutations in genes encoding structural exosome subunits cause tissue-specific diseases makes defining the role of the RNA exosome within specific tissues critically important to understand the basis of these diseases. The *Drosophila* system provides ideal tools to examine tissue-specific gene function. Mutations in the RNA exosome subunit 3 gene (*EXOSC3*) cause Pontocerebellar Hypoplasia Type 1b, an autosomal recessive neurodegenerative disease. The disease-causing mutations identified are missense mutations in evolutionarily-conserved amino acids. The tissue-specific defects these changes cause are challenging to understand based on current models of RNA exosome function with only limited analysis of the complex in any multicellular model *in vivo*. The goal of this study is to provide insight into how mutations in *EXOSC3* impact the function of the RNA exosome leading to disease. EXOSC3 is an evolutionarily conserved subunit, termed Rrp40 in *Drosophila.* To begin to determine the functional consequences of the specific amino acid substitutions that cause disease in humans, we developed a novel model of RNA exosome-linked disease in *Drosophila* utilizing CRISPR/Cas9 gene editing to introduce disease-linked mutations in *Rrp40* (*EXOSC3*). Preliminary data examining disease-linked amino acid substitutions in Rrp40 of differing severity reveal behavioral and morphological phenotypes that align with disease severity found in patients. Next, we performed RNA-Seq analysis to define the spectrum of RNA targets affected. Principal component analysis revealed that PCH1b-linked alleles in flies segregated into distinct molecular groups based on their gene expression profiles. Gene expression patterns among the PCH1b mutants highlight large-scale differences in lncRNAs and mRNAs in each PCH1b-mutant providing a molecular basis for the phenotypes observed. These data provide evidence that the *Drosophila* model can be used to provide insight into tissue-specific function of the RNA exosome *in vivo* and explore the functional consequences of amino acid substitutions linked to disease phenotypes.

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668 Molecular consequences of a U12 snRNA mutation causing cerebellar ataxia

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Impaired function of the minor spliceosome, the machinery responsible for the splicing of the rare U12-type introns, underlies a group of human diseases with mutations in the minor spliceosome-specific small nuclear RNA (snRNA) and protein components (1). Here, we present a mechanistic characterization of a recessive U12 snRNA mutation (84C>U) reported to cause an early-onset form of cerebellar ataxia (2). To study the molecular consequences of the U12 snRNA mutation, we used base editing to create cell lines harboring the 84C>T mutation in the *RNU12* gene. We show that, in the context of human cell lines, the 84C>U mutant snRNA displays 60–70% reduced steady-state levels compared to the wild-type snRNA. RNA half-life measurements show that the lower steady-state levels result from accelerated decay of the mutant snRNA. Depletion of components of the RNA exosome and the nuclear exosome targeting (NEXT) complex stabilize the mutant snRNA, suggesting that the mutant is recognized as aberrant and targeted for decay in the nucleus. Edited cell lines homozygous for the 84C>T mutation display the molecular hallmark of minor spliceosomal diseases, increase in U12-type intron retention levels, as well as usage of cryptic U2-type splice sites

1. Verma et al. (2018) *Semin Cell Dev Biol* 79:103–112
2. Elsaid et al. (2017) *Ann Neurol* 81(1):68–78

669 A novel zebrafish model system provides insight into the pathology of U8 snoRNA variants associated with Leukoencephalopathy with Calcifications and Cysts

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Leukoencephalopathy with Calcifications and Cysts (LCC), or Labrune syndrome, is a rare and fatal neurological disorder of the cerebral small blood vessels. Characterized by the radiological triad of cerebral white matter disease, intracranial calcifications and cysts, LCC was recently shown to be an autosomal recessive genetic disorder caused by biallelic mutations in the gene SNORD118, encoding the box C/D U8 small nucleolar RNA (snoRNA)[1].

Here we report the first vertebrate model for U8 snoRNA function. Zebrafish U8 snoRNA mutants were found to exhibit defective rRNA biogenesis and activation of the tumour suppressor p53, which monitors ribosome biogenesis. Human pre-U8 snoRNA (pre-hU8), but not mature U8 snoRNA, rescued the zebrafish U8 mutant, demonstrating an evolutionary conserved function. Functional assessment of multiple LCC patient variant U8 alleles revealed that patients inherit one null and one functional, presumably hypomorphic, allele. We show that the 3´ extension of pre-hU8 is critical for U8 biological function, and that 29 of 33 patients contain a variant within the 3´ extension, or in nucleotides at the 5' end of pre-hU8 predicted to interact with this 3' extension. Disease associated variants in the 5' end of pre-hU8 disrupt 3' end pre-hU8 processing and structure, supporting the prediction that the 5' and 3' ends of the pre-hU8 interact to allow proper maturation of the pre-hU8 snoRNA. Restoring base-pairing between the 5' and 3' ends of the pre-hU8 rescues pre-hU8 3' end processing. Taken together, these data characterize the function consequences of the disease associated U8 snoRNA variants and propose a model where the 5' and 3' ends of the pre-hU8 interact to allow correct processing of the pre-hU8 to the mature form.

[1]Jenkinson et al. (2016). Mutations in SNORD118 cause the cerebral microangiopathy leukoencephalopathy with calcifications and cysts. Nature Genetics. 48: 1185-1192. https://doi.org/10.1038/ng.3661

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670 Muscleblind mitigates FUS toxicity by modulating stress granule dynamics and restoring SMN levels

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Amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) are devastating human neurodegenerative diseases that share overlapping clinical and pathological features. The majority of ALS/FTD (80-90%) cases are sporadic in nature and only a small subset (10-20%) of cases are familial. Pathogenic mutations in several genes such as SOD1, TDP-43, FUS, Matrin 3, Profilin 1 and C9orf72 have been linked with ALS/FTD pathogenesis. Disease-causing mutations in these proteins suggest potential defects in RNA metabolism.

We developed mammalian meuronal and fly models of ALS by expressing normal and disease-causing mutations in FUS protein. Our models recapitulate key features of human disease including neurodegeneration, morphological defects, protein mislocalization and behavioral defects. Imterestingly, we found that the RNA-binding ability of FUS is essential causng for the neurodegenerative phenotypes in vivo. We performed an unbiased genetic screen using a Drosophila model of ALS and discovered muscleblind, an RNA-binding protein, as a novel modifier of FUS-mediated neurodegeneration in vivo. Muscleblind regulates cytoplasmic mislocalization of mutant FUS and subsequent accumulation in stress granules, dendritic morphology and toxicity in mammalian neuronal and human FUS patient iPSC-derived motor neurons. Interestingly, genetic modulation of endogenous muscleblind was sufficient to restore reduced SMN protein levels in axons expressing pathogenic mutations in FUS, suggesting a potential mode of suppression of FUS toxicity. Upregulation of SMN protein suppressed FUS toxicity in vivo indicating a link between FUS and SMN. Our data provide in vivo evidence that muscleblind is a dominant modifier of FUS-mediated neurodegeneration by regulating pathways involved in ALS pathogenesis.

671 Small non-coding RNA transcriptome signatures of chondrocyte ageing

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Introduction Osteoarthritis (OA) is linked to ageing but the mechanisms are incompletely understood. Small non-coding RNAs (SNCRNAs) are key regulators of gene expression (microRNAs), rRNA modification and protein translational capacity (snoRNAs). Short RNA fragments derived from parental RNAs, such as tRNA cleavage fragments, are novel regulatory RNAs that inhibit translation in response to stress. In this current study we profile SNCRNAs in normal young and old chondrocytes in order to elucidate age-specific changes.

Materials and Methods RNA isolated chondrocytes from n=5 young (mean age ±SD; 4±1 years) and n=5 old (18±3.5 years) horses was subject to small RNA sequencing; Illumina MiSEq platform generating 2x150 bp paired-end reads. Reads were aligned to the EqCab 3.0 genome. Additional analysis identified novel snoRNAs and tRNA fragments. Differential gene expression (DE) analysis was conducted using edgeR. Ingenuity Pathway Analysis (IPA) identified microRNA targets and pair data with our previous RNASeq study undertaken on young and old cartilage determining microRNA interactome in ageing.

Results 50 DE SNCRNAs were identified in ageing; 16 microRNAs, 8 snoRNAs, 2 novel snoRNAs, and 24 tRNAs (FDR<0.05). tRNA fragments were from two distinct populations of approximately 50nts (tRNA halves) and 30nts (tRNA fragments) and these increased in ageing with 96 tRNA fragments DE (FDR<0.05). DE microRNAs input into IPA were involved in cell death, movement, growth and proliferation pathways. After applying prioritisation towards likely miRNA-mRNA targets, a regulatory network of 16 miRNAs targeting 75 previously identified DE mRNAs was created. Subsequent pathway analysis identified genes within the pathways joint inflammation, activation and mineralisation of connective tissue and OA.

Discussion One key characteristics of OA is an imbalance between protein anabolism and catabolism. Ageing leads to a loss of protein homeostasis. This data evidences specific microRNAs that may be potent regulators of gene expression at different levels during cartilage ageing. tRNA fragments increase in response to stress and reduce protein synthesis. We identified for the first time in tRNA fragments DE in cartilage ageing that may, to some extent, result in the loss of protein homeostasis evident in OA.

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672 MBNL splicing regulators contribute to microtranscriptome composition in myotonic dystrophy

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Myotonic dystrophy type 1 (DM1) is the most common, an autosomal-dominant adult onset muscular dystrophy caused by expansion of CTG repeats in the 3' UTR of the dystrophia myotonica-protein kinase (DMPK) gene. The pathogenic agent is the RNA containing expanded CUG repeats (CUG^{exp} RNA) expressed from the mutated allele. The best-characterized consequences of CUG^{exp} RNA is sequestration and impaired function of the RNA binding proteins: the muscleblind-like (MBNL) protein family (MBNL1, MBNL2, MBNL3) and Elav-like family member 1 (CELF1). MBNLs regulate various RNA-processing steps including alternative splicing, polyadenylation, RNA stability and mRNA intracellular localization. Alternative splicing and alternative polyadenylation disruptions, observed in DM1 patients, are effects of MBNLs sequestration on CUG^{exp}. So far, the mechanism of deregulation of microRNAs (miRNAs) in DM1 is unknown. To address this issue and to recognize MBNL-depended deregulations in microtranscriptome in DM, we performed deep sequencing of small RNA fraction isolated from various cell models with genetic or functional *MBNL* knock-out, as well as from skeletal muscles of DM1 mouse model (HSA^{LR}), *MBNL* knock-out mouse model and from DM patients. We identified several miRNAs sensitive to the level of MBNL proteins and we revealed that disruptions of miRNAs expression resulted from MBNL deficiency contribute to the microtranscriptome changes observed in DM1 patients. We determined that miRNA alterations developed as a consequence of MBNL loss, have mainly transcriptional origin. On the other hand, we confirmed a direct involvement of MBNL1 in posttranscriptional biogenesis of many miRNAs, such as miR-23b miR-27b and miR-24-1 coming from the same genetic cluster. We showed that these miRNAs are not produced from unspliced pre-mRNA precursors but are only excised from spliced mature RNA variants with alternatively included exons containing individual miRNA sequences. Since, it was demonstrated that miR-23b targets MBNL1 and MBNL2, we suggest that there is auto regulatory loop in which miR-23b repress MBNL, lower level of MBNL cause production of lower amount of pri-miR-23b followed by decrease of miR-23b, thus the level of MBNL is not down-regulated efficiently and the protein level is normalized.

673 Protein interactors of antisense $(C_4G_2)_n$ RNA repeats from *C9orf72* repeat expansion mutation

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The effects of repeat expansion mutation in *C9orf72* gene are still not entirely known, even though the mutation is the main genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). As one possible mechanism sense (G_4C_2) and antisense (C_4G_2) RNA transcripts of the expanded repeat are proposed to sequester different RNA binding proteins, therefore impeding their normal cellular functions. Several proteins have been identified so far as the interacting proteins of RNA foci formed by RNA transcripts of mutation. Mostly proteins interacting with sense RNA were identified and their interaction studied, while much less is known about antisense RNA repeats. In our work we focused on antisense repeats. We have set up RNA pull-down assay using long, biologically relevant RNA constructs ($32x C_4G_2$). Constructs contained S1m aptamer on one side, which enabled binding of the constructs to the streptavidine magnetic beads. Several proteins have been identified using this method in combination with mass spectrometry. Proteins involved in protein synthesis and cytoskeleton stability were among main interactors. All the proteins were also tested for interaction with sense RNA ($48x G_4C_2$) in vitro. Interactions were also tested in *C9orf72* mutation-positive patient derived cells. We were able to confirm interaction of proteins identified in in vitro experiments with sense and antisense RNA foci in these cells. Further evaluation of these interactions will be important in defining their role for disease development and progression.

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674 Brain organoids to study circRNA function in the pathogenesis of brain diseases

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Understanding how the human brain functions in health and disease is one of the greatest challenges of modern science. Limited availability of human samples and ethical limitations hinder the study of the brain with methods from genomics and genetics. Recently, three-dimensional human brain organoids have emerged as a cutting-edge, genetically-tractable experimental system to study human brain development and function *in vitro.*

Using patient iPSC-derived brain organoids as an experimental model, we are investigating early molecular mechanisms underlying neurodegenerative diseases such as Leigh syndrome and Huntington's disease. Combining latest cutting-edge technologies such as single cell RNA sequencing (scRNA-Seq), total/ circRNA RNA sequencing with immunohistochemistry and measurements of synaptic activity we aim to detect any early disease associated changes in the cell composition, gene expression, tissue morphology and electrophysiological activity in order to identify any new potential targets for the diagnostic and treatment. We are particularly focused on the expression and function of disease-associated circRNAs as novel potential players in disease pathogenesis. We will report on this work in progress.

675 Identification and characterization of the biological roles of long non-coding RNAs in early stage breast cancer, ductal carcinoma in situ (DCIS)

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Breast cancer is the most common cancer in women worldwide, with incidence rates increasing and survival rates varying widely depending on early diagnosis and access to treatment. Ductal carcinoma in situ (DCIS) is a non-obligate precursor to invasive ductal carcinoma (IDC). Often non-palpable and asymptomatic, DCIS is most commonly identified by mammography. If untreated, approximately 30 - 40% of DCIS patients will go on to develop IDC; however, it is not currently possible to detect differences in DCIS subtypes to determine which patients will likely progress to IDC. Clearly, better molecular characterization of DCIS progression is necessary. Since an increasing number of studies have linked long, non-coding RNAs (lncRNAs) to various cancers, we have specifically selected to examine lncRNAs as novel DCIS biomarkers and to characterize their biological roles in cells.

Here we present our targeted sequence capture and RNA sequencing (RNAseq) results from two DCIS patient-derived cell lines, one DCIS xenograft-derived cell line and a small patient cohort (n=5) of DCIS affected and normal-matched tissue. We identified four uncharacterized lncRNAs in our cell line dataset, LINC00473, MIR210HG, LOC729970 and LOC100134040, with altered expression, that are associated with adverse breast cancer patient outcomes in The Cancer Genome Atlas. Using a similar approach, we identified a novel, DCIS-associated lncRNA, MEG8 in our cohort of DCIS patients. Next, we compared the overlap between the DCIS cell lines and DCIS patients datasets to confirm those candidates. Results of current work on the investigation of the biological function of identified lncRNAs will also be presented. Ultimately, our goal is to validate a unique and specific lncRNA signature in DCIS, leading to the development of diagnostic tools that could change how early breast cancer is detected worldwide. Moreover, the functional characterization of DCIS-associated lncRNAs will open new windows for novel therapeutic targets in breast cancer treatment.

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676 Inducing Protein Synthesis Errors through mutant tRNAs promotes tumor growth in mice

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The hypothesis that protein synthesis errors are increased in cancer has long been supported by indirect evidence, such as deregulation of tRNAs, aminoacyl-tRNA synthetases, tRNA modifying enzymes and activation of protein quality control mechanisms. However, direct measurements of this event in tumors were still absent. We show here that human colon tumors and xenograft tumors produced in mice by two epithelial cancer cell lines mistranslate 2- to 4-fold more frequently than normal tissue and that tumor-derived cell lines retain that characteristic independently of the tissue of origin. Interestingly, certain amino acid substitutions were more frequent than others and misincorporations changed between tumor stages, highlighting that the occurrence of these errors shifts during tumor evolution To clarify the role protein synthesis errors may play in tumor biology, we expressed mutant Ser-tRNAs that misincorporate Ser-at-Ala (frequent error in tumors) and Ser-at-Leu (rare event in tumors) in NIH3T3 cells and investigated how they responded to the proteome instability generated by the amino acid misincorporations. There was high tolerance to both misreading tRNAs, but the Ser-to-Ala misreading tRNA was a more potent inducer of cell transformation, stimulated angiogenesis and produced faster growing tumors in mice than the Ser-to-Leu misincorporating tRNA. In fact, using the mutant Ser-to-Ala tRNA induced similar tumor growth kinetics to that of K-rasV12 expressing cells, by activating several UPR branches and AKT signaling. Most surprisingly, the relative expression of both misreading tRNAs increased during tumor growth, suggesting that protein synthesis errors are advantageous in cancer contexts. These data highlight new features of protein synthesis deregulation in tumor biology.

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677 Noncoding RNAs in *M. tuberculosis*-infected macrophages

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Mycobacterium tuberculosis (Mtb) infection reveals complex and dynamic host-pathogen interactions, leading to host protection or pathogenesis. Using a unique transcriptome technology (deepCAGE), we explored differentially expressed long non-coding RNAs (lncRNAs) of IFNγ (M1) or IL-4/IL-13 (M2) stimulated macrophages during Mtb infection in a time-kinetic manner. We obtained a total 151 differentially expressed lncRNAs species. It is evident that pre-stimulations distinctly enhanced the number of differentially expressed lncRNA, as well as protein-coding genes. Interestingly, Venn diagram analysis revealed that only 33 differentially expressed lncRNAs (21.9%) were commonly altered, which is in contrast with 49.5% in protein-coding genes. Another interesting feature was that the majority of differentially expressed lncRNAs were down-regulated, which is in contrast with that major differential expression of protein coding genes were up-regulation. Further, there was no drastic transient up-regulation nor down-regulation in differentially expressed lncRNAs, which is also different from those in protein-coding genes. Finally, we revealed positive expression correlation between differentially expressed lncRNAs and their nearest protein coding genes.

We also explored miRNA expression profile in Mtb-infected macrophages. MiR-143 and miR-365 were highly induced in Mtb-infected M2 macrophages. Knockdown of miR-143 and miR-365 using antagomiRs decreased the intracellular growth of Mtb HN878, reduced the production of IL-6 and CCL5 and promoted the apoptotic death of Mtb-infected M2 macrophages. Computational target prediction identified c-Maf, Bach-1 and Elmo-1 as potential targets for both miR-143 and miR-365. Functional validation using luciferase assay, RNA-pulldown assay and Western blotting revealed that c-Maf and Bach-1 are directly targeted by miR-143 while c-Maf, Bach-1 and Elmo-1 are direct targets of miR-365. Knockdown of c-Maf using GapmeRs promoted intracellular Mtb growth. Our work reports a host detrimental role of miR-143 and miR-365 during Mtb infection and highlights for the first time the role and miRNA-mediated regulation of c-Maf, Bach-1 and Elmo-1 in Mtb-infected M2 macrophages.

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678 EFTUD2/Snu114 missense variants associated with Mandibulofacial dysostosis Guion-Almeida type disrupt both protein function and splicing of EFTUD2/Snu114 pre-mRNA

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Several rare human disorders, characterised by craniofacial abnormalities, are now known to share genetic susceptibility across a number of core spliceosome genes. In the majority of these disorders, the disease variants lead to inactivation of one allele and are proposed to be disease-causing by haploinsufficiency. Of particular interest is the disorder Mandibulofacial Dysostosis Guion-Almeida type (MFDGA) that results from mutations in the U5 snRNP gene EFTUD2/Snu114. There are currently more than 100 different MFDGA-associated mutations in EFTUD2 that range from whole allele or multi-exon deletion to single nucleotide missense variants. In each case, the mutations are predicted to lead to a loss of function in the affected allele. Whilst the contribution of large deletions within EFTUD2 to allele loss of function is self-evident, the mechanisms of how missense mutations are disease-causing have not been characterised functionally.

Using a combination of bioinformatic-software prediction, yeast functional growth assays and a mini-gene splicing assay, we have characterised how MFDGA-missense mutations can lead to decreased expression of EFTUD2. We found that only 4/18 missense variants were likely to cause defective EFTUD2 function through altered protein function. Furthermore, we identified that of the remaining missense variants, 5 altered the normal splicing pattern of EFTUD2 pre-mRNA either through an increase in exon skipping or activation of a cryptic splice site leading to the introduction of a premature termination codon (PTC). Comparison of results with bioinformatics predictors for each missense variant revealed a disparity amongst different software packages and, in many cases, an inability to correctly predict the changes in splicing seen by mini-gene interrogation, ultimately highlighting the need for laboratory-based validation of bioinformatic predictions.

Overall, we have revealed how missense variants in EFTUD2 can influence both EFTUD2 protein function and pre-mRNA splicing to cause MFDGA and provide support for the growing evidence that missense variants can influence splicing and should be routinely tested for splicing defects.

679 An ALS-causing mutation in FUS leads to deficits in translation in vivo

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Mutations in FUS are associated with amyotrophic lateral sclerosis (ALS), a neurodegenerative disease leading to motor neuron death. FUS is localised mainly to the nucleus, however it also has various roles in the cytoplasm, including translation. The majority of the ALS-causing mutations cluster in the C-terminal nuclear localisation signal (NLS) of the protein, causing its increased cytoplasmic localisation, the degree of which correlates with disease severity. Whether the mutations may cause ALS through loss of nuclear functions of FUS, or by gain-of-cytoplasmic-functions is unknown. We investigated the effect of FUS mutants on cytoplasmic functions by assessing translation in vivo.

We used a FUS-ALS model with a point mutation that leads to a frameshift in exon 15 and subsequent deletion of the NLS (FUS-D14) and compared it with a loss-of-function model (FUS-KO), both crossed with a RiboTag mouse. By expressing the RiboTag (HA-Rpl22) under the ChAT promoter, we were able to specifically isolate ribosome-associated transcripts from spinal motor neurons and thereby monitor their translational landscape. Surprisingly, translation of only 40 and 45 motor-neuron specific transcripts was altered in FUS-D14 or FUS-KO, respectively. 6 of these transcripts were commonly altered in both FUS-D14 and FUS-KO, suggesting they reflect a loss of nuclear FUS function. However, changes in 34 transcripts were specific for FUS-D14, suggesting that they result from a toxic gain-of-function. These transcripts are enriched in targets of FMRP, as defined by previous iCLIP experiments. FMRP is thought to act by repressing translation, and is known to directly interact with FUS. We used polysome profiling to study the relative association of FMRP and FUS with translation machinery. We observed decreased association of mutant FUS-D14 and FMRP with polysomes in FUS-ALS. This observation was validated by proximity ligation assay in cultured embryonic motor neurons, where the interaction between FMRP and ribosomal protein RPL26 is reduced in FUS-D14 expressing neurons.

In conclusion, we have identified a possible gain-of-function mechanism of FUS-ALS mutation in vivo and in vitro. This finding provides insight in to the disease pathology and function of FUS in the cytoplasm.

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680 Single-color spatially multiplexed RNA in situ hybridization to reveal tumor heterogeneity

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In situ approaches, such as immunostaining and DNA in situ hybridization (ISH), are standard methods for the analysis of tumor tissue sections. While they provide detailed spatial information about a small number of genes, gene aberrations, or protein content, recent developments have additionally extended in situ analysis to transcripts. Using RNA-ISH multiplexing of several targets in a single tissue is commonly limited by the density of the transcript of interest and the spectral separation of fluorophores for detection.

Here, we present a novel method for single-color multiplexed RNA-ISH experiments on formalin-fixed paraffin-embedded tissue sections. To locally deliver ISH-probes to the tissue in regions of a few hundred micrometers (~100 sectioned cells), we applied an open space microfluidic approach - the microfluidic probe. Using the principle of simultaneous delivery and aspiration of the probe solution, ISH is performed locally allowing for spatial multiplexing on the same tissue while utilizing a single amplification and detection channel. Therefore, multiplexed detection of the transcript of interest with positive and negative internal controls on a single tissue section becomes possible. We used this approach to propose a semiquantitative measure of gene expression. Furthermore, we analyzed the expression levels of the biomarkers estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 in breast tumor tissues using multiplexed single-color or dual-color detection. While the number of different transcripts detected in fluorescence experiments is limited only by spectral separation of the fluorophores, the here established spatial multiplexing method can even be used in brightfield detection. This may facilitate its applicability in clinical settings. We thus provide a novel multiplexed method for the detection of transcripts in tissue sections, which may a find a wide applicability in the cell biological, neurobiological, and oncological context.

681 Targeting RNA Pol II Pausing to Alter Human Gene Expression

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This project studies RNA polymerase II pausing in human cells, and we found that it is highly regulated by nucleic acid structure and affects gene expression. While it is known that in model organisms, cancer, and stem cells, the RNA Pol II synthesizes RNA with punctuated pauses, the pattern of RNA Pol II pausing is less well characterized in other human cells. To consider manipulation of RNA Pol II pausing as a therapeutic approach to change gene expression, we need to understand its regulation in normal cells. Here we examined RNA Pol II pausing in five individuals and three human cell types. Using Precision nuclear Run-On and Sequencing assay (PRO-seq), we isolated nascent RNA with actively transcribing RNA polymerase, then mapped the locations of the polymerases. We find paused RNA Pol II in the promoters of over 7,000 human genes. RNA Pol II pauses highly consistently across individuals and cell types. At over 1,300 sites, RNA Pol II pauses at the same nucleotide locations. These results show that the polymerase pauses in a programmed manner. We found 7 factors including the two known pausing protein complexes, DSIF and NELF, and 5 cis-regulatory elements that characterize these pause sites. This includes a 9-mer motif where at over 65% of the sites, RNA Pol II pauses at a cytosine. To assess how RNA Pol II pausing affects gene expression, we sequenced the mRNA of the same samples. Studying the pause sites where our subjects are heterozygous, we confirmed that RNA Pol II pauses ~4-times more at a cytosine compared to another base, and this allelic pausing leads to significantly lower expression of the cytosine-bearing transcripts. By analyzing the thousands of samples collected by the Genotype-Tissue Expression Consortium, we corroborated that polymerase pausing explains allele-specific gene expression. Then, by site-directed mutagenesis, we showed that we can increase gene expression by changing the pause base from cytosine to thymine. In this presentation, I will describe how we manipulate the regulators of RNA Pol II pausing to alter the expression levels of specific genes and the implications of this finding for treatment of human diseases.

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682 Do redox-regulated microRNAs play a role in age-related muscle wasting?

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There is currently a disproportionate increase in age-related health issues, with one of the major problems being the age-related loss of muscle mass and function - sarcopaenia. Redox and epigenetic factors are key regulatory pathways associated with ageing. MicroRNAs, stable RNAs with half-life >24h, regulate muscle homeostasis posttranscriptionally. Oxidative modification of microRNAs could result in the regulation of non-native targets. Redox balance is disrupted during ageing and the accumulation of oxidised, most likely pathogenic, microRNAs in muscle leads to their disrupted specificity for regulating protein content.

We have validated microRNAs/mRNAs/proteins networks affected by ageing in muscle and have shown that modifying microRNA expression improves muscle function, but there is currently no research into the function of oxidised microRNAs in ageing.

Integrating epigenetic/redox experimental approaches with functional studies, we have shown that a set of microRNAs undergoes oxidation during ageing. We are validating key oxidised microRNAs and targets in human and mouse muscle. Moreover, we have shown that inhibiting one of the oxidised microRNAs in muscle of old mice positively affects myofibre size and muscle strength.

Key words: microRNAs, sarcopenia, redox

683 Disease modelling of core pre-mRNA splicing factor haploinsufficiency

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The craniofacial disorder Mandibulofacial Dysostosis Guion-Almedia type (MFDGA) is caused by haploinsufficiency of the U5 snRNP gene *EFTUD2/SNU114*. However, it is unclear how reduced expression of this core pre-mRNA splicing factor leads to craniofacial defects. Here we use a CRISPR-Cas9 nickase strategy to generate a human *EFTUD2*-knockdown cell line, and show that reduced expression of *EFTUD2* leads to diminished proliferative ability of these cells, an increased sensitivity to endoplasmic reticulum (ER) stress and the mis-expression of several genes involved in the ER stress response. Furthermore, RNA-Seq analysis of the *EFTUD2*-knockdown cell line revealed transcriptome-wide changes in gene expression, with an enrichment for genes associated with processes involved in embryonic development and development of the craniofacial region. Additionally, our RNA-Seq data identified widespread mis-splicing in the *EFTUD2* mutant cell line. Analysis of the functional and physical characteristics of these mis-spliced pre-mRNAs highlighted conserved properties, including length and splice site strengths, of retained introns and skipped exons in our disease model identifying enriched processes and pathways associated with the affected genes, including cell death and survival, the cell cycle, cell and organ morphology and embryonic development. Together, these data support a model in which haploinsufficiency in *EFTUD2* leads to the mis-splicing of a distinct subset of pre-mRNAs with a global effect on gene expression, including altering the expression of key ER stress response genes and genes involved in the development of the craniofacial region. The increased burden of unfolded proteins in the ER resulting from global mis-splicing would exceed the capacity of the defective ER stress response, inducing apoptosis in cranial neural crest cells that would result in craniofacial abnormalities during development.

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684 Deciphering translation dysregulation mechanisms across multiple models of ALS

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Amyotrophic Lateral Sclerosis (ALS) is a synaptopathy accompanied by the presence of cytoplasmic aggregates containing TDP-43, an RNA binding protein linked to ~97% of ALS cases. Using a *Drosophila*model of ALS based on TDP-43, we have previously shown that the localization and translation of futsch mRNA, which encodes a microtubule stabilizing protein (MAP1B) is altered. This results in synaptic instability at the Drosophila neuromuscular junction (NMJ), and sequestration of Futsch/MAP1B protein in motor neuron cell bodies in both flies and patient spinal cords. In addition, overexpression of TDP-43 or G4C2 hexanucleotide repeat (HRE) expansion in fly motor neurons results in decreased expression of the synaptic vesicle chaperone, Hsc70-4 at the NMJ. Mechanistically, mutant TDP-43 sequesters*hsc70-4*mRNA and impairs its translation. Electrophysiology, imaging, and genetic interaction experiments reveal TDP-43 and G4C2 HRE dependent defects in synaptic vesicle endocytosis. Notably, expression of Hsc70-4's ortholog, HSPA8 is also reduced in patient derived iPSC motor neurons harboring TDP-43 or C9orf72 mutations. These deficits can be partially restored in flies by overexpression of Hsc70-4 or proteins involved in synaptic vesicle cycling, suggesting a common disease pathomechanism for TDP-43 and G4C2 HRE ALS. Finally, RNA immunoprecipitations coupled with ribotagging and RNA fractionation experiments are uncovering additional candidate translation targets of TDP-43 that encode synaptic and metabolic proteins currently under investigation in flies and patient samples.

685 Transcriptome analysis based on RNA sequencing in understanding pediatric myelodysplastic syndrome

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Objectives Notable differences set myelodysplastic syndrome (MDS) in children apart from the group of malignant myeloid disorders bearing the same denominator in adults. First, the traditional classification of MDS in adults using cytomorphology and number of myeloblasts as main variables is of limited utility in children. Second, the clinical presentation of childhood MDS is heterogeneous and there is broad overlap with inherited bone marrow failure disorders making the differential diagnosis a challenging puzzle. Last but not least it has become evident that the mutational landscape of childhood MDS is composed of lesions other than those found in the eldery, supporting fundamental differences in pathogenesis. More than 50% of adult patients with MDS carry somatic mutations in spliceosome genes encoding proteins involved in the 3' splice site recognition and U2 snRNP function. In contrast, relatively little is known about recurrently affected genes and pathways in childhood MDS and their contribution to disease pathogenesis. In spite of technological advances, a diagnostic marker for these syndromes does not yet exist.

Methods In this study we performed, for the first time, an RNA-sequencing (RNA-Seq) analysis of pediatric MDS patients. For comparison, we also included healthy donor pediatric patients. All RNA samples from patients with MDS and normal donors were obtained from bone marrow blood samples.

Results RNA-Seq analysis revealed that 3200 transcripts were differentially expressed between pediatric patients with MDS and healthy donors. This analysis identified different signaling pathways and multiple genes that were differentially spliced in childhood MDS as compared with healthy donors. Genes selected for their involvement in the mitochondrial machinery, the cell-cell, protein ubiquitination, mRNA processing and splicing were significantly upregulated in MDS patients.

Conclusions In this project we focused on the analysis of differentially expressed (DE) genes in childhood MDS based on RNA-sequencing. Based on these results, we selected biologically relevant genes that were differentially expressed in order to validate them by using the droplet digital PCR. We now confirmed the genomic landscape of pediatric MDS is substantially different than adult MDS, and these findings may provide new treatment options for children with MDS.

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686 Molecular and functional characterization of snoRNP protein partners

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The human genome contains more than two hundred C/D box small nucleolar RNAs (snoRNAs) genes. C/D snoRNAs associate with a set of four core proteins to catalyze 2'-O-methylation of ribosomal RNAs (rRNA) and small nuclear RNAs (snRNAs). Interestingly, several lines of evidence suggest that snoRNAs are involved in unrelated cellular processes. First, the human genome encodes numerous orphans C/D snoRNAs that do not possess obvious complementarities with canonical target RNAs. Second, recent studies have shown that peculiar C/D snoRNAs may affect cell metabolism and proliferation. Finally, specific snoRNAs are deregulated in pathologies such as the Prader-Willi syndrome or in several cancers.

In front of this initially unsuspected complex picture, we emit the hypothesis that unappreciated protein factors associate with snoRNPs in normal as well as pathological conditions. We searched for candidates by a combination of co-immunoprecipitation (IP), RNA-IP, CLIP and mass spectrometry analyses. We identified that GNL3/Nucleostemin, a GTP-binding protein already known to play a role in ribosome biogenesis, interacts with assembly protein complexes involved in C/D snoRNP biogenesis. We also identified proteins interacting mostly, if not exclusively, with mature snoRNPs. For these two categories of candidates, we aim to identify the function of the interactions in relation with snoRNP biogenesis, composition, stability, localization and function and, conversely, whether the interaction modulates candidate protein functions.

687 Biophysical characterization of FUS liquid droplets.

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Almost one third of eukaryotic proteins are predicted to have long (> 30 residue) disordered regions[1]. The function of these eukaryotic-specific segments is poorly understood despite their highly abundance among disease-related proteins. Interestingly, intrinsically disordered regions (IDRs) are over-represented in RNA binding proteins, even though no interpretation for this observation exists. Apart from RNA recognition, most of these proteins share an important additional feature, formation of phase separated compartments both *in vivo* and *in vitro* under physiological conditions. Arguably, one of the best characterized such protein is FUS which the unstructured low complexity region was shown to form liquid droplets under stress conditions inside of the cell as well as with recombinant protein *in vitro*[2]. Normally FUS is localized predominantly inside the nucleus, where it participates in many important pathways like transcription regulation, RNA splicing and maturation. Loss of FUS function, by regulation or mutagenesis, has been associated with neurodegenerative diseases like amyotropic lateral sclerosis (ALS)[3]. Currently structural studies of FUS droplets were done either on solid state sample or with single centimeter sized phase separated compartment. Here we report a method to spectroscopically (NMR and EPR) study the dynamics ,diffusion and interactions of proteins inside the actual droplets. They are formed inside the mesh of agarose gel, which prevents sedimantation and fusion over experimental time. Using this technique we were not only able to analyze with atomic resolution protein-protein but also protein-RNA interactions. Strikingly, the low complexity degenerate region of FUS exhibits specific preference for G-quadruplex RNA sequences. While it is known that FUS interacts with telomeric G-quadruplex RNAs, here we show that this interaction can promote phase separation *in vitro* and this is not due to oligomerization of the G-quadruplexes rather stacking of protein aromatic sidechains on the solvent exposed bases of the G-quadruplex monomers.

[1] Ward J. J. et al., (2004 *JMB*. **337** (3): 635-45.

[2] Kato M. et al., (2012) *Cell*. **149** (4): 753-67.

[3] Murakami T. et al., (2015) *Neuron.* **88** (4): 678-90

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688 The enigmatic role of the exon junction complex component CASC3

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In metazoans, the exon junction complex (EJC) is a central component of spliced messenger ribonucleoprotein particles (mRNPs). EJCs are assembled by the spliceosome and deposited upstream of exon-exon boundaries in the nucleus. The heterotetrameric core of the EJC is composed of the proteins EIF4A3, MAGOH, RBM8A (Y14) and CASC3 (BTZ, MLN51). EJCs contribute to different steps of post-transcriptional gene expression including splicing regulation, translation and nonsense-mediated mRNA decay (NMD). CASC3 has been reported to be a core component of the EJC and to be crucial for assembly, the splicing regulating function of the EJC and NMD. However, there has already been evidence that CASC3 functions differently from other EJC components. To elucidate the cellular role of CASC3, we have established human HEK 293 cell lines in which CASC3 was inactivated by means of CRISPR-Cas9 genome editing. We show that in these cells the composition of the EJC and the interaction of peripheral proteins with the EJC are unchanged. However, using RNA-Seq we find that certain transcripts and transcript isoforms are upregulated in the CASC3-edited cells. Among these upregulated transcripts and isoforms are known as well as many new NMD substrates, suggesting that CASC3 is required for the efficient execution of NMD, at least for a certain number of transcripts. Using reporter mRNAs, we investigated the mechanism by which CASC3 mediates the degradation of these transcripts. Taken together, our results challenge a function of CASC3 as an assembly factor and core component of the EJC. However, our data also show that CASC3 is involved in the degradation of NMD substrates and therefore uncover the primary molecular function of CASC3 in human cells.

689 SART3 binding to post-splicing snRNPs suggests a molecular mechanism for spliceosome recycling

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Human spliceosome is a complex and dynamic machine composed of hundreds proteins and five small nuclear RNAs (snRNAs). Core spliceosomal proteins are organized around these snRNAs to form small nuclear ribonucleoproteins (snRNPs) that are named according to their respective snRNA: U1, U2, U4, U5 and U6. During activation of the spliceosome, snRNPs are remodeled to catalyze the splicing reaction. Post-spliceosomal snRNPs then need to be recycled to their original shape before a next round of splicing. Currently, there are very few data available about snRNP recycling and factors involved in this process. Protein SART3 has been proposed to function as a U4/U6 snRNP recycling factor, however a molecular mechanism of its function is not known. Here, we have analyzed SART3 interactions with spliceosomal snRNPs and discovered unexpected binding between SART3 and core U2 snRNP. Using MS2 system, we immunoprecipitated specifically U2 particles stalled prior to spliceosome assembly. These pre-spliceosomal U2 snRNPs do not associate with SART3 suggesting that SART3-U2 snRNP interaction does not occur during U2 snRNP biogenesis but is restricted to the spliceosome or recycled snRNPs. To characterize SART3-bound complexes, we applied two-step immunoprecipitation to specifically enrich particles containing both SART3 and U2 snRNPs. We show that in addition to U2 snRNP, immunopurified SART3-U2 complexes also contain U5 and U6 snRNAs. Together, our data imply that SART3 interacts with post-catalytic spliceosomes composed of U2, U5 and U6 snRNPs. We speculate that SART3 binds to a post-splicing complex to facilitate the release of U6 snRNA and chaperones U6 during its recycling.

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690 Investigation the role in mRNA export of the actin binding protein, Moesin

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Accurate and precise control of gene expression is critical for cell survival in order to respond to cellular stress and environmental stimuli. Gene activity is tightly regulated at the level of transcription and translation but mRNA export which links the two processes also plays key role in gene regulation. During RNA export, several specific proteins are recruited to the transcribed RNA molecule where they form an RNA-protein complex, called messenger Ribonucleoprotein Particle (mRNP). In our laboratory we are studying the function of Moesin, the single cytoskeletal actin-binding ERM protein in *Drosophila melanogaster*. ERMs (Ezrin, Radixin and Moesin) form a highly conserved group of proteins and carry out many crucial cytoplasmic functions including reorganization of the actin cytoskeleton, cell survival, membrane dynamics or cell migration. In our work we demonstrated that the Moesin protein is present also in the nucleus where it shows clear co-localization with mRNA export factors. In a functional assay we observed the accumulation of total mRNA in the nucleus upon RNAi against *moesin* in cultured cells and *in vivo* as well, demonstrating that the inhibition of Moesin function impairs mRNA export. As the detailed molecular mechanism underlying Moesin's nuclear activity is still not known, we aim to identify the nuclear protein interaction partners of Moesin. Mass spectrometry analysis verified by protein co-immunoprecipitation suggests that Moesin's function is related to the NXF2-mediated mRNA export pathway as a possible new binding partner of the Mediator complex. Moesin also showed colocalization with Mediator proteins on Drosophila larval giant chromosomes. In vitro assays will further confirm the protein interactions in order to better understand the role and significance of Moesin in mRNA export.

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691 Characterization of new assembly intermediates during box H/ACA snoRNP biogenesis.

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The conserved box H/ACA RNPs consist of one box H/ACA RNA and 4 core proteins: Dyskerin, NHP2, NOP10 and GAR1. A subset of these RNPs catalyze the formation of pseudouridine residues on various RNAs. Others participate in the nucleolytic processing of pre-ribosomal RNA and in the synthesis of telomeric DNA. Moreover, during the past years, dozens of H/ACA RNAs of unknown function have been described. The assembly of the H/ACA RNPs is a stepwise process which requires several assembly factors: SHQ1, NAF1 and the R2TP complex. SHQ1 interacts with Dyskerin in the cytoplasm as soon as it is synthesized, thereby protecting it from aggregation and degradation, and preventing Dyskerin from illicit binding to RNAs. SHQ1 is released with the help of the R2TP complex in the nucleus. NAF1 interacts with the RNA polymerase II and allows the co-transcriptional assembly of a pre-particle containing the nascent RNAs, Dyskerin, NOP10, NHP2 and NAF1. NAF1 keeps the H/ACA RNP inactive and needs to be replaced by GAR1 to produce mature and functional H/ACA RNPs in the Cajal bodies.

We have characterized the proteins associated with GFP-tagged versions of NHP2, GAR1 and NAF1 in HEK 293 cells, by immunoprecipitation combined to SILAC quantitative mass spectrometry. We have confirmed by IP and Duolink experiments that the identified associations also occur with endogenous proteins. Based on a clustering analysis of all the data obtained, we propose the formation of additional assembly intermediates during the biogenesis of box H/ACA RNA. Moreover, our data reveal new putative factors for box H/ACA RNP assembly.

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692 Single mRNP analysis by super-resolution microscopy and fluorescence correlation spectroscopy reveals that small mRNP granules represent mRNA singletons

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Small cytoplasmic mRNP granules are implicated in mRNA transport, translational control and decay. Employing Super-resolution Microscopy and Fluorescence Correlation Spectroscopy, we analyzed the molecular composition and dynamics of single cytoplasmic YBX1_IMP1 mRNP granules in live cells. Granules appeared elongated and branched with patches of IMP1 and YBX1 distributed along mRNA, reflecting the attachment of the two RNA-binding proteins in cis. Particles form at the nuclear pore and are spatially segregated from translating ribosomes, so the mRNP is a repository for mRNAs awaiting translation. Individual mRNPs contain a single mRNA and 5 to 15 molecules of YBX1 and IMP1, which is in agreement with the average number of mRNA-binding sites calculated from CLIP analyses. We conclude that small cytoplasmic mRNP granules are mRNA singletons, thus depicting the cellular transcriptome. Consequently, expression of functionally related mRNAs in RNA regulons is unlikely to result from coordinated assembly.

693 Structural dynamics in the essential human telomerase three-way junction

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Cells preserve genomic integrity and prevent the DNA damage response at the ends of chromosomes by maintaining specialized chromatin structures called telomeres. Telomere DNA is synthesized by the reverse transcriptase telomerase, a multi-subunit ribonucleoprotein (RNP) enzyme. Telomerase RNA contains several structurally conserved domains that contribute to RNP biogenesis and function. One of these domains is the conserved region 4 and 5 (CR4/5), which folds into a three-way junction (3WJ), binds directly to the telomerase catalytic protein subunit, and is absolutely required for telomerase function in vitro and in vivo. Generally, 3WJs have been shown to be highly dynamic and capable of adopting multiple tertiary conformations. In the case of the CR4/5 domain, two dramatically different structures have recently been reported for the 3WJ of a fish model organism in the absence or presence of its cognate protein. These structures highlight the ability of a particular RNA sequence to fold into exceedingly distinct structures. However, precisely how the intrinsic folding properties of the CR4/5 domain contribute to functional RNP assembly remains to be elucidated. In the present work, we characterize the human CR4/5 domain using high throughput chemical mapping, the recently developed mutate, map, and rescue strategy, and single molecule FRET techniques. Our data suggests that a functionally critical stem in the 3WJ is not stably folded in solution. Upon telomerase protein binding the structural equilibrium of the helix is shifted. Engineering telomerase RNA such that the structural equilibrium is altered results in defects in telomerase assembly and function. Taken together, these studies shed light on mechanisms of telomerase RNA folding and RNP assembly, as well as more fundamental principles of RNA structural dynamics and function.

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694 Modeling the oscillating cellular stress response to hepatitis C virus infection

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Stress granules (SGs) are cytosolic aggregates of stalled translation pre-initiation complexes that form under various stress conditions, including virus infection. Using long-term live-cell imaging based on confocal microscopy, we have shown that infection with hepatitis C virus (HCV), a major causative agent of chronic liver diseases, induces an oscillating host cell stress response characterized by cycles of SG assembly and disassembly. On the mechanistic level, protein kinase R is activated by viral double-stranded RNA and phosphorylates the eukaryotic translation initiation factor 2 alpha (eIF2alpha) leading to host translation repression and SG formation. This process is antagonized by GADD34, the regulatory subunit of protein phosphatase 1, which allows the rapid dephosphorylation of eIF2alpha leading to SG disassembly and thus reactivation of translation. With help of dynamic and quantitative mathematical modeling, we addressed the cell-to-cell variability and differences observed in SG oscillation patterns. We identified the molecular determinants involved in HCV-induced SG oscillations and predicted that rapid SG oscillations correlate with prolonged survival of infected cells despite extended stress duration. Taken together, our study provides an example of host adaptation to stress, which might be exploited by chronic virus infection.

695 Functional interactions of the metalloprotein YbeY, involved in ribosomal metabolism, with the putative metal efflux protein YbeX

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YbeY is a putative ribosomal endoribonuclease which has been implicated, among other things, to be involved in quality control of 70S ribosomes, in 17S pre-rRNA maturation and in ribosomal degradation. However, controversy reigns over its mode of action, substrates, co-factors, and interaction partners. Proposed interactors of YbeY include ribosomal protein S11, Era, YbeZ, and SpoT.

In many bacteria ybeY is located in the *ybeZYX-Int* operon, where *ybeZ* encodes a PhoH subfamily protein with NTP hydrolase domain and *ybeX* encodes a putative Cobalt/Magnesium efflux protein. While deletion of ybeY *Escherichia coli* homologue has a reduced growth rate during normal exponential phase growth, *ΔybeX* and *ΔybeZ* exhibit no effect on exponential growth. Nevertheless, *ΔybeY* and *ΔybeX* have largely overlapping phenotypes, including accumulation of 17S pre-rRNA and an approximately 1 kb 16S rRNA cleavage product (cleaved from the 3' side), sensitivity to heat shock, and to the protein synthesis inhibitors chloramphenicol and erythromycin. Overexpression of the YbeX partially rescues the growth phenotype of *ΔybeY*. In the case of *ΔybeX*, accumulation of aberrant rRNAs is associated with unequal colony size and slow outgrowth from the stationary phase, resulting in a prolonged lag phase. We also found that reducing the Mg+2 concentration in a minimal growth medium leads to lower growth plateaus for the ΔybeX strain. Taken together our results indicate a functional metal-dependent interaction between *ybeY* and *ybeX*.

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696 Ataxin-2 RNA granules: assembly and clearance of intracellular foci implicated in ALS and SCA2

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Ataxin-2 (Atx2) is a ubiquitous protein involved in multiple cellular functions. Poly-Q expansions in Atx2 have been implicated in the progression of the neurodegenerative diseases, Amyotropic Lateral Sclerosis (ALS) and Type-2 Spino-Cerebellar Ataxia (SCA2). Atx2 mediates translational regulation of several mRNAs (e.g. PERIOD, CaMKII) via binding to the 3'UTRs. Using *Drosophila* S2 cells, we have studied the dynamics of Atx2 granulesformed upon oxidative stress (arsenite exposure) or upon Atx2 overexpression. These granules contain several proteins known to be present in the canonical stress granules (SGs) and related granules can also be induced by expression of ALS-causative pathogenic GR50 or Fus proteins. The formation of these granules is dependent on a C-terminal intrinsically disordered region (IDR) in the Atx2 protein and we have shown that deletion of the IDR not only inhibits granule formation but also inhibits ALS-like degenerative phenotypes in Drosophila. Thus, understanding how SGs assemble and disassemble is both fundamentally and clinically important. Components of the cellular protein quality control machinery have been implicated in the triage of SGs. Thus, to elucidate the factors important for the assembly and clearance of Atx2 granules, we have employed proteomic and transcriptomic approaches. Mass spectrometry analyses unravel the complex interactions that Atx2 undertakes with a wide variety of proteins during normal and stress conditions. Interestingly, we find that Atx2 is transiently modified by oxidative stress and interacts with far more proteins under stress as compared to normal conditions and these proteins are important components of diverse cellular pathways. RNA-Seq reveals that mRNAs corresponding to several heat shock proteins and components of the ubiquitin-proteasome system are differentially regulated. Future work will unravel the mechanistic details of Atx2 stress granules dynamics and their relevance to physiology, pathology and therapy.

697 TSSC4 is a novel U5 snRNP-specific protein important for snRNP biogenesis

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Splicing is catalysed by a multi-megadalton complex called the spliceosome, which needs to be assembled *de novo* on each intron to be spliced. The spliceosome is formed from pre-assembled small nuclear ribonucleoproteins particles (snRNPs), each containing a small nuclear RNA and a specific set of proteins. Three of these snRNPs enter the splicing reaction as a pre-formed U4/U6•U5 tri-snRNP. During splicing the tri-snRNP undergoes extensive rearrangement, individual snRNPs are released and recycled before entering a next splicing reaction. Using SILAC, we have recently identified a protein called TSSC4 to interact with U5-specific proteins Prpf8 and Eftud2. Here, we show that TSSC4 is a new U5 snRNP specific protein, which also associates with components of the NTC complex. TSSC4 knockdown in HeLa cells impairs snRNP biogenesis and consistently with this result we observe increased accumulation of snRNP components in Cajal bodies, including a mono U5-specific protein CD2BP2. Finally, we provide evidence that TSSC4 is important for stable interaction between U5-specific proteins Snrnp200 and Prpf8. Together we conclude that TSSC4 as a novel U5 snRNP specific factor important snRNP biogenesis and speculate that TSSC4 also acts during recycling phase of the spliceosomal cycle.

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698 Disease-causing point mutations in FMRP's RNA binding domains affect RNP-granule stability in vivo.

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Loss of the gene encoding for Fragile X Mental Retardation Protein (FMRP) is the monogenetic cause of Fragile X Syndrome (FXS), the most commonly inherited form of intellectual disability. Typically, FXS results from a CGG-trinucleotide repeat expansion within FMR1's 5'UTR resulting in transcriptional silencing of Fmr1 during early development, although there are incidences of FXS occurring in patients with point mutations within FMRP's RNA-binding domains (RBDs). One of FMRP's prominent functions within neurons is shuttling translationally repressed mRNAs to distal neurites. FMRP is thought to suppress translation via directly binding to scanning polysomes and stalling them during elongation. Genetic models of FXS show that loss of FMRP results in dysregulated translation of its target mRNAs at distal dendrites, especially in response to neuronal signaling. Interestingly, missense mutations within the RBDs KH1, KH2, and the KH-like domain KH0, cause a range of defects in normal FMRP function, from disrupting FMRP's ability to bind known mRNA targets and protein complexes, to abolishing its role in translational suppression via abrogating its association with polysomes. Perhaps not surprisingly, FMRP is found within a variety of RNA-protein (RNP) complexes within neurons, including RNA Processing bodies (P-bodies) and stress granules which are involved in mRNA transport and decay and translation repression, respectively. Although FMRP's function within these granules has yet to be elucidated, here we present evidence that FMRP contributes to the size and stability of these cytoplasmic assemblies, specifically via it's RBDs. Surprisingly, missense mutations within the KH1 and KH2 domains have a severe impact on granule structure and dynamics, showing rapid redistribution of FMRP into photobleached granules compared to wt, indicating these domains are important for maintaining RNA and/or protein interactions in vivo. Additionally, FMRP contains a large intrinsically disordered region (IDR) which is itself sufficient to form FMRP-granules, and by adding back three of FMRP's RBDs (KH1, KH2, and RGG-box), there is a complete rescue of the wt granule phenotype in vivo. This study will bring invaluable insight into how RBPs and IDR domains found in proteins that undergo phase separation are contributing to defects in learning and memory.

699 A Complete Structural View of Pre-mRNA Splicing by the Spliceosome

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Pre-mRNA splicing is executed by the spliceosome, a supramolecular complex with exceptional dynamics in its composition and conformation. The fully assembled spliceosome can be isolated in at least eight major functional states: precursor of the precatalytic spliceosome (pre-B), pre-catalytic spliceosome (B), activated complex (Bact), catalytically activated complex (B*), catalytic step I complex (C), step II catalytically activated complex (C*), post-catalytic complex (P), and intron lariat spliceosome (ILS). Each splicing cycle, involving branching and exon ligation, results in the removal of the intervening RNA sequences between two target exons. The cryo-EM structures of the spliceosome at all states with atomic and near-atomic resolutions shed lights on the organization principle of the active site and spliceosomal components, as well as its molecular mechanism. In the last four years, we captured structures of all functional states have made it clearer the mechanisms of spliceosomal assembly, activation, catalysis and disassembly. Together, a complete cycle of pre-mRNA splicing can be recapitulated in atomic details.

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700 Combinatorial control of *Spo11* alternative splicing by modulation of RNA polymerase II dynamics and splicing factor recruitment during meiosis

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Homologous recombination and chromosome segregation in meiosis rely on the timely expression of two splice variants of the endonuclease SPO11, named a and b. However, in spite of its physiological importance, the mechanism underlying *Spo11* alternative splicing in meiosis is still unknown. By screening the activity of factors that are predicted to bind the alternatively spliced region of *Spo11*, we identified hnRNPF and H as key regulators of this splicing event in mouse spermatocytes. Although neither hnRNP was up-regulated in meiosis concomitantly with the switch in splicing, their recruitment to *Spo11*pre-mRNA was favored by selective modulation of RNA polymerase II (RNAPII) phosphorylation and processivity in proximity of the regulated exon. Furthermore, antisense oligonucleotides masking the hnRNPF/H binding sites recapitulated exon 2 skipping and SPO11a splicing, suggesting that hnRNPF/H act by competing out positive splicing regulators. Remarkably, knock-in mice lacking SPO11a proceeded through meiosis with defective X-Y chromosomes segregation, generating aneuploid gametes. Thus, our work reveals that modulation of RNAPII dynamics in concert with hnRNPF/H recruitment exerts a combinatorial control of the timely-regulated *Spo11*splicing during meiosis, which is essential for gamete ploidy.

701 Development of a *S. cerevisiae*-based system to study the mechanism of backsplicing

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Backsplicing, a specific form of alternative splicing, results in the formation of circular RNAs (circRNAs). Although a few mechanisms of circRNA generation were proposed, it is still unclear what determines the choice of back- vs linear splicing.

In order to better understand the mechanism of backsplicing we developed an assay to detect the presence of circRNAs in *Saccharomyces cerevisiae*[1]. Even though backsplicing is fairly inefficient in this organism, we chose it as our model, because its powerful genetic system allows for detailed mechanistic analysis.

In our assay, fragments of a two-intron gene, *SUS1*, were cloned in frame with the *ACT1-CUP1* reporter, allowing for the simultaneous detection of circRNAs by RT-PCR, and linear splicing efficiency assessment by growth on copper-containing plates.

The results obtained so far on constructs containing mutations in 5' splice sites and branch sites of the two *SUS1* introns are consistent with the "lariat precursor model" of circRNA generation proposed by Barrett et al.[2]. However, we detected additional circRNA products composed of exon 2 spliced to a cryptic 3' splice site in the 1st *SUS1* intron. We have found two sequence elements affecting backsplicing efficiency. (1) The above-stated exon 2-cryptic 3'SS is dependent on a UACUAAC-like sequence present in the 5'UTR. (2) Exon 2 of Sus1 contains a double-helical stem topped by a three-way junction[3], deletion of which increases efficiency of backsplicing, suggesting that the exon 2 structure inhibits its circularization. Thus, in *S. cerevisiae* backsplicing may proceed on unspliced pre-mRNAs, generating patterns more complicated than previously described.

References:

1. Wang, P. L. *et al.* Circular RNA is expressed across the eukaryotic tree of life. *PLoS One* **9,** (2014).
2. Barrett, S. P., Wang, P. L. & Salzman, J. Circular RNA biogenesis can proceed through an exon-containing lariat precursor. *Elife* **4,** 1-18 (2015).
3. AbuQattam, A., Gallego, J. & Rodríguez-Navarro, S. An intronic RNA structure modulates expression of the mRNA biogenesis factor Sus1. *RNA* **22,** 75-86 (2016).

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702 Cwc15 stabilizes the spliceosomal catalytic interactions

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To better understand structural rearrangements between the catalytic steps of splicing, we performed a structural search of the cryo-EM spliceosomal structures to identify proteins in very close proximity to the catalytic center, which are likely to affect splicing catalysis.

We focused on a poorly characterized protein, Cwc15, which through its disordered N-terminal domain interacts directly with U6 snRNA at the catalytic center. To explain the role of Cwc15 in splicing, we used the yeast *S. cerevisiae* model with Prp16 as a marker for spliceosomal rearrangements between the first and second steps of catalysis. *prp16-302* allele is defective in the exit from the first step conformation, and this results in cold-sensitivity at 18ºC.

We demonstrated that *cwc15*Δ allele suppresses the cs defects of *prp16-302* allele, restoring growth at 18ºC. A similar effect is observed with *cwc15* allele lacking the N-terminal disordered tail. This suggests that the N-terminal disordered tail of Cwc15 stabilizes catalytic interactions at least during the first step of catalysis. Furthermore, splicing of a two-intron gene, *SUS1*, is inhibited in *cwc15*Δ strain, accumulating unspliced pre-mRNA and yielding reduced levels of spliced mRNA. We suggest that interactions between the N-terminal Cwc15 tail and U6 snRNA modulate splicing at least during the first step.

Since modulation of splicing is proposed as one of the mechanisms altering gene expression in the stress response (Pleiss et al., 2007), we are also monitoring a potential Cwc15 involvement in response to various stresses (e.g. oxidative, heat, etc.), aiming to understand a mechanistic link between splicing and the stress response.

703 Combinatorial recognition of 3′ splice sites by UHM splicing factors

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Splicing factors SF1 and U2AF cooperatively bind the 3' splice sequence as an early step of spliceosome assembly. Within this complex the C-terminal domain of U2AF65 presents a particular structural motif called UHM (U2AF Homology Motif) allowing direct interaction with a short N-terminal domain of SF1 called ULM (UHM Ligand Motif). Interestingly similar domains and putative interactions are shared with a set of proteins involved in splicing. In particular the splicing factor SF3b155, which is recurrently found mutated in cancer, presents seven potential ULM binding sites for UHM splicing factors. Indeed splicing factors U2AF65, CAPER alpha, SPF45 and PUF60 bind SF3b155 at least in vitro. SF3b155 thus appears as a potential molecular hub in a network of splicing factors interactions. In this context, we shall present our progresses in deciphering the potential heterogeneity in spliceosome assembly linked to the ability of SF3b155 to interact with various partners and its consequences for the choice of splice sites in normal cell and in disease.

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704 Withdrawn

705 Exonic UACUAC motif affects splicing of defective introns at early steps of spliceosome assembly

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At the early steps of spliceosome assembly, U1 and Msl5/SF1 (yeast/human orthologs) recognize 5'SS (/GUAUGU) and BS (UACUAAC) motifs, respectively, and form important cross-intron interactions during the intron-definition phase of spliceosome assembly. In metazoa, similar interactions occur across the exon during the preceding exon-definition phase. We have found that in yeast, the UACUAC motif inserted in the 5' exon strongly improves splicing of introns with suboptimal 5'SS, in which the U1:5'SS interaction is destabilized. Such UACUAC motifs improve splicing when placed upstream of the 5'SS (in the 5' exon), but not downstream of it (in the intron). Exonic UACUAC motifs generally improve splicing of 5'SS and BS mutants defective at the early steps of spliceosome assembly.

In the presence of *msl5* alleles, the UACUAC exonic motifs fail to improve splicing of the 5'SS and BS intron mutants, indicating that Msl5 binding is responsible for the observed effect. In particular, short (18 or 35 aa) deletions within the 63 aa disordered C-terminal Msl5 tail strongly inhibit the functionality of exonic UACUAC motifs, suggesting that this tail is involved in stabilization of the nearby U1 binding. Previously, the 5'SS motif (GUAUGU) placed downstream of the BS was shown to similarly correct splicing of introns with suboptimal 5'SS (Libri et al., 2000); we have confirmed and extended these findings. Importantly, in all cases, the function of inserted 5'SS or BS motifs requires proper polarity relative to the nearby BS or 5'SS, respectively. Only the BS-5'SS polarity, characteristic of the exon-definition phase (and not the 5'SS-BS polarity, typical of the intron-definition phase) supports improvement of splicing of suboptimal introns.

Thus, Msl5 binding within the closely positioned BS-5'SS motifs improves splicing of introns with suboptimal 5'SS or BS signals, presumably by stabilizing Msl5-U1 interactions. The polarity of Msl5-U1 binding is critically important for function and resembles the polarity of exon definition interactions. These results allow us to develop an attractive system in yeast to study the still poorly understood exon-definition complexes and their subsequent transition to intron-definition spliceosomes. Understanding of exon definition interactions may help explain regulation of both alternative splicing and circular splicing processes.

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706 A missense DDX38 mutation linked with retinitis pigmentosa

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During splicing, snRNPs and pre-mRNA undergo a series of association-dissociation steps and eight RNA helicases play essential roles in controlling these conformational rearrangements. A large number of human diseases are consequence of splicing errors. Surprisingly, most mutations in ubiquitously expressed spliceosome components exhibit a tissue specific phenotype. For example, mutations in several snRNP-specific proteins cause retinitis pigmentosa (RP), a major cause of blindness. A missense mutation in the RNA splicing helicase DDX38, which results in the Gly332Asp substitution was associated with early-onset autosomal recessive RP. Our aim is to determine how RP mutation affect DDX38 function. We provide evidence that the DDX38 helicase interacts with several components of the active spliceosome and that the RP mutation does not inhibit these interactions. We further show that the knockdown of DDX38 as well as the expression of RP-related mutant protein affect splicing efficiency of several ubiquitously expressed and retina specific genes. Moreover, we showed that the knockdown of DDX38 and expression of the mutant variant enhance usage of cryptic splice sites. We hypothesize that DDX38 has role in RNA splicing quality control and that RP-related mutation affects this function.

707 DDD01004659- a novel modulator of pre-mRNA Splicing-->

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Previously, together with the group of Reinhard Lührmann and the University of Dundee Drug Discovery Unit (DDU), we used a high throughput *in vitro* splicing assay (adapted from Samatov et. al., 2012), to screen a curated library of >75,000 small, drug-like compounds (see; www.eurasnet.info). This identified several novel classes of small molecule splicing inhibitors, including 'DDD40800' (Pawellek et al., 2014). In cells, treatment with 'DDD40800' leads to a change of ~3000 splicing events, cell cycle arrest and the disruption of Cajal bodies. Thermal proteome profiling (TPP) revealed STRAP as a potential cellular target of 'DDD40800'. We now have screened ~100 derivatives of DDD40800, identifying 12 new compounds that inhibit splicing *in vitro* and *in vivo*. One of these drug-like, small molecules, 'DDD1004659', is more potent than DDD40800. DDD1004659 inhibits splicing *in vitro* by blocking one or more early steps of spliceosome formation, alters alternative pre-mRNA splicing *in cellulo*, (e.g. adenovirus E1A and MCL1 gene transcripts) and disrupts nuclear Cajal bodies. DDD1004659 inhibited cell cycle progression in multiple human cell lines, with arrest in S/G2&M phase in a time and dose-dependent manner and formation of abnormal mitotic cells and micronucleated cells. CESTA experiments indicate that DDD40800 and DDD1004659, while related in structure, likely have different protein targets. Experiments are in progress to characterise in more detail how DDD1004659 modulates pre-mRNA splicing.

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708 Role for pre-mRNA secondary structure in efficient splicing

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In the first stages of RNA splicing, sequence-distant 5' splice sites and branch point sequences must be recognized and co-localized with high specificity over cryptic alternatives; the mechanism for this splice site selection remains mysterious. In some cases, RNA secondary structure has been implicated in this process, for instance by bringing together the correct 5' splice site and branch site sequences spatially via base pairing in the Dscam gene of *Drosophila melanogaster* or the RPS17b intron of *Saccharomyces cerevisiae*. Here we ask whether secondary structure properties can act as general mechanisms for promoting efficient splicing. We look across *Saccharomyces cerevisiae* introns and use a variety of secondary structure prediction methods to generate consensus ensembles for intron and control sequences, finding numerous features in intron sequences more often than expected, including 5' splice site and branch point co-localization, the lack of branch point sequence protection, and the formation of zipper stems between key splicing sequences. These results motivate the development of multidimensional chemical mapping and functional experiments to experimentally test whether these specific pre-mRNA structural features influence splicing efficiency. By better understanding the role of pre-mRNA secondary structure, we can move closer to a predictive model for splice site selection.

709 Regulation of miR-17-92 cluster splicing by hnRNP A1

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In eukaryotes, the majority of genes are transcribed as pre-mRNAs, which are formed by introns and exons. The splicing process involves the excision of introns and exon ligation, resulting in mature transcripts (mRNA). This process is performed by a complex machinery named spliceosome, which is composed by five small nuclear RNAs (snRNAs) and more than 100 proteins. The regulation of the splicing process has already been associated with the presence of proteins that associate transiently to spliceosome components and/or pre-mRNAs. The performance of proteins that modulate splicing may directly interfere in the interactions between the spliceosome components and the RNAs. Heterogenous nuclear ribonucleoproteins (hnRNPs) are proteins that can be associate with spliceosome complex. These proteins have a regulatory role in the splicing sites and are frequently involved in mediating alternative splicing. In the human genome, over 70% of microRNAs are located in introns, therefore, it is possible that splicing is important for its maturation. Importantly, miRNAs participate in many regulatory processes related to cell control and apoptosis. Such processes are of extreme importance on the progression of cancer, and for this reason, many studies have already demonstrated that miRNAs are associated with the process of tumorigenesis. Increased expression of *miR-17-92* cluster has already been related to the development of many pathologies, such as thyroid cancer, lung cancer and lymphoma. Previous studies have shown hnRNP A1 is important for the maturation of some miRNAs of this cluster. Our working hypothesis is that hnRNP A1 is associated with introns containing miRNAs, especially the miRNAs of miR17-92 cluster. Our results show that overexpression of hnRNP A1 in HEK293T and BCPAP cells increase expression of miR-17, miR-18 and miR-19a in comparison to control, but decrease expression levels of miR-92. Interestingly, cells overexpressing hnRNP A1 presented higher proliferation, migration and invasion rates, indicating this protein can positively regulate cellular process involved in cancer development.

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710 The tRNA Splicing Endonuclease of *Haloferax volcanii* - Transcription Repression with CRISPRi and Potential Substrates

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tRNA molecules are transcribed as precursors and have to undergo a number of processing steps to become functional tRNAs. One of the enzymes involved in tRNA maturation is the splicing endonuclease that recognizes and cleaves the bulge-helix-bulge (BHB) motif present in the introns of tRNAs in archaea and eukarya. After splicing, tRNA halves are ligated by an RNA ligase to become mature tRNAs. The RNA ligase also circularizes the excised intron. Using different approaches, we aim to investigate all biological functions and substrates of the splicing endonuclease in the halophilic archaeon *Haloferax volcanii*.

We successfully repressed transcription of the essential splicing endonuclease encoding gene *endA*, using the endogenous CRISPR-Cas system type I-B in our model organism *Haloferax volcanii* for CRISPRi. This resulted in reduction of the *endA* transcript down to 1.1 % compared to the control strain, causing a growth defect in CRISPRi cells. Unspliced $tRNA^{Trp}$ precursors accumulated and a reduction of mature $tRNA^{Trp}$ levels was observed.

To investigate the substrate specificity of the splicing endonuclease, we generated recombinant protein and successfully carried out *in vitro* processing experiments using the known substrate $tRNA^{Trp}$. We could also confirm processing of the 16S rRNA precursor. Furthermore, additional BHB motifs were identified in the genome by bioinformatics analysis of RNA-seq data and tested using the established *in vitro* processing assay. In addition, RNA from CRISPRi and control cells were used in RT-PCR experiments to reveal whether these motifs are recognized *in vivo* by the splicing endonuclease. If these motifs are substrates for the splicing endonuclease, we expect unprocessed RNAs to accumulate in CRISPRi cells. Moreover, we aim to identify circular RNAs in *Haloferax.* This will give us more insight into the abundance of circRNAs in *Haloferax* in general and how repression of the *endA* expression influences their occurrence.

711 Probing the functional role of *S. pombe* splicing factor SpPrp16, an ATP dependent RNA helicase in splice-site recognition

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Nuclear pre-mRNA splicing occur precisely at conserved sequence elements at and around the splice-sites resulting in exon ligation and removal of introns as lariat RNA. Prp16 is a DExD box RNA helicase splicing factor that in budding yeast promotes critical spliceosomal rearrangements that allow the second step catalytic reaction. Pre-mRNA splicing studies in *Schizosaccharomyces pombe*, where transcripts have several short introns with more degenerate consensus elements, will enable us to understand the co-evolution of spliceosome functions with regard to splice-site recognition by intron definition. Prp16 functions are largely unexplored in *Schizosaccharomyces pombe*. Here, we present functional studies on the essential *spprp16*$^{+}$ gene, through our studies on several missense alleles: *spprp16T643K*, *spprp16D712R*, *spprp16Q812A*, *spprp16D612A*, *spprp16F528S* and *spprp16G515A* in the helicase domain. We show the functional conservation of SpPrp16 C-terminal region, as a chimeric Prp16 protein that is fused to N-terminal domain of budding yeast ScPrp16 can complement the budding yeast temperature sensitive recessive mutant *scprp16-2*. Splicing of several fission yeast cellular introns is abrogated in the mutant *spprp16F528S* while cellular splicing is unperturbed in the *spprp16G515A* mutant. Biochemical assays were performed using bacterially purified proteins comprising the helicase domains from the wild type and the mutant fission yeast SpPrp16 proteins. The in-vitro RNA unwinding activity of wild type SpPrp16 and SpPrp16F528S, SpPrp16G515A and SpPrp16T643K mutant proteins have been correlated to their in vitro ATP hydrolysing and RNA binding ability. Taking leads from transcriptome deep sequencing data based global splicing profile in *spprp16*$^{+}$ and *spprp16F528S* strains, we experimentally investigated the contribution of splice site and snRNA interactions and observed global splicing dependence on SpPrp16 is dictated by the strength of splice site-snRNA interactions for individual introns in cellular transcripts. Taken together these studies help us infer that the global dependence on Prp16 for splicing is dictated by the strength of splice site-snRNA interactions in the genome. In vitro studies together with the in vivo splicing studies observed for transcripts hints the involvement of some spprp16 residues in indispensable spliceosomal interactions and others in its intrinsic enzymatic activity.

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712 Fluctuations in inositol polyphosphates alter substrate selection for the first step of splicing

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Recent cryo-electron microscopy (cryo-EM) has allowed the visualization of the structure of the spliceosome at many different stages in the splicing process. Two different groups recently noted the presence of the small molecule inositol hexakisphosphate (IP_6), also known as phytic acid, in a positively-charged cavity in the N-terminal domain of Prp8, close to the 5'-exon–U5 snRNA loop I helix (Fica et al., 2017; Zhan et al., 2018; Wan et al., 2019). IP_6 is an inositol ring with a phosphate group at all six carbons that is synthesized through sequential phosphorylation of inositol triphosphate (IP_3). IP_6 can be further phosphorylated to IP_7 and IP_8 and functions both as a signaling molecule and as a co-factor for many enzymes, including ADARs and Gle1. Thus far, there have been no investigations into the role of IP_6 in splicing.

Deletion of genes encoding the kinases and phosphatases in the inositol polyphosphate cycling pathway (i.e., *ARG82*, *DDP1*, *IPK1*, *KCS1*, *OCA1*, *OCA2*, *SIW14*, *VIP1*) alters the levels of polyphosphate isoforms. Here, we show that deletion of specific polyphosphate enzymes improves the first catalytic step of splicing for splicing reporters that are impaired for first-step catalysis. Consistent with a role in modulation of spliceosome dynamics, two of the genes show negative genetic interactions with the *prp8*-R1753K allele that improves the first step and exhibit temperature-sensitive phenotypes that are rescued by *prp8*-161 and -162 alleles that improve the second step. In addition, three other genes in the IP pathway rescue the cold sensitivity of *prp8*-R1753K. These data suggest that modulating the levels of inositol polyphosphate in yeast may provide an opportunity to fine-tune splice site selection.

Fica SM, Oubridge C, Galej WP, Wilkinson ME, Bai XC, Newman AJ, Nagai K. 2017 Nature 542:377-380. doi: 10.1038/nature21078.

Zhan X, Yan C, Zhang X, Lei J, Shi Y. 2018 Cell Res. 28:1129-1140. doi: 10.1038/s41422-018-0094-7.

Wan R, Bai R, Yan C, Lei J, Shi Y. 2019 Cell 177: S0092-8674(19)30155-2. doi: 10.1016/j.cell.2019.02.006.

713 Two conserved A-C mismatches in yeast U6 snRNA's internal stem-loop have multiple and opposing functions

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Pre-mRNA splicing is accomplished by the spliceosome, a large and dynamic protein-RNA enzyme whose central catalytic component is U6 small nuclear RNA. To enter the splicing cycle, U6's internal stem-loop (ISL) unwinds to allow U4/U6 annealing. Later, it partially reforms during the exchange of U4 for U2 in the active site, where it coordinates catalytic metals. Two conserved A-C mismatches in the ISL, A62-C85 and C67-A79 in yeast, promote ISL dynamics. Substitutions that stabilize either mismatch result in cold-sensitive (cs) growth, presumably by slowing U4/U6 annealing and/or perturbing the active site. The severity of the cs phenotype of U6-62/85 mutants, however, also depends on sequence: a pyrimidine/purine (YR) orientation is invariably more cs than the corresponding purine/pyrimidine (RY) pair, despite symmetric stacking interactions with neighboring nucleotides. A C-G pair at U6-62/85 (U6-CG) is lethal even at 30 or 37 °C, yet U6-GC is less cs than U6-UA. We propose that stable U6-62Y/85R mutants cause at least one defect in addition to stabilizing the ISL. We tested our hypothesis that the YR orientation exacerbates cs growth by disrupting the U6-62/U4-57 pair in U4/U6 Stem I and found that Stem I stability only partially explains the YR-specific defect. To further investigate, we selected and identified spontaneous genetic suppressors of the cs growth of U6-GC and U6-UA strains. Our study yielded one mutation expected to stabilize U4/U6 (U4-A16G) and many mutations expected to destabilize the free U6 snRNP. Of note were a complete deletion of the C-terminal domain in U6's binding partner Prp24 (Cdel47) and null mutations in *LSM7*. Lsm7 is one of seven protein subunits of the Lsm2-8 ring, which binds U6's 3′ end. These results are consistent with reported interactions between Prp24's C-terminal SNFFL-box motif and Lsm2-8 (Rader and Guthrie, 2002; Montemayor et al., 2018), and imply Lsm7 mediates this interaction. Intriguingly, lsm7Δ and Prp24-Cdel47 each increase rather than suppress the cs phenotype caused by stabilization of the C67-A79 pair (U6-A79G), suggesting the ISL's two A-C mismatches have opposing functions. We will present our progress on two genetic selections: a genome-wide selection for U6-A79G-suppressors and a *PRP8*-targeted selection for U6-UA-suppressors.

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714 Disruption of autoregulatory feedback mechanisms of the minor spliceosome core di-snRNP proteins 65K and 48K, leads to cell cycle defects.

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The minor (U12-type dependent) spliceosome excises a rare group of introns that are characterized by a highly conserved 5' splice site (5'ss) and branch point sequence. Intron recognition is facilitated by the U11/U12 di-snRNP, which is stabilized by seven integral proteins; 65K, 48K, 59K, 35K, 31K, 25K and 21K, that are unique to the minor spliceosome. Previous work in our lab identified an autoregulatory feedback mechanism for two of the core proteins associated with the di-snRNP, U11/U12-65K and U11-48K, involving a highly conserved U11-snRNP binding splicing enhancer element (USSE), present in intron 4 of *SNRNP48*(*48K*) and 3'UTR of *RNPC3*(*65K*). Binding of the U11 snRNP to the USSE enhances recognition of upstream cryptic 3'splice sites (3'ss) by the major spliceosome. Activation of the cryptic 3'ss in *SNRNP48*, leads to the inclusion of a 'poison' exon that targets the mRNA for NMD, whereas in *RNPC3*, it results in production of a long 3'UTR isoform that is retained in the nucleus. The extensive conservation of the USSE and consequent regulation of the 48K and 65K proteins suggests an essential cellular function for this regulatory mechanism.

To investigate the consequences of disrupting these autoregulatory mechanisms, we employed CRISPR-Cas9 genome editing to excise or mutate the USSE and flanking conserved sequences in HEK 293 and eHAP1cells. As expected, mutations that disrupt the USSE led to significant upregulation of mRNA and protein particularly in homozygous mutations. USSE deletion in *RNPC3* led to increased nuclear size and G2 arrest with 4N DNA content, whereas in *SNRNP48*, there was a more drastic phenotype of giant multinucleated cells including spindle and cytoskeletal abnormalities. Due to ongoing compensatory mechanisms in these cells, we generated conditional knockout alleles to monitor initial cellular and molecular alterationsthat lead to the observed cell cycle defects. Preliminary data obtained from these conditional knockout lines exhibit increased cell death, growth delays and noticeable size differences in nuclei, which are consistent with previous observations. Our findings suggest that the precise maintenance of the levels of 65K and 48K proteins of the minor spliceosome, is essential for normal cell cycle progression.

715 Alternative splicing of Gephyrin, a code for the diversity of inhibitory synapses

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Brain function relies on a balance between neuronal excitation and inhibition established through a myriad of specific synapses. How the diversity and specificity of inhibitory synapses take place remain unclear although it is proposed to lean on the molecular complexity of their components. The Gephyrin (GPHN) gene is the main molecular organizer of inhibitory synapses in mammalian. Unconventional isoforms, produced by irregular splicing, were identified as risk factors for neuropsychiatric pathologies including schizophrenia, Autism Spectrum Disorders (ASDs), and more recently in epileptogenesisis. We have conducted a deep analysis of *Gphn* expression in mouse brain using long read sequencing. Remarkably, our data reveal an unexpected diversity of *Gphn* transcripts (over than 277), while only two transcript isoforms are currently reported in public database (Ensembl). We observe that the newly identified *Gphn* transcripts are regulated during brain development and expressed in a tissue specific manner. In addition, we found some of these novel transcripts associated to the translation machinery and peptides corresponding to new splice junctions where identified by mass spectrometry, strongly supporting that many of the novel transcripts encode for new GPHN protein isoforms. To get inside the functional aspect of this diversity, we examined in primary neuronal cells the localization and function at the synapse of novel GPHN protein isoforms arising from transcripts regulated during development. We demonstrate that new GPHN isoforms own different clusterization properties at the post-synaptic membrane, thereby supporting their capability to modulate the synapse strength. In addition, we found that inhibitory synapses are endowed with a specific combination of GPHN protein domains highlighting the different incorporation of GPHN isoforms in synapses. We identified pattern of GPHN expression associated with specific synapses targeted a different subcellular localization/region of the neuron (dendrites, soma, and axon) and associated with various specific inhibitory receptors. We propose that GPHN isoforms arising from alternative splicing regulation elaborate a code involved in the diversity and the specificity of inhibitory synapses.

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716 Regulation of splicing integrin α6 during development and differentiation

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Alternative splicing is an important mechanism for creating protein diversity. Integrins are significant in many aspects of cell biology, including cell signalling and interaction with the cell matrix. by monitor splicing patterns for paralogues and orthologues of integrin subunit alpha 6 (ITGA6) to see alternative splicing events, ITGA6 has two different cytoplasmic C-termini (a6A and a6B) that shift 100% between stem cells and fibroblasts. These two forms are extremely conserved in paralogues and orthologues of ITGA6 alternative splicing. The a6A and a6B integrins had been differentially implicated in the expression in the function of breast cancer and cancer stem cells.

It has already shown that enhancing ITGA6 exon 25 is under the control of three separate splicing factors MBNL1, RBFOX2 and ESRP. However, PTBP was discovered as a novel regulator for ITGA6 splicing that inhibited the exon of ITGA6 in cancer cell line. We aim to identify the mechanism of splicing of this ITGA6 alternative exon, including identifying the PTB binding site that regulates ITGA6. A minigene system was established to study the regulation of the ITGA6 alternative exon. The ITGA6 minigene positively responded to siRNA mediated depletion of splicing factors in the same way as the endogenous gene, indicating this minigene was a good model. The alternative exon of ITGA6 was activated by MBNL1 and was inhibited by PTBP, leading to more production of ITGA6B. Using this minigene plasmid it was confirmed that PTBP inhibited alternative splicing of ITGA6. Through a series of in silico analyses, a binding site for PTB was identified downstream of the regulated exon. Surprisingly, loss of this PTB binding site actually repressed this splicing event. These data suggest that PTB both activates this alternative splicing event through direct RNA-protein interactions, but also more strongly represses this exon, possibly through protein interactions with other regulatory factors.

717 A novel approach to detect the influence of RNA-binding proteins on pre-mRNA processing

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The processing of nascent pre-mRNA consists of many steps, with the key role of exon splicing and primary transcript polyadenylation. Both processes are crucial to amplify the variability of cellular mRNA and protein pools. Several studies indicate the existence of RNA-binding proteins (RBPs) that act on splicing as well as 3' end processing but the specificity of these regulatory effects to a restricted cell types or conditions is yet to be discovered. In the following study we present a novel method to identify RBPs that could shape the pre-mRNA maturation. Applying our approach to RBP knock-down experiments we were able to identify both novel and well-known RBPs which seem to be regulators of splicing and/or polyadenylation. Our tool reports the exact sequence motifs on which the RNA-binding proteins act and allows us to gain insight into the nature of their action.

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718 KDM3A regulates alternative splicing of cell-cycle genes

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Changes in the cellular environment result in chromatin structure alteration, which in turn regulates gene expression. To learn more about this connection, we focus here on the H3K9 de-methylase KDM3A. Using RNA-seq, we found that KDM3A regulates the transcription and alternative splicing of genes associated with cell-cycle and DNA damage. To study the role of KDM3A in cell-cycle, we silenced it and found that the G2/M DNA damage check-point is hindered. In addition we found that as part of the cell response to DNA damage, KDM3A undergoes phosphorylation by PKA at serine 265. Our results suggest that KDM3A phosphorylation following DNA damage promotes an expression as well as alternative splicing pattern that allow the cell to repair the damage before continuing to replicate. Focusing on several alternative splicing events regulated by KDM3A, we are looking into the method by which KDM3A regulates cell-cycle progression following DNA damage. On a more mechanistic level we identified an adaptor system orchestrated by KDM3A to connect chromatin via HP1b to alternative splicing through the spliceosome component SF3B1. Our work demonstrates the effect that environment has on alternative splicing via the chromatin factor KDM3A.

719 Missense variations in *RBM10* cause a new syndrome with intellectual disability

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RNA binding protein 10 (RBM10) is a RNA binding protein involved in the regulation of alternative splicing and mRNA stabilization. Loss of function mutations in the *RBM10* gene cause the congenital disease, TARP syndrome, with severe malformations and early childhood death.

In a Danish family with history of X-linked intellectual disability a missense variant inherited through healthy women was identified in the *RBM10* gene of two affected males. We have collected multiple patients with intellectual disability and missense variants in the *RBM10* gene, demonstrating that missense variants in the RBM10 gene can cause disease.

We hypothesize that the missense variants, unlike loss of function variants, result in the production of RBM10 protein with an altered function. Many of the identified missense variants cluster in the RNA recognition motif 2 (RRM2) of RBM10. We hypothesize that these variants result in a RBM10 protein with an altered RNA binding profile, resulting in missplicing of many genes, thereby causing a new syndrome with intellectual disability, that differs from the known and more severe TARP syndrome, which is caused by loss of function mutations.

To explore our hypothesis, we are currently testing the effect of the missense variations by overexpression experiment. The missense variants are introduced into a RBM10 expression vector by in vitro mutagenesis. The effect of the patient mutations on the protein stability is investigated by western blotting analysis. Cell proliferation is investigated in cells expressing RBM10 protein with patient mutations.

Moreover, we have collected RNA from cells from affected patients and controls which will be analyzed by RNA sequencing. We expect that our study will contribute to explain the observed clinical phenotype and provide general knowledge of RBM10 function.

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720 Changes of phosphorylation drive alternative splicing modulation by mild heat shock in human cells

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In human cells, stress conditions globally affect transcriptional and post-transcriptional regulations of gene expression including alternative splicing of pre-mRNAs and lncRNAs. However, the mechanisms underlying these modulations remain poorly understood. Concerning mild heat stress (41 to 43°C), relocalization of splicing regulators such as SR or hnRNP proteins within nuclear or cytoplasmic stress granules has been proposed to participate to alternative splicing modulation, but so far this has only been verified for one splicing reporter: the adenoviral E1A gene.

In order to better understand the mechanisms underlying splicing alterations in response to mild heat-stress, we performed a genome-wide transcriptome analysis on mild heat shocked HeLa cells using Human Junction ArraYs (HJAY) from Affymetrix. Our results highlight significant alternative splicing variations of about 450 pre-mRNAs. We then tested the contribution of the formation of nuclear or cytoplasmic stress granules to heat shock induced splicing alterations and established that these events do not contribute to splicing alterations nor to the return to normal splicing pattern after a long recovery time. In agreement with this result, none of the SR or hnRNP protein depletions we performed modulate alternative splicing in the same way as mild heat shock did. We also demonstrated the HSF1-independence of heat-induced splicing modulations. Finally, in collaboration with Juan Valcarcel's lab, we observed a similarity between profiles of alternative splicing changes induced by mild heat shock and the knockdown of some spliceosome core components including SF3B1. We further confirmed that heat shock modulated splicing events were affected in a similar way by SF3B1 knockdown. Surprisingly, we did not detect any effect of heat shock on total SF3B1 protein level. Instead we observed that changes of SF3B1 phosphorylation status and nucleocytoplasmic distribution were associated with mild heat shock. Importantly, treatment of HeLa cells with phosphatase inhibitors prior submission to heat shock totally inhibited heat-induced splicing modulations. Altogether our results established that alternative splicing modulation by mild heat shock is a matter of phosphorylation changes occurring on the core spliceosome machinery rather than on alternative splicing regulators.

721 Exon Junction Complexes Suppress Spurious Splice Sites to Safeguard Transcriptome Integrity

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Productive splicing of human precursor messenger RNAs (pre-mRNAs) requires the correct selection of authentic splice sites (SS) from the large pool of potential SS. Due to the degenerate nature of the splice consensus sequences, the cell is faced with the challenging task to discriminate between authentic and so-called cryptic SS, which exhibit consensus motifs but are not intended to be used. Therefore, many different mRNA-binding proteins assist the spliceosome in the accurate detection of introns and SS. Once the spliceosome has completed the exon ligation step, the generated exon-exon architecture normally needs to be preserved in order to ensure proper mRNA functionality. Thus, a specific mechanism must exist, which marks the position of exon-exon junctions and prevents the usage of cryptic SS located in spliced exons.

Here, we find that many aberrant exonic SS are efficiently silenced by the exon junction complex (EJC), a multi-protein complex that is deposited on spliced mRNA near the exon-exon junction. Upon depletion of EJC proteins, cryptic SS located in exons are de-repressed, leading to the mis-splicing of a broad set of mRNAs. Our work establishes that the EJC recruits an auxiliary complex containing the splicing regulator RNPS1 to exert a spatially confined exon inclusion effect. Consequently, RNPS1 functions mainly in the vicinity of previously spliced introns, so that cryptic SS usage within already-ligated exons is prevented. Because the EJC itself is deposited on the RNA during splicing, this mechanism functions as a positive feedback loop to reinforce authentic SS and establishes a hierarchy of preferential SS usage. Thus, the EJC protects the transcriptome of mammalian cells from inadvertent loss of exonic sequences and safeguards the expression of intact, full-length mRNAs.

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722 *Arabidopsis thaliana* alternative splicing regulation trought light induced changes in transcriptional elongation

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Plant adaptation to changing enviromental conditions like light is crutial for their survival, appropiate growth and development. Changing light conditions trought chloroplast retrograde signaling can affect alternative splicing of subset of *Arabidopsis thaliana* transcripts. Mechanism by which retrograde signals controls alternative splicing is still not fully understood.

Using Pol II CHIP and single molecule intron tracking we prove that light promotes RNA polymerase elongation on affected genes whereas in darkness elongation is lower. Changes in elongation rate in turn can affect alternative splicing trought kinetic coupling mechanism. Our data provide evidance that light induced retrograte signals regulates alternative splicing trought control of RNA polymerase II.

723 Structural basis for 5′-splice site splicing correction induced by a small molecule

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Potential drugs that promote a specific switch of SMN2 exon 7 splicing have been recently discovered (N. A. Naryshkin et al., *Science* **345**, 688-693, 2014) providing an alternative therapeutic strategy to cure Spinal Muscular Atrophy (SMA), the leading genetic cause of infant mortality. We have previously identified that the molecule acts at the pre-mRNA level and modulates 5'-splice site recognition (M. Sivaramakrishnan et al., *Nat. Commun*. **8**, 1476, 2017). Understanding the mode of action of the splicing modifier at the atomic level is of main fundamental interest as it represents the first gene-specific RNA-processing drug acting on 5'-splice site recognition and could set the rules to develop new therapeutic molecules for other diseases. Here, we elucidated the mechanism of action of a specific small molecule splicing modifier. We first identified the site of action of the splicing modifier SMN-C5 at the interface between U1 snRNP and the exon-intron junction of SMN2 exon 7 on the pre-mRNA. Second, we solved the solution structures of the RNA helix formed by U1 snRNA and the 5'-splice site of SMN2 exon 7 with and without the splicing modifier. The structure of the complex revealed the pharmacophores required to target the 5'-splice site duplex. In the case of SMN-C5, nitrogen containing rings anchor the molecules by contacting both strands across the major groove. The target specificity originates from the central ring of the molecule that inserts between two pyrimidines of U1 snRNA and stabilizes the unpaired adenine at the last position of the exon by a direct hydrogen bond. Our structures of the apo and bound forms reveal that SMN-C5 triggers a conformational switch in the RNA. The unpaired adenine at the last position of the exon experiences the most striking change since it is pulled into the RNA base stack helix by the splicing modifier. This conformational change facilitates the binding of U1-C and consequently promotes spliceosome assembly. As SMN-C5 transforms the weak 5'-splice site of SMN2 exon 7 into a stronger one, we named this switch "5'-splice site bulge repair" and validated this mechanism experimentally in human cells.

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724 Development of Splice Switching Small Molecules as Inducers of Apoptosis

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The Bcl-2 protein family are essential gatekeeper regulators of apoptosis.[1]One such protein, Bcl-x is of particular interest as a therapeutic target. It has two splicing isoforms; pro-apoptotic Bcl-x_S and the anti-apoptotic Bcl-x_L; the latter of which is upregulated in a variety of cancers. Thus, exogenous regulation of Bcl-x splicing, which biases the pathway towards the pro-apoptotic Bcl-x_S isoform could provide a new a novel mechanism for cancer therapy.[2,3]

In this poster we present a suite of small molecules that induce switching of the splicing pathway of Bcl-x in favour of the production of the pro-apoptotic Bcl-x_S isoform[4]A focused structure-activity-relationship profile revealed key functional requirements for splice-switching activity of this suite of ellipticine compounds. Furthermore, we present a one-pot, modular route for the synthesis of ellipticine analogues that will allow us to access an extensive library of small molecules, that can be used to probe the mechanism of binding.

References

(1) Yip, K. W.; Reed, J. C. Bcl-3 Family Proteins and Cancer. *Oncogene*, **2008**, 27, 6398 - 6406.

(2) Naryshkin, N. et al. SMN2 Splicing Modifiers Improve Motor Function and Longevity in Mice with Spinal Muscular Atrophy. *Science,* **2014**, 345, 6197.

(3) Sivaramakrishnan, M. et al. Binding To SMN2 Pre-mRNA-Protein Complex Elicits Specificity For Small Molecule Splicing Modifiers. *Nat. Commun*, **2017**, 8.

(4) Weldon, C. et al. Identification Of G-Quadruplexes In Long Functional RNAs Using 7 Deazaguanine RNA. *Nucleic Acids Res*. **2018**, 46, 886 - 896.

725 SRSF2 regulation of MDM2 reveals splicing as a therapeutic vulnerability of the p53 pathway

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MDM2 is an oncogene and critical negative regulator of tumor suppressor p53. One of the ways in which p53 is regulated is through alternative splicing of *MDM2* transcripts under conditions of genotoxic stress. One of these transcripts, *MDM2-ALT1*, comprises terminal exons 3 and 12 and is the most commonly observed isoform in response to stress. Previously, we identified SRSF1 as a negative regulator of *MDM2* exon 11. Here we report that splicing regulator SRSF2 antagonizes the regulation of SRSF1 by facilitating the inclusion of exon 11 through binding at two conserved exonic splicing enhancers. Furthermore, overexpression of SRSF2 promotes the inclusion of *MDM2* exon 11 under genotoxic stress, whereas knockdown induces the expression of *MDM2-ALT1*. Additionally, we induced *MDM2-ALT1* using splice-switching oligonucleotides against SRSF2 binding sites in exon 11. Lastly, we report that SRSF2 regulation of MDM2 splicing is conserved from mouse to man as murine *Mdm2* is processed similarly to the human transcript. Using CRISPR-Cas9 we mutated one conserved SRSF2 binding site in *Mdm2* exon 11. This was sufficient to both significantly increase the expression *MDM2-ALT1* homolog *Mdm2-MS2* and proliferation of NIH 3T3 cells over controls. Taken together, these findings underscore the relevance of *MDM2* alternative splicing in cancer and suggests that p53 can be regulated by artificially regulating *MDM2* splicing.

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726 SUVA: splicing site usage variation analysis from RNA-seq data reveals the functionality of low-frequency cancer-associated alternative splicing

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Alternative splicing (AS) increases the diversity of proteomes, while aberrant AS is related to many human diseases including cancers. Most of the current AS analysis tools are annotation-dependent and powerless to analyse complex AS events (ASEs). We developed a new method called SUVA (Splice sites Usage Variation Analysis) that defined ASE by splice site usage without prior annotations. For analysis of transcriptome data of paired tumour-adjacent tissues from 42 cancer patients and simulated data, SUVA showed higher sensitivity and accuracy in detecting AS events than other methods, especially for the complex AS events. Notably, after pooling the top 100 significant cancer associated AS events (CASEs) in each patient detected by SUVA, 1860 CASEs derived from 535 genes could separate the tumour and adjacent tissues. Furthermore, by classifying the frequency of CASEs across the patients, low frequency CASEs were not only well validated experimentally but also enriched in cancer-related pathway. These results indicated that the CASEs are more diverse and functionally important in cancers than previously appreciated, especially for the long neglected LF-CASE. In conclusion, SUVA is a powerful pipeline independent of annotations, which could be broadly used to detect and quantify complex AS patterns in various transcriptome data with high-confidence.

727 Alternative RNA splicing controls cancer cell plasticity

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Although changes in alternative splicing have been observed in cancer, their functional contributions still remain largely unclear. We have used the cancer stem cell (CSC) marker CD44 as a prototype and showed that the CD44 splice isoforms exhibit strikingly diverse functions in breast cancer. Alternative splicing of the CD44 gene generates two families of splice isoforms that are termed CD44s and CD44v. The CD44s splice isoform is essential for cell-death resistance and positively associates with the Breast CSC gene signatures, whereas the CD44v isoform plays a critical role in cell proliferation and inversely correlates with the breast CSC gene signature. Functionally, splice isoform switching of the CD44 gene causally controls cancer stem cell states. By manipulating CD44 alternative splicing, cancer cells undergo phenotypic changes between the non-CSC and CSC states. We have also identified the RNA-binding proteins as critical splicing factors that perform antagonized roles to tightly regulate the change of cell states through modulating alternative splicing. These results suggest that RNA splicing regulation provides functional gene versatility that is essential for distinct cancer cell states and thus cancer phenotypes.

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728 The patterns of *Alu* exonisation in human cancers

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Alu elements are primate-specific retrotransposons and account for more than 10% of the human genome. Previous studies have reported the emergence of novel exons from *Alu* elements where single point mutations can generate new splice sites. There is evidence that some *Alu* exons can be translated, contributing to the protein diversity, whereas others have been shown to promote the degradation of transcripts via nonsense-mediated decay (NMD) as a consequence of the introduction of premature stop codons. The mechanisms and consequences of *Alu* exonisation are not completely dissected.

Alternative splicing regulation depends largely on the interaction of the RNA binding proteins (*trans*-acting factors) with the sequence elements (*cis*-elements) contained in the exonic or intronic regions. In this study, we took advantage of the available cancer data in The Cancer Genome Atlas (TCGA) to investigate the patterns of *Alu* exonisation in disease to further understand their regulatory mechanisms. We characterise the exon inclusion profiles of more than 3000 *Alu* derived exons within different cancer cohorts.

We also identified single nucleotide variants within the *Alu* elements and neighbouring regions that might influence on the exonisation patterns on a patient-level. The connection between *cis*-elements and further identification of *trans*-acting factors in the regulatory context of *Alu* exons will allow us to understand general aspects of alternative splicing and evolution.

729 RBM4 is essential for brain development via its role in alternative splicing regulation

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The RNA-binding motif 4 (RBM4) protein is an alternative splicing regulatory factor whereby it participates in cell differentiation and development. We have previously reported that RBM4 regulates alternative splicing of several signaling factors essential for neurogenesis and neuronal migration. Our previous study has demonstrated that RBM4 antagonizes the activity of PTBP1 to promote alternative exon 7/8 inclusion in Dab1, a pivotal regulator of the reelin signaling cascade, and hence influences neuronal migration during cortical lamination.

To better understand the physiological function of RBM4, we generated conventional Rbm4 double knockout (dKO) mice. Rbm4 dKO results in defects in the cerebellar foliation pattern.

Rbm4 deficient cerebellum exhibited thickened external granule layer at P10 and stunted growth of purkinje cell dendrites at P30, suggesting a potential role of RBM4 in signaling control of early cerebellum development. Moreover, Rbm4 dKO mice exhibited hyperlocomotion and reduced anxiety, and therefore may serve as a behavioral disorder model. To determine how RBM4-mediated splicing regulation impacts cerebellar development is still underway.

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730 Pre-mRNA bound U2 snRNP contains novel protein components

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Much of pre-mRNA splicing occurs while the primary transcript is associated with chromatin. To assess how interactions of spliceosome components and splicing regulators might differ in this cellular compartment from the soluble nucleoplasm, we examined proteins and snRNAs pelleting with chromatin and other high molecular weight (HMW) nuclear material. These can be efficiently extracted from the pellet after extensive digestion of RNA and DNA. We found that fragments of the HMW-extracted U2 snRNA remains protected from degradation in a stable RNP complex. To isolate this complex, we generated isogenic 293Flp-In cell lines conditionally expressing Flag-tagged SF3A3, a U2-interacting protein. Anti-Flag affinity-purified complexes containing U2 RNA fragments also contained many known components of the 17S U2 snRNP, including SF3a and SF3b, U2SURP/SR140, DHX15/PRPF43, and RBM17/SPF45. Other components, such as the U2-specific A', and B" proteins, the Sm core proteins, and the U2-associated factors 65 and 35 were only detected in trace amounts.

In addition to these well characterized U2 components, we also identified novel proteins associated stably with the HMW-extracted U2 snRNP. These include the tumor suppressor factors RBM5, and RBM10, but not the related protein RBM6, and the cell cycle and apoptosis regulator CCAR1, but not its putative paralog CCAR2. Unlike previously characterized U2 snRNPs, these complexes also contain pre-mRNA fragments, which we are currently mapping.

In sum, our data demonstrate that branch-site recognition may involve a more complex machinery than previously recognized. Two of the novel U2 snRNP proteins, RBM5 and RBM10, are well-known splicing regulators. The interaction of these proteins with the U2 snRNP provides new clues to how they can alter splice site choice.

731 Characterization of the protein-RNA network leading to the different splicing outcome of SMN1 and SMN2 exon 7 using CLIR-MS/MS

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Crosslinking of segmentally isotope-labeled RNA coupled with tandem mass spectrometry (CLIR-MS/MS) is a new method developed in collaboration with the Aebersold lab. This technique allows the characterization of RNA-protein interactions at single nucleotide and single amino-acid resolution. So far, CLIR-MS/MS has been successfully applied to biological systems using purified recombinant protein and RNA components assembled in vitro [1]. In addition to rapidly provide structural information on protein-RNA interactions, we believe that this method could also be used to investigate more complex systems in a cell extract context.

Using this method, we aim to characterize protein-RNA networks involving splicing regulators on their exon target in the context of the splicing of the exon 7 of SMN. The two genes encoding the human SMN protein (SMN1 and SMN2) differ only by few nucleotides, notably a silent C to U mutation at position 6 of exon 7 of SMN2 which is known to induce the skipping of this exon [2]. The splicing outcome of SMN exon 7 depends on a molecular competition between the binding of splicing activators and inhibitors on this exon and surrounding introns. The C to U change was proposed to force the recruitment of a splicing inhibitor (hnRNP A1) at this position instead of the splicing activator SRSF1 and to be the event that initiates the change of exon 7 splicing [3,4]. We started to setup CLIR-MS/MS experiments in HeLa nuclear extract in order to understand the effect of this protein exchange on the binding of other splicing regulators and on their interactions with the splicing machinery. We therefore established a purification protocol allowing enrichment of specific pre-spliceosome complexes assembled on the SMN1 and SMN2 pre-mRNAs and are currently characterizing these complexes with mass spectrometry and electron microscopy. We aim at confirming already characterized protein-RNA interactions and discovering new ones, which, in combination with complementary structural methods, should give further insights in the understanding of the mechanism regulating SMN exon 7 alternative splicing.

1. Dorn et al., Nat. Methods (2017)
2. Lorson et al., PNAS. (1999)
3. Cartegni et al., Nat Genet. (2002)
4. Kashima et al., Nat Genet. (2003)

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732 Weak exons are hotspots for disruptive splicing perturbations

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Alternative splicing is an important component of disease. Up to one third of heritable disease alleles are thought to affect splicing. The current wealth of clinical deep sequencing data has revealed an overwhelming number of variants of uncertain significance that cannot be interpreted at the same pace as they are discovered. In the process of developing a machine learning algorithm to predict the effects of variants on splicing, we discover that little information comes from the variants themselves. Instead, splicing depends mostly on exonic features. We identify “hotspot exons” as a class of exons that is susceptible to mutations and other splicing perturbations. Hotspot exons are characterized by low splice site usage, and we show that their splicing strength depends on the strength of their flanking exons. When hotspot exons are flanked by other weak exons, their inclusion levels rise. Splice sites are under purifying selection to maintain their combined splice site usage above a threshold to yield sufficient levels of functional mRNA transcripts. The low splice site usage of a hotspot exon is compensated for by selection for higher splice site usage in the remaining exons. Hotspot exons can narrow the search space for clinical and functional genomics efforts by prioritizing variants for interpretation or potential drug targets for diseases caused by errors of splicing.

733 SmD3-b splicing factor modulates plant immunity response

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SmD3 is one of the core components of the spliceosomal small nuclear ribonucleoprotein (snRNP) complex. *Arabidopsis thaliana* has two SmD3 homologs with a conserved function in splicing, but only the *smd3-b* null mutant displays severe pleotropic phenotypes.

To evaluate the role of *Arabidopsis* SmD3-b in response to biotic stress we investigated sensitivity of the *smd3-b* mutant to *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 infection as well as its effectors flg22, elf18 and coronatine. Our results show that the mutant exhibits enhanced susceptibility to *Pst* accompanied with marked changes in the expression of key pathogenesis markers. mRNA levels of major biotic stress response factors were also altered upon treatment with pathogenesis effectors. Our genome-wide transcriptome analysis of *smd3-b* mutant infected with *Pst*, verified by northern and RT-qPCR, revealed that the lack of SmD3-b protein deregulates defense against *Pst* DC3000 infection on the transcriptional and post-transcriptional (splicing and alternative splicing) levels. Also callose deposition, additional marker of plant immunity, was strongly induced by elf18 and flg22 in the mutant. In turn, flg22-triggered production of apoplastic reactive oxygen, species that accompanies and also regulates response to pathogen, was reduced. Moreover, we observe increased activation of MAPKs in the absence of SmD3-b, which most likely affects the function of multiple pathogen-related transcription factors through their earlier or stronger phosphorylation. Together, our data strongly support a regulatory role of SmD3-b in plant immune response via modulation of mRNA splicing of key pathogenesis factors.

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734 Formation of cryptic last exons by the splicing factor SFPQ

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The splicing factor SFPQ is a ubiquitously expressed member of the major spliceosome that plays a role in transcriptional regulation, alternative splicing, and transport of RNAs into neuronal axons. In zebrafish, *sfpq* mutants show defects in axon outgrowth and brain boundary formation, and the homozygous null mutant is lethal by four days post fertilization. In order to understand the molecular mechanisms of SFPQ function during embryonic development, we performed RNA-seq analysis on *sfpq* mutant embryos and looked for abnormalities in splicing. We discovered a novel form of alternative splicing and polyadenylation in which a cryptic exon is formed, followed by truncation of the transcript. These cryptic last exons (CLEs) appear preferentially in long introns of genes with neuronal function. Here we show that CLEs form as a direct result of loss of SFPQ binding, that the resulting short peptides cause developmental defects in the *sfpq* mutant, and that the CLEs act as a novel form of transcriptional regulation. These results greatly expand our understanding of SFPQ function in addition to describing an important new regulatory mechanism.

735 Regulation of tandem acceptor splice site usage in the context of proximate exons

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Tandem acceptor splice sites (TASS) that are located 3 nt apart represent a frequent evolutionary conserved alternative splicing event. It is estimated that the motif is alternatively spliced in at least 5 % of human genes. Our previous work on TASS led us to the notion that both intronic-proximal (upstream) and intronic-distal (downstream) 3´ss choice can be related to other splicing events occurring on the same pre-mRNA. In order to elucidate the control mechanisms of NAGNAG choice in the context of two closely located exons, we investgated splicing of two minigenes consisting of two model exons each, cloned between vector-specific exons. The minigenes contained *AFAP1L2* exons 14 and 15, of which the downstream exon harboured AAGCAG motif; and *CTSD* exons 7 and 8, with the downstream exon adapted to contain the TAGTAG motif. The middle introns between the model exons spanned 82 and 92 bp, respectively.

In the *CTSD* minigene, the distal NAG usage was markedly lower when the upstream exon was skipped (by 30 %), compared to both exons inclusion. On the contrary, in the *AFAP1L2* minigene the distal NAG was used comparably in both transcripts. Employing thorough mutagenesis of the 'Ns' in the NAGNAG motifs showed that the distal NAG usage related most to the -3 position (with recpect to the distal NAG), T and C being much more favorable than A or G. The same applied for the proximal NAG usage, yet this was best correlated with its predicted strength using MaxEnt predictor. In addition, the influence of middle intron length on the splice site choice has been tested, yet with ambiguous results. These data have brought some clues to regulation of tandem acceptor splice site recognition. Hopefully we will be able to explore the underlying mechanism more deeply in the near future.

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736 Genome-wide siRNA screening revealed an interplay between the exosome and first steps of pre-spliceosome assembly

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In our research, we are focused on identification of novel factors implicated in RNA metabolism that can be functional interactors of Dis3, an essential component of exosome complex, which play a fundamental role in RNA processing and degradation processes.

We have conducted genome-wide synthetic lethal siRNA screening that revealed a complex network of Dis3 interactions with different cellular pathways. Precise analysis of those interactors revealed enrichment in genes responsible for transcription, splicing and 3' end formation. Among the strongest positive interactors were all components of SF3a complex and some of SF3b complex. This intriguing observation implied that Dis3 dysfunction is in one way or another specific for alterations in splice site recognition as both complexes are major constituents of the U2 snRNP. SF3a complex is a substantial integral component of the pre-spliceosome and is required for complex A and B assembly. As SF3a interacts with U1, U2 snoRNAs and pre-mRNA, it plays crucial role in initial steps of spliceosome assembly. Not only establishing a strong interaction with the U2 snRNP and intron, but also by taking part in the step of bringing two splice sites together and shielding the 5′SS from the active site.

Transcriptome analysis demonstrated that depletion of SF3A1, one of three SF3a complex components, lead to extensive alterations in multiple classes of alternative splicing events (ASE). Remarkably, the only group of ASE that was reshaped in the presence of Dis3 dysfunction was intron retention. Detailed investigations of transcripts with altered intron retention revealed high enrichment in transcripts coding ribosomal proteins (RPs). This implies that functional SF3a complex play a role in maintaining an appropriate level of housekeeping genes, as t is known that in yeast and *C. elegans* limited subset of RP genes is regulated at splicing level. Also, we show that Dis3 dysfunction alters RNPII speed, indirectly decreasing intron retention in a subset of genes.

Concluding, we determined for the first time broad alterations in mRNA splicing after SF3A1 depletion in human cells. Moreover, our work revealed a functional link between splicing and exosome-mediated RNA decay pathway that regulates specific subset of genes.

737 Allotopic expression of *nad7* rescues the Arabidopsis *slow growth3* mutant

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Plant pentatricopeptide repeat (PPR) proteins are mostly involved in chloroplast or mitochondrial RNA metabolism. Loss-of-function in the PPR proteins is often associated with strong phenotypes in plant growth and development. However, direct evidence that correction of the molecular defects in the organelles can restore the plant phenotypes has yet to be demonstrated in a *ppr* mutant. To study genes that are important for plant growth, we have isolated a collection of *slow growth* (*slo*) mutants in Arabidopsis. One of the *slo* mutants, *slo3*, is defective in a nuclear gene encoding a mitochondrion-localized PPR protein. Analysis of mitochondrial RNA metabolism revealed that the *slo3* mutant was impaired in the splicing of *nad7* intron 2. This molecular defect may cause a reduction in complex I activity and eventually affect plant growth and development in the *slo3* mutant. Since mitochondrial transformation is still a challenging technique in plants, we used an alternative approach to demonstrate that transformation of correctly spliced *nad7* into the nuclear genome and targeting the Nad7 subunit into mitochondria can restore the complex I activity and the growth defects of the *slo3* mutant. Together, these results provide direct evidence that the strong growth and developmental phenotypes of the *slo3* mutant are caused by defects in mitochondrial *nad7*. Given that many *ppr* mutants have strong phenotypes, and the lack of an efficient mitochondrial transformation protocol, the technique developed here can be used to provide direct evidence for the function of a mitochondrial gene and may eventually be important for applications in agricultural biotechnology.

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738 When alternative splicing meets Wnt signaling

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The Wnt signaling is an evolutionarily conserved pathway that plays a crucial role in development, tissue homeostasis, and cancer. Although genetic mutations within Wnt pathway genes leading to deregulation of Wnt pathway and carcinogenesis have been described in several types of cancer, such mutations are uncommon in lung cancer. In this work, we identify a novel splicing-dependent mechanism for the activation of Wnt signaling in lung cancer. We found that the key component of Wnt signaling, Dishevelled gene, undergoes splicing changes in non-small cell lung cancer (NSCLC). A higher ratio of long to short Dishevelled isoforms was detected in lung tumor tissue, compared to normal tissue, and predicts a poor survival in patients. To understand whether this splicing event contributes to tumorigenesis, we generated lung cancer cell lines overexpressing these two isoforms, respectively, or expressing only one isoform through genome editing. Our results indicate that the long isoform has a higher oncogenic activity in promoting cell proliferation, migration, invasion, and metastasis than the short isoform both in vitro and in vivo. Mechanistically, the long isoform is more potent in activating Wnt signaling than the short one. Phosphorylation of the long isoform by a Wnt-activated kinase promotes its nuclear translocation, leading to increased Wnt signaling. Collectively, we reveal a novel mechanism for activating Wnt signaling in NSCLC through splicing-dependent phosphorylation of Dishevelled protein.

739 Dynamic effects on co-transcriptional splicing regulation and transcriptional changes induced by fast depletion of Ptbp1 in mES cells

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The polypyrimidine tract-binding protein (Ptbp1/PTB) is expressed at relatively high levels in non-differentiated cells where it represses multiple neuron- and muscle-specific alternative splicing events. Despite being initially considered a splicing repressor, it is now known to play a more complex role in splicing regulation functioning as a repressor or an activator depending on the context. In order to gain further insights into Ptbp1 biology, we established a mouse embryonic stem cell line that allows Ptbp1 to be rapidly depleted through auxin-inducible protein destabilization. We argued that this system may help us identify direct targets of Ptbp1 and understand its role in co-transcriptional pre-mRNA splicing. Our system currently allows Ptbp1 levels to be reduced to ~50% within 2 hours and to <10% in 4 hours of auxin treatment. To examine the effect of Ptbp1 depletion on co-transcriptional splicing, we analysed auxin- and control-treated cells using native elongating transcript (mNET-seq), chromatin-associated and total RNA sequencing. Strikingly, Ptbp1 downregulation resulted in significant accumulation of intron-retained isoforms in chromatin- and pol II-associated fractions, suggesting that this RNA-binding protein may be required for optimal removal of introns from nascent transcripts. We will present results of these and further experiments focused on molecular mechanisms and biological significance of this newly identified splicing regulation program.

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740 Npl3-mediated alterations of nuclear RNA export affect splicing fidelity

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To better understand the mechanistic consequences of inefficient spliceosome assembly, we carried out an open genetic screen for alleles that improve splicing of 5'SS-G5a introns, defective in base pairing to both U1 and U6 snRNAs.

Genomic sequencing yielded alleles of *npl3* and *mtr10,* encoding two functionally linked proteins, Mtr10 and Npl3. Npl3 is a primarily nuclear, shuttling SR-like mRNA binding protein implicated in many steps of mRNA biogenesis, including splicing and export, and Mtr10 is its karyopherin.

Preponderance of the selected *mtr10* mutations create truncated proteins whereas the *npl3* allele carries a L219S mutation within the RRM2 domain and a 49 aa deletion within the C-terminal RGG domain. Both *mtr10* and *npl3* suppressors, as well as additionally generated *npl3* mutants carrying various deletions in the RGG domain, disrupt nucleo-cytoplasmic shuttling of Npl3, resulting in reduced nuclear Npl3 levels and improved splicing of suboptimal introns. Interestingly, inhibition of nuclear export by *mex67* alleles also improves splicing of suboptimal introns.

We hypothesize that the observed splicing effects result from two mechanisms. First, destabilization of Npl3-Mtr10 interactions (by *npl3* and *mtr10* alleles) leads to inefficient re-import of Npl3 to the nucleus. Second, defective interactions of Npl3 with the export machinery (*npl3-RGG* mutants and *mex67* alleles) inhibit RNA export. Both these mechanisms give more time for spliceosome assembly on suboptimal introns before their export to the cytoplasm. Thus, splicing and export compete for RNA substrates.

Both *npl3* and *mtr10* mutants improve splicing of a variety of mutant introns and improve splicing of WT *SUS1* reporter transcript (carrying non-canonical splice sites), suggesting that they may also affect splicing of endogenous introns containing non-canonical splice sites. To study this, we carried out RNA-seq analysis of RNA from selected alleles. Preliminary analysis suggests that alterations of nuclear RNA export result in transcription down-regulation of genes involved in rRNA processing and ribosome biogenesis, and in transcription up-regulation of stress-related genes, resembling effects of stress response in yeast.

Our results indicate that reduction of nuclear Npl3 levels leads to a decreased stringency of splice site selection, uncovering a previously unknown role of Npl3 in the modulation of splicing specificity.

741 SRRM3 regulates a functional program of microexons in endocrine pancreas

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Pancreatic beta cells play a key role in maintaining glucose homeostasis by secreting insulin. Dysfunction or loss of beta cells results in diabetes, a worldwide-growing epidemic. While extensive analyses have uncovered transcriptional regulatory programs that control specification, differentiation and maturity of beta cells and showed that diabetes-associated variants often affect cell-specific enhancers, little is known about the role of post-transcriptional mechanisms regulating gene expression in beta cell development, function and disease. We have used public RNA-seq data from human, mouse and rat to compare splicing profiles across tissues and cell types to identify alternative exons specifically included in beta cells and other hormone-producing cells of the pancreas. Our analysis identified a highly conserved program of 187 alternative exons with high inclusion in endocrine pancreas but absent or reduced in the exocrine fraction or in pancreatic progenitors. Endocrine exons showed GO enrichment in pathways involved in endocrine functions such insulin secretion, type II diabetes or vesicle-mediated transport. Interestingly, this endocrine splicing program is extensively embedded in larger neuronal and retinal programs, suggesting overlapping regulation. Strikingly, almost half of endocrine exons are 3-27 nucleotides microexons previously reported to be specifically regulated by the splicing factor SRRM4 in neural tissues. We found that endocrine cells express low but significant levels of the SRRM4 paralog SRRM3. RNA-seq analysis and functional assays of SRRM3-depleted human and rat beta cells lines revealed that SRRM3 regulates the inclusion of most endocrine microexons, with consistent effects on insulin secretion and beta cell responses to stress. Our findings indicate that endocrine cells share common splicing programs with neurons that can play important roles in the acquisition or maintenance of endocrine functions.

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742 Obesity-related alternative splicing in adipose tissue

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Obesity is a major public health problem worldwide with rising prevalence in both developed and developing countries. Obesity has a multifactorial etiology, with heritability ranging from 50-90%, leaving the remaining variance to be explained by environmental factors and gene-environment interactions. To identify the obesity-related alternative splicing events, we utilized the RNA-sequence data from the population-based cross-sectional METSIM study. To this end, we used the STAR 2-pass approach to map the reads, followed by the rMATS to identify differential splicing events. To increase the reliability of the Percent Spliced In (PSI) estimate we have focused only on the high coverage splicing junctions.

Fifty-eight middle-aged obese males(BMI≥30 kg/m²) were compared to the 58 middle-aged males(BMI<25 kg/m²)to identify a change of PSI of five basic types of alternative splicing (skipped exon, alternative 5′ and 3’splice sites, mutually exclusive exons and retained introns). We detected 3516 differential splicing eventswith an FDR < 5% and |ΔPSI| > 1%. The majority of changes in splicing pattern were identified in the mutually exclusive exon category, a group previously implicated in human diseases. KEGG pathway enrichment analysis revealed,that the splicing pattern of the genes related toribosome and oxidative phosphorylation pathways was significantly different between lean and obese subjects.These results were consistent with an altered metabolic state observedin the obese individuals, including increased protein synthesis and increased energy demands.Finally, GO analysis showed that amongst the differentially alternatively spliced genes, genes related to RNA splicing are enriched, suggesting that in obesity the splicing machinery itself is targeted for alternative splicing.

Taken together our results suggest that aberrant splicing may be an important player in the development and progression of obesity.

743 LNAs in the sequencing space

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Splice-switching oligonucleotides can be designed to elegantly manipulate alternative splicing such that it brings a therapeutic benefit in the context of disease. The strategy can be applied in many ways - both directly to target and restore aberrantly spliced RNA caused by mutations, indirectly by targeting adjacent RNA to restore open reading frames or to introduce de novo isoforms coding for novel proteins with particular activities. Recently, oligonucleotide drugs, based on the splice-switching approach, have been marketed for treatment of patients with severe rare genetic diseases.

Locked nucleic acid (LNA) is a class of nucleic acid analogues possessing a very high affinity and excellent specificity toward complementary DNA and RNA. Here, we have utilized LNA technology for two different purposes. Firstly, we have taken advantage of LNA to design highly potent splice-switching oligonucleotides. Splice-switching oligonucleotides act in the nucleus by blocking the spliceosome from interacting with specific intron/exon/intron pre-mRNA sequences. This way it is possible to generate specific and desired mRNA isoforms. Secondly, we have used LNA to increase our overall drug screening efficiency. We have developed a method based on NGS technology, where we utilize gene-targeting LNA oligonucleotides with a 5'-biotinylation as a tool, to investigate the performance of particular splice-switching oligonucleotides by ultra-deep RNA sequencing.

In this, we will present NGS data that illustrates how we are now able to not only efficiently rank-ordering splice-switching oligonucleotides according to potency, and also assess splicing fidelity. Once the method has been fully implemented, we believe our approach will provide a significant step forward for drug discovery of splice-switching oligonucleotides.

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744 The Kreb's cycle enzyme fumarase regulates pre-mRNA splicing through ubiquitin-like protein Hub1

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The ubiquitin-like protein Hub1 functions in pre-mRNA splicing by binding non-covalently to the spliceosomal proteins Snu66 and Prp5. We have found that Schizosaccharomyces pombe Hub1 also binds to a mitochondrial enzyme of the citric acid cycle, fumarase (Fum1). The enzyme binds to a conserved surface of Hub1 centered at a solvent-exposed tryptophan residue. This surface is absent in S. cerevisiae Hub1; however, an introduction of tryptophan at analogous position restores its affinity with Fum1. Hub1-Fum1 complex precipitates in vitro indicating mutually inhibitory activities of the two partners. Supporting the potential inhibitory activities, elevated levels of cytosolic Fum1 in S. pombe led to Hub1-dependent growth and splicing defects. Since fumarase is frequently mutated in multiple diseases in humans, some of the disease-causing mutants may have altered Hub1-dependent pre-mRNA splicing

745 Genome-Wide Kinetic Analysis of pre-mRNA Processing in *C. elegans*

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In previous studies, we analyzed order of intron excision in some of alternatively spliced pre-mRNAs in *Caenorhabditis elegans*. We found that a downstream intron was excised first for select of neuron-specific exon 7a of the *unc-32* gene, whereas an upstream intron was excised first in a splicing regulator mutant *unc-75*, resulting in switching to exon 7b. This example suggested that splicing rates can vary and splicing regulators switch the splicing patterns by changing the order of intron removal. However, genome-wide dynamics of pre-mRNA processing such as timing and order of intron excision *in vivo* are still to be elucidated.

Here, we aimed to analyze global kinetics of nascent pre-mRNA processing in two approaches, metabolic labeling and *in vitro* labeling of nascent RNAs. First, we sequenced metabolically labeled nascent RNAs. The processing rates of *cis*-splicing, *trans*-splicing and 3'-end cleavage were calculated based on declining patterns of unprocessed reads. We revealed that the splicing rates varied on a genome-wide scale and even within a gene. We validated predicted orders of excision for some pairs of neighboring introns. Furthermore, alternative regulation models can be predicted by comparing nascent transcriptome of wild type worms and splicing regulator mutants. Second, we labeled RNAs being transcribed using Nuclear Run-On (NRO) assay and performed long-read sequencing. This approach enables us to elucidate the timing of co-transcriptional splicing as a function of the Pol II position at single transcript levels. We unexpectedly found that most neighboring introns are both spliced or both unspliced, suggesting coupling of the splicing events. The splicing rates correlated with splice site sequences and intron lengths. Interestingly, 'slow' introns were likely to be closer to 5'-end of genes. This position effect might be explained by coupling of multiple splicing events implied in our long-read sequencing.

Nascent RNA-seq also enabled genome-wide estimation of RNA half-lives. We found that the estimated mRNA stability significantly correlated with codon usage as has recently been demonstrated in *E. coli*, *S. cerevisiae* and zebrafish. Totally, our study revealed the genome-wide dynamics of pre-mRNA processing and mRNA stability in *C. elegans*.

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746 The U2AF1 splicing factor controls cell-fate determination in a dose-dependent manner via transcription regulation

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Alternative splicing (AS) plays a central role during cell-fate determination. However, how the core spliceosomal factors (CSFs) are involved in this process is poorly understood. Here, we report the down-regulation of the U2AF1 CSF during stem cell differentiation. To investigate its function in stemness and differentiation, we downregulate U2AF1 in human induced pluripotent stem cells (hiPSCs), using an inducible-shRNA system, to the level found in differentiated ectodermal, mesodermal and endodermal cells. RNA sequencing and computational analysis reveal that U2AF1 down-regulation modulates the expression of development-regulating genes and regulates transcriptional networks involved in cell-fate determination. Furthermore, U2AF1 down-regulation induces a switch in the AS of transcription factors (TFs) required to establish specific cell lineages, and favors the splicing of a differentiated cell-specific isoform of DNMT3B. Our results thus show that U2AF1 is involved in the transcriptional regulation of stem cells, establishing a link between transcription and AS during cell-fate determination.

747 Dual roles of XAB2 in the regulation of gene expression

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Previous studies revealed that XAB2 acts as a multi-functional protein participating biological processes including transcription, homologous recombination, pre-mRNA splicing, mRNA export and mitosis. Here we showed that POLR2A as a major target gene down-regulated after XAB2 deficiency. XAB2 depletion led to severe splicing defect of POLR2A that resulted in substantial loss of POLR2A at RNA and protein levels, which further impaired global transcription and promoted cellular senescence. Treatment of splicing inhibitor Madrasin could also induce reduction of POLR2A in similar way. Using iTRAQ we characterized several proteins involved in mRNA surveillance including Dom34 with elevated expression after XAB2 knockdown. We found that decreased expression of POLR2A caused by XAB2 depletion could be rescued by inhibition of translation or Dom34 knockdown, apparently by stabilizing its mRNA. Domain mapping revealed that TPR motifs 2-4 and 11-12 of XAB2 were critical for POLR2A expression. Furthermore, we found depletion of XAB2 or POLR2A induced cell senescence by decreasing pRB and increasing p53/p21. Surprisingly, re-expression of POLR2A after XAB2 depletion alleviated cellular senescence. These results suggest dual roles of XAB2 in the regulation of gene expression, first XAB2 deficiency could impair pre-mRNA splicing including the splicing of POLR2A, then the drop of POLR2A further reduce global transcription, which results in cell senescence.

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748 Withdrawn

749 Withdrawn

750 Identification of RBPMS as a smooth muscle master splicing regulator via proximity of its gene with super-enhancers

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Cell-specific alternative splicing (AS) programs are primarily regulated by RNA binding proteins (RBPs). It has been suggested that a subset of RBPs act as master regulators of cell-specific AS programs and that these master regulators could be identified via the proximity of their genes to transcriptional super-enhancers[1]. Using this approach we identified RBPMS as an AS master regulator in differentiated vascular smooth muscle cells (VSMC). RBPMS is highly expressed in the differentiated contractile VSMCs and downregulated during dedifferentiation. RBPMS was found predominantly in the nucleus and expressed as two major isoforms, RBPMS-A and B, which vary in their extreme C-termini. mRNA-Seq was carried out for RBPMS knockdown in differentiated PAC1 cells and RBPMS-A overexpression in dedifferentiated PAC1 cells. RBPMS promoted numerous differentiated splicing patterns and was solely responsible for nearly 20% of the AS changes during PAC1 phenotypic switching. Moreover, RBPMS overexpression was sufficient to promote some splicing events that are usually only observed in fully differentiated tissue SMCs. RBPMS targets were enriched for superehancer-association in SMC tissues and for a network of proteins involved in the actin cytoskeleton and focal adhesions, critical machineries in both the differentiated contractile and proliferative motile SMC states. RBPMS also controlled splicing and activity of other regulators of AS (MBNL1, MBNL2) and mRNA stability (LSM14B), as well as the key SMC transcription factor Myocardin. Notably, RBPMS promoted production of a MYOCD isoform that more potently activates the contractile phenotype, acting antagontistically with QKI which is more highly expressed in proliferative cells. RBPMS directly regulated target exons with a positional bias; upstream and exonic sites associated with repression and downstream sites associated with splicing activation. Strikingly, RBPMS-A showed higher activity than RBPMS-B, particularly for splicing repression. RBPMS therefore matches many of the expected criteria of a SMC master regulator of AS[1]. Our future studies aim to understand the role of the RBPMS regulated splicing program in controlling SMC phenotype in health and disease as well as RBPMS splicing mechanism.

1. Jangi & Sharp, Cell, 159, 487-98 (2014)

751 Comparative transcriptomics analysis reveals a conserved alternative splicing program during primate neurodifferentiation

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Alternative splicing is a central feature of eukaryotic gene expression, and is essential for realizing the functional diversity of the genome. It is well established that alternative splicing plays an out-sized role cell fate specification, and that neurodifferentiation in particular is a process rich in alternative splicing dynamics. Despite 40 years of research the extent to which alternative splicing dynamics in neurodifferentiation and early brain development are conserved, even among primates, is still relatively unknown. Moreover, the functional consequences of many if not most specific events remain poorly understood. To address these knowledge gaps, we contribute a comparative transcriptomics analysis using RNA sequencing data derived from an in vitro cortical neurosphere formation time courses experiment in four close primate species – human, chimpanzee, orangutan, and macaque. Our analyses reveal a number of important features, foremost the fact that alternative splicing dynamics are largely conserved among all examined species. We further report that alternative splicing events that are predicted to alter coding regions are more dynamically conserved than those restricted to untranslated regions and/or noncoding genes, and that increased predicted protein-protein interaction degree is a predictor of better conservation. Finally, we highlight a handful of alternative splicing events with both clear neurodifferentiation-associated dynamics and clear species-specific behavior. These observations broadly support previous assertions that a principle consequence of differentiation-associated alternative splicing dynamics is the alteration of expressed protein sequences and the consequential rewiring of protein-protein interaction networks. Our observation of species-specific dynamics also provides new avenues into the exploration of species-specific phenotypes.

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752 Regulated Splicing in a Dramatically Reduced Splicing System

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The splicing of pre-messenger RNA is carried out by a large ribonucleoprotein complex, the spliceosome, composed of small, nuclear RNAs and associated proteins. Thought to have evolved from group II self-splicing introns and their attendant maturases, spliceosomes today are highly divergent. In humans, for example, there are two largely orthogonal spliceosomes that carry out splicing on different sets of transcripts, and are regulated by a vast network of pathways and splicing factors to allow specific transcripts to be spliced in particular ways in different tissues, developmental stages, or cell cycle stages. At the other extreme are the highly reduced spliceosomes of organisms such as *Cyanidioschyzon merolae*, an acidothermophilic red alga that lacks the U1 snRNP and has only 27 annotated introns in its genome. Since the catalytic core of the spliceosome is highly similar over 100s of millions of years of evolution, it has been hypothesized that most of the variation in complexity is due to the presence or absence of regulatory factors that respond to signalling pathways. We were consequently surprised to discover that *C. merolae* harbours homologues of a small number of alternative splicing factors such as SR and hnRNP proteins. To investigate whether splicing may in fact be regulated, despite the dearth of introns and the absence of alternative splicing possibilities, we measured splicing under a variety of environmental conditions such as depletion of nitrogen, phosphorus, or carbon. In each case, we observed transcript-specific, condition-specific increases in splicing of a small number of intron-containing genes. While expression of intron-containing genes did not vary over these conditions, the transcription levels of splicing regulatory proteins changed considerably. These observations suggest that *C. merolae* has distinct signalling pathways that regulate the activity of the spliceosome in a transcript-specific manner. Given the small number of regulatory proteins and intron-containing genes, we hope to be able to work out the entirety of these pathways and how they allow *C. merolae* to better respond to environmental challenges.

753 The role of the U12-dependent spliceosome in cellular differentiation.

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Previous studies in our laboratory have identified an auto/crossregulation system that regulates minor spliceosome cellular abundance via core 48K and 65K proteins [1]. This regulatory program uses evolutionarily highly conserved atypical splicing enhancers denoted as USSE that are located in the introns of U11/U12-65K and U11-48K genes. Recognition of the USSE by U11 snRNP leads to the formation of non-functional mRNA isoforms that are either degraded by nonsense-mediated decay (NMD) in the case of U11-48K following the inclusion of a mini exon or trapped in the nucleus in the case of U11/U12-65K, due to a long 3'UTR [1, 2]. More recently, work in our laboratory has shown that the USSE-mediated downregulation of 65K is strongly activated during neuronal differentiation [2]. To address the general significance of U11/U12-65K regulation, we examined several differentiation models including iPSC differentiation to bi-potential gonad and pancreatic cells, neuronal differentiation with SH-SY5Y cells and myoblast differentiation with C2C12 cells. We find that in each of these systems the levels of U11/U12-65K protein are downregulated in USSE-dependent manner due to a switch from the productive mRNA isoform containing a short 3'UTR to a nonproductive nuclear isoform with a long 3'UTR.

To address the significance of the U11/U12-65K downregulation in SH-SY5Y neuronal differentiation model, we blocked the USSE element with an antisense morpholino oligonucleotide, that prevents the downregulation of the U11/U12-U65K protein. We find that neurite outgrowth appeared to be inhibited and there was an enrichment of fibroblast-like cells rather than mature neurons. Additionally, blocking the 65K-USSE in later stages of neuronal differentiation led to a retraction of already established synaptic connections. Therefore, our preliminary data suggest that 65K USSE–mediated regulation, and consequently the control of minor spliceosome abundance, appears to be important during differentiation as well as neuronal maintenance and survival.

1. Niemelä (2015) RNA Biol. 12:1256–1264.
2. Verbeeren (2017). PLoS Genetics, 13(5):e1006824.

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754 Alternative Splicing Modulation and Growth Inhibition of Colorectal Cancer Cells by a Novel Class of Compounds

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Alternative splicing (AS) greatly contributes to proteomic diversity and its deregulation is often associated with tumorigenesis. Specifically, increased expression of a splicing regulatory factor SRSF10 in colorectal cancer (CRC) promotes the production of a pro-tumorigenic splice variant of the apoptosis-related gene BCLAF1. BCLAF1 splicing is altered by a small molecule (1C8) which affects the function of splicing regulatory factor SRSF10. We screened a library of 500 1C8 derivatives to identify novel compounds that can more efficiently inhibit the pro-tumorigenic variant of BCLAF1 and growth of CRC cells. We selected the most potent compounds and studied their effect on BCLAF1 splicing in CRC or normal colon epithelial cell lines and organoids. Our compounds specifically decreased production of the pro-tumorigenic BCLAF1 variant in CRC cell lines and organoids. In reporter assays, the selected compounds inhibited the splicing regulatory activity of exogenously expressed SRSF10. The selected compound GPS167 inhibited phosphorylation of SRSF10 in CRC cells and increased its interactions with CLK1. GPS167 cosedimented with CLK1- and SRSF10-containing fractions from a nuclear extract. RNA-Seq analysis of CRC cells treated with the compounds identified a group of genes with shifts in AS patterns. The AS events from this group showed similar shifts in CRC organoids upon treatment with GPS167. A subset of these AS events also exhibited similar shifts upon knockdown of SRSF10, consistent with the view that the compounds affect SRSF10 function. The selected compounds inhibited growth of CRC cell and organoid lines and induced expression of apoptotic markers. Thus, our selected compounds modulate AS patterns and inhibit CRC cell growth. The effect of our compounds on SRSF10 activity suggests inhibition of an extensive pro-tumorigenic AS network regulated by SRSF10 in CRC.

755 SAM68 interaction with U1snRNP regulates alternative splicing

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Src-associated in mitosis, SAM68, modulates nuclear RNA processing events such as alternative splicing (AS) and polyadenylation of transcripts including mechanistic target of rapamycin (*mTor*) that are implicated in key developmental processes. However, the involvement of SAM68 in modulating spliceosome assembly during transcript maturation has not been explored till date. Herein, we report SAM68, as the first non-snRNP interactor of U1A, the core component of U1snRNP. The tyrosine rich, YY region of SAM68 mediates a direct interaction with the U1A-RRM1 motif and deletion of this region in SAM68 abrogates U1snRNP recruitment triggering premature intronic polyadenylation in *mTor* intron 5. In addition, we also uncovered a subset of genes whose AS is modulated by the functional interaction of SAM68 and U1snRNP. Taken together, our results provide the first mechanistic study by which SAM68 modulates alternative splicing decision, by affecting U1snRNP recruitment at 5' splice sites.

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756 Structural basis of alternative splicing regulation by MBNL

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Alternative splicing is a complex process governed by a network of interactions between multiple protein factors and cis-acting elements specifically arranged within primary and secondary structures of mRNA precursors. An adequate stoichiometry of these components in time allows for a proper and directed development of living organisms. Muscleblind-like proteins (MBNL1, MBNL2, MBNL3) are the splicing factors, which exhibit spatiotemporal pattern of expression differing between three paralogs. MBNL1 and MBNL2 coordinate fetal-to-adult splicing transition in most tissues including muscles, while MBNL3 resides in muscle primary cells taking part in a muscle regeneration process. All MBNLs promote alternative exon inclusion or exclusion depending on a position of a specifically recognized binding motif 5'-YGCY (Y - C or U) within pre-mRNA.

In our research, we are studying the contribution of the sequence composition and RNA secondary structure determinants of MBNL-binding sites to the mechanism of MBNL-mediated splicing regulation and reciprocal interplay between MBNL paralogs. We are also focused on gaining a deeper insight into the network of interactions between MBNL and other cis- and trans-acting elements and their significance in the mechanism of alternative splicing regulation.

Applied biochemical, biophysical and in cellula assays led us to define the specific composition of YGCY sequence motifs and features of RNA secondary structure within which the motifs are embedded, which play essential roles in the mechanism of MBNL-governed alternative splicing. They either augment or deteriorate MBNL binding affinity and splicing activity. We also observed that the organization of RNA regulatory elements bound by MBNLs is an essential factor differentiating the splicing activity of MBNL1 and MBNL3 in different cell lines. Our results suggest that due to an RNA substrate-influenced interaction between the MBNL paralogs, a developmentally and tissue-controlled ratio of MBNL paralogs, and isoforms is indispensable for a proper switch from fetal to adult splicing patterns. Lastly, having performed in silico and in cellula analysis we emerged RNA helicases as modifiers of MBNL-dependent splicing pattern.

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757 SRSF1 modulates PTPMT1 alternative splicing to regulate lung cancer cell radioresistance

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Splicing dysregulation plays critical roles in tumorigenesis. However, the involvement of alternative splicing in resistance of cancer cells to radiotherapy remains elusive. Here, we screened splicing factors that might participate in radioresistance regulation, and found that SRSF1 is involved in radioresistance in cancer cells. The level of SRSF1 is elevated in ionizing radiation treated lung cancer cells, whereas knockdown of SRSF1 sensitizes cancer cells to irradiation. Mechanistically, SRSF1 modulates various cancer-related splicing events, particularly the splicing of PTPMT1, a PTEN-like mitochondrial phosphatase. Reduced SRSF1 favors the production of short isoforms of PTPMT1 upon ionizing radiation, which in turn promotes phosphorylation of AMPK, thereby inducing DNA double-strand break to sensitize cancer cells to irradiation. Additionally, the level of the short isoforms of PTPMT1 is decreased in cancer samples, which is correlated to cancer patients' survival. In conclusion, our study provides mechanistic analyses of aberrant splicing in radioresistance in lung cancer, and establishes SRSF1 as a potential therapeutic target for sensitization of patients to radiotherapy.

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758 Biochemical Characterisation of RNA Binding by ZFR – An Essential Zinc Finger Protein Associated with Splicing

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Alternative splicing of pre-RNA is an important mechanism used by eukaryotes to regulate their gene expression and to expand the protein isoform repertoire. The human Zinc finger RNA-binding protein (ZFR) is found throughout the metazoans and loss of this protein is lethal in mice. ZFR has recently been identified as an inhibitor of the type I interferon response upon infection by regulation of alternative pre-mRNA splicing of the histone variant macroH2A1 [1]. ZFR has three zinc finger RNA binding domains and is known to dimerise with Nuclear Factor 45 (NF45) through a domain associated with zinc fingers (DZF) [2].

In order to gain a mechanistic understanding of the role of ZFR in alternative splicing, it is important to characterise its RNA binding specificity. We produced recombinant human ZFR by co-expression with mouse NF45 and purified constructs containing or lacking the three zinc-finger domains. Constructs that lack the zinc finger domains have weak RNA binding activity compared to constructs with zinc fingers. These two constructs were used in RNA Bind-n-Seq experiments [3], which suggested a propensity for ZFR to bind structured RNAs. *In vitro* verification of binding of RBNS obtained motifs indicate that ZFR has a strong preference for dsRNA over ssRNA, suggesting that in vivo binding sites that determine alternative splicing sites are likely to contain substantial secondary structure.

[1] Haque, N, Ouda, R, Chen, C, Ozato, K, Hogg, JR (2018). ZFR coordinates crosstalk between RNA decay and transcription in innate immunity. Nat Commun, 9, 1:1145.

[2] Wolkowicz, UM, Cook, AG (2012). NF45 dimerizes with NF90, Zfr and SPNR via a conserved domain that has a nucleotidyltransferase fold. Nucleic Acids Res., 40, 18:9356-68.

[3] Lambert, N, Robertson, A, Jangi, M, McGeary, S, Sharp, PA, Burge, CB (2014). RNA Bind-n-Seq: quantitative assessment of the sequence and structural binding specificity of RNA binding proteins. *Mol. Cell*, 54, 5:887-900.

759 RBM10 functions as a tumor suppressor in lung cancers by mediating splicing of eukaryotic translation initiation factor 4H

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Alternative splicing (AS) influences the expression of most eukaryotic genes and is tightly regulated in both special and temporal fashion. The dysregulation of AS is one of the molecular hallmarks of cancer, with hundreds of AS events shifted in cancer cells. The gene encoding splicing factor RBM10 is frequently mutated in lung adenocarcinoma (LUAD), however its underlying molecular mechanism remains elusive. Here we identified RBM10 mutations in East Asian lung adenocarcinoma (LUAD) patients as the cancer driver, and compared them with those in Western LUAD patients in The Cancer Genome Atlas (TCGA). The majority of RBM10 mutations are loss-of-function mutations in LUAD patients from both populations, despite their dramatically distinct patterns of oncogenic mutations. Furthermore, we found that silencing of RBM10 suppresses the proliferation and survival of LUAD cells, as well as the progression of exnograft tumor. To gain additional molecular insights, we conducted RNA-Seq experiments in lung epithelial cells with RBM10 knockdown and LUAD cells with overexpressed RBM10, as well as LUAD tissues with wild type or mutated RBM10 (with matched adjacent non-tumor tissues). We found that RBM10 exerts suppressive functions in LUAD by regulating splicing of many key genes involved in gene transcription and RNA processing. In addition, several RBM10-regulated RNA splicing events in LUADs are significantly associated with patient survival. Specifically, loss of RBM10 promotes the inclusion of exon 5 in the translation initiation factor EIF4H, and the EIF4H splice variant containing exon 5 is accumulated in LUAD patients. Blocking EIF4H exon 5 inclusion dramatically inhibits LUAD cell proliferation, survival and tumorigenesis. Collectively, this study demonstrates tumor suppressive activity of RBM10 and therapeutic potential of key RBM10 target genes.

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760 The role of TCF7L2 alternative splicing in tumor progression and its modulation by splice-switching antisense oligonucleotides (SSOs)

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TCF4 (TCF7L2) is a member of the T-cell Factor/Lymphoid Enhancer Factor (TCF) family of transcription factors, nuclear mediators of the Wnt/β-catenin signaling pathway. TCF7L2 is known as a regulator of liver gluconeogenesis and metabolism. However, its role in cancer initiation and progression has not been studied in depth. The TCF7L2 gene consists of 17 exons, which are known to display a complex pattern of alternative splicing (AS), resulting in TCF4 proteins with different C-termini.

We have identified TCF7L2 as a target of the oncogenic splicing factor hnRNP A2/B1, which promotes breast cancer metastasis when up-regulated. TCF7L2 undergoes AS in metastatic breast tumors and knockdown of hnRNP A2/B1 in breast cancer cell lines induces skipping of exons 13-15. Breast cancer cells overexpressing the short TCF7L2 isoform (excluding exons 13-15) formed less tumors and had fewer metastases when injected into mice than cells overexpressing the long isoform (including exons 13-15). RNA-seq of breast cancer cells overexpressing TCF7L2 long and short isoforms identified an enrichment of cytokines and immune system modulators affected by the long and short TCF7L2 isoforms.

Analysis of TCGA data, identified higher inclusion of TCF7L2 exons 13-15 in glioblastoma samples. Thus, we hypothesize that modulating TCF7L2 splicing can inhibit glioblastoma growth and survival. We designed a splice switching antisense oligonucleotide (SSO) screen to target the intron 14/exon 15 splicing junction of TCF7L2. A SSO that most efficiently caused skipping of exons13-15 was tested in both breast and glioblastoma cancer cells. Treatment with TCF7L2 SSO resulted in inhibition of anchorage-independent growth, proliferation and survival of breast and glioblastoma cells in culture. We are presently testing this SSO in vivo.

Our hypothesis is that the AS of TCF7L2 is a crucial step in tumorigenesis, specifically in glioblastomas and metastatic breast cancer, and modulation of its splicing might inhibit tumor progression.

761 From pathogenesis to therapy of triplet repeat expansion diseases

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Polyglutamine (polyQ) diseases are neurological disorders caused by CAG repeat expansion in ORFs of specific genes. RNAi-based targeting of mutation site is an attractive therapeutic option for such diseases. Previously we designed CAG repeat-targeting miRNA-like siRNAs and showed preferential decrease of mutant protein level in various polyQ disease cellular models, e.g. HD (Huntington's disease). Now we have investigated activity and mechanism of these allele-selective siRNAs in HEK293-based cell lines and HD iPSC-derived human neural progenitors.

We developed Flp-In T-REx 293 inducible models in which NanoLuciferase is stably expressed with the first exon of huntingtin with normal or mutant repeat tract. These cell lines were used for precise investigation of kinetics of the silencing process upon CAG repeat-targeting siRNAs treatment. The interplay between translational inhibition and deadenylation was examined by polysome fractionation and poly(A) tail-length measurement. Moreover, we generated similar cell models with additional 4xMS2 aptamers, which enabled us to investigate the protein composition of miRISCs forming on expanded CAG repeats upon allele-selective siRNA treatment. Furthermore, by using single-molecule fluorescent *in situ* hybridization we have investigated *HTT* transcripts abundance and localization in HD neural progenitors after treatment of cells with selected siRNAs.

Our study shows that during the inhibition of mutant *HTT*, CAG repeat-targeting siRNAs cause translational repression, which precedes slight mRNA decay. This effect is similar to the activity of miRNAs, especially those acting in a cooperative manner and targeting ORF regions. These siRNAs offer allele-selective therapeutic strategy for several polyQ diseases.

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762 SINEUPs: a functional class of lncRNAs that activates translation as a novel strategy for gene therapy of neurological disorders

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SINEUPs represent a new platform to increase endogenous protein levels of target mRNAs for therapeutic purposes. They are antisense long non-coding RNA (lncRNAs) that stimulate translation of sense mRNAs. Their activity depends on the combination of two domains: the overlapping region, or binding domain (BD), dictates SINEUP specificity, while an embedded inverted SINEB2 element acts as effector domain (ED) controlling the enhancement of mRNA translation. Their modular structure can be employed to artificially engineer their BD and design synthetic SINEUPs to specifically enhance translation of virtually any target gene of interest. They usually increase target protein expression of 2-3 fold, thus representing a more physiological effect compared to other DNA or RNA-based approach. Moreover, they are active only on cells that express target mRNAs, thus limiting the side effects. As representative examples, SINEUP-GDNF RNA increases endogenous GDNF protein levels both in-vitro and in-vivo. AAV9-mediated delivery in the striatum of WT mice led to an increase of endogenous GDNF protein for at least six months and the potentiation of DA system's functions while showing no side effects. Furthermore, SINEUP-GDNF was able to ameliorate motor deficits and neurodegeneration of DA neurons in a PD mouse model. SINEUP-frataxin RNA increases endogenous frataxin protein levels restoring mitochondrial activity in Freidreich's Ataxia patient's cells. Our data indicate that SINEUPs could represent a new strategy to increase endogenous protein levels in a more specific and physiological manner and a novel therapeutic approach for haploinsufficiencies.

763 Insights into the structural basis of nonspecific binding of RNA by small molecules

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Non-coding RNA (ncRNA) is an emerging class of attractive drug targets in a wide array of human diseases and pathogens. While there has been some success in targeting RNA with anti-sense oligonucleotides (ASOs), there is a growing interest in developing small molecule inhibitors to avoid the delivery limitations presented by ASOs. The largest class of known RNA-binding small molecules is the aminoglycosides, many of which are FDA-approved antibiotics. However, aminoglycosides exhibit many side effects due to known, well-studied non-specific binding among RNA. There are non-aminoglycoside RNA-targeted small molecules being developed, and it is unknown whether or not these compounds also display non-specific binding properties. To answer this question, we obtained four non-AG small molecules that have been shown to bind different RNA targets with high selectivity, as demonstrated by activity in cell-based assays. We found that all four compounds bind to HIV1-TAR and HIV1-RRE by NMR and fluorescence-based in vitro binding assays. Furthermore, these compounds inhibited TAR-dependent transactivation in a cell-based assay of HIV1-TAR function, while also demonstrating off-target effects consistent with non-specific binding. Our results are consistent with non-specific binding of these RNA-targeted drugs. We then asked if the mechanism of non-specific binding was the same for these compounds as it is for aminoglycosides. To answer this, we created a structural database of all RNA-small molecule hydrogen bonding interactions in the Protein Data Bank. We found that the RNA atoms most frequently contacted by aminoglycosides in these structures are the same atoms most frequently contacted by non-aminoglycoside small molecules. These contacts differ from the contacts seen in the pool of known selective RNA-small molecule interactions, represented by synthetic RNA aptamers selected for binding to a specific small molecule. Taken together, our results suggest the existence of common hydrogen bonding modalities that give rise to nonspecific binding, and that rigorous tests of selectivity are called for when developing lead compounds against RNA elements.

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764 RNA *trans*-splicing for the treatment of HIV infection

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Despite remarkable advances in therapy leading to near normal lives for people with HIV on antiretroviral therapy there are still a large number of new cases of HIV infection each year and with extensive usage drug resistance to most of the currently available medications is emerging. We previously showed that delivery of an incomplete *Herpes simplex virus* thymidine kinase (HSV-tk) gene that splices *in trans* on to an HIV transcript to produce an intact coding RNA leads to expression of HSV-tk in infected cells and renders them susceptible to ganciclovir induced killing. Optimal killing was seen targeting the D4 splice site in the virus and *trans* spliced chimeric RNA transcripts could be detected. Selective killing of around 80% of HIV-producing cells was detected without any killing of uninfected cells with the most efficient construct targeting D4. We have now engineered these constructs into gene vectors based on lentiviruses for delivery to cells *in vitro* and eventually *in vivo*. Lentiviral delivery of therapeutic vectors into tissue culture models of HIV infection was investigated by quantitative PCR (qPCR) on extracted genomic DNA and induction of HSV-tk *trans*-gene expression was confirmed by qRT-PCR. We have experimented with various modifications of promoter to drive the *trans*-gene and taken other steps to optimise delivery. Results of optimisation experiments and progress towards an RNA targeted *trans*-splicing approach to achieve selective killing of HIV infected cells will be presented.

765 Universal RNAi triggers for specific inhibition of mutant huntingtin, ataxin-3, ataxin-7 and atrophin-1 expression.

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Expansion of CAG repeats within the coding region of associated genes is responsible for nine inherited neurodegenerative disorders including e.g., Huntington's disease (HD), spinocerebellar ataxias (SCAs) and dentatorubral-pallidoluysian atrophy (DRPLA). Despite many years of research aimed at developing an effective method of treatment, these diseases remain incurable and only their symptoms are controlled. The purpose of this study was to develop effective and allele-selective genetic tools for silencing the expression of mutated genes containing expanded CAG repeats. For polyglutamine (polyQ) disease genes, the regions differentiating the alleles that can be selectively targeted are single-nucleotide polymorphisms (SNPs) linked to the repeat expansions and the repeat region itself. The first strategy has however some limitations, because SNPs are present only in the selected group of patients. More universal strategy is based on the difference between the repeat tract length in the normal and mutant alleles. Here we show that CAG repeat - targeting short hairpin RNAs preferentially reduce the level of mutant huntingtin, ataxin-3, ataxin-7 and atrophin-1 proteins in patient-derived fibroblasts and may serve as universal allele - selective reagents for polyQ diseases.

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766 Transcriptional activation of HIV by structure-switching the 7SK RNA

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Transcription in eukaryotic cells is highly regulated, controlled in part by the positive transcription elongation factor complex, P-TEFb. The non-coding 7SK RNA binds P-TEFb and selectively sequesters the complex, preventing transcriptional activation. The cycle of binding and release of P-TEFb is important for both transcriptional activation in general and, because Tat protein-dependent transcription from the HIV-1 promoter is uniquely sensitive to P-TEFb, disruption of 7SK-dependent P-TEFb sequestration represents a plausible strategy for HIV latency reversal. Using single molecule in-cell chemical probing, we have identified a conformational switch in the structure of the 7SK RNA that modulates P-TEFb release and consequent transcriptional activation. Structural characterization of the 7SK conformations enabled intelligent design of antisense oligonucleotides (ASOs) that promote formation of either of the two 7SK RNA conformations. In a human cell-based assay that measures gene expression from the HIV long terminal repeat (LTR) promoter, we show that structure-switching 7SK-targeting ASOs can enhance Tat-dependent reporter expression. Induced structure-switching of the 7SK non-coding RNA enables transcriptional reprogramming in cells and represents a potential strategy to reverse latency and contribute to a functional cure for HIV.

767 Identification of RNA binding proteins involved in the acquisition of resistance to chemotherapy treatment in Pancreatic Ductal Adenocarcinoma (PDAC).

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer. Most patients presentwith advanced diseaseat diagnosis, which only permits palliative chemotherapeutic treatments and their mean survival is <1 year. Both innovative chemotherapeutic treatments and targeted therapies have shown little efficacy so far. Thus, improvement in PDAC management represents a clinical priority.

Dysregulation of RNA processing pathways in cancer cells is emerging as a distinct feature capable to precisely stratify patients and an exploitable therapeutic vulnerability. To identify novel biomarkers that are capable to distinguish between tumors with potentially different responses to treatments and to test the potential of targeting RNA processing dysregulation as novel therapeutic approachfor PDAC, we performed a bioinformatics screening to search for splicing factorsassociated with PDAC prognosis by querying "The Cancer Genome Atlas (TCGA)" database. We selected 202 genes among those encoding RNA binding proteins (RBPs)and other proteins (i.e. kinases) that are involved in nuclear RNA processing and found 12 genes that displayed a significant correlation with progression free survival (PFS)in surgically resected PDAC patients. Out of these candidates, we focused our attention on MEX3A because itwas previously shown to mark intestinal stem cells that are refractory to chemotherapeutic treatments.

Increased expression of MEX3A correlated with higher disease stage in PDAC patients. Knockdown of MEX3A in PDAC cells impaired cell cycle progression and enhanced sensitivity to chemotherapeutic treatment. RNA sequencing analyses of MEX3A-depleted cells highlighted hundreds of genes whose expression is sensitive to MEX3A, with significant enrichment in cell cycle genes and stemness pathways genes. Our findings uncover a new pathway possibly involved in the acquisition of chemoresistance by PDAC cells, which may be suitable for therapeutic targeting in PDAC patients.

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768 Designing and investigating the action mechanism of PNA antimicrobials against *Salmonella enterica* serovar Typhimurium

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The rise of antibiotic-resistant infections is a globally recognized threat. There is an urgent need for new classes of antimicrobials, which would be more species-specific and therefore reduce the development and spread of antibiotic resistance. One such class are peptide nucleic acids (PNAs) - synthetic polymers with a pseudo-peptide backbone, to which nucleobases are attached.

To improve their uptake, PNAs are conjugated with short cell penetrating peptides (CPPs). CPPs are efficiently recognised and taken up by both mammalian cells and bacteria, which makes them potent delivery agents for PNAs against intracellular bacteria such as *Salmonella enterica* serovar Typhimurium.

In this project, we aim to design and determine the antimicrobial potential of several CPP-PNA constructs against acpP, the essential bacterial gene involved in fatty acid biosynthesis. Here we present our attempts to elucidate the uptake mechanism and action of several acpP-PNA constructs in *Salmonella*. We determine minimum inhibitory concentrations for each construct and analyse the transcriptome changes, induced by the PNA treatment in *Salmonella*. We perform induced mutagenesis experiments to uncover genes potentially involved in CPP-PNA transport across the bacterial double membrane. We also analyse a pool of essential genes in *E.coli*, *Salmonella* and *P.aeruginosa* and suggest potential target genes for designing species-specific antisense antimicrobials. This study is a proof of concept for selective microbiome editing using RNA-based therapeutics.

769 Prophylactic and therapeutic potential of NS1 shRNA against influenza infection

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Viruses have evolved strategies to counteract host defense by targeting its components. Influenza virus nonstructural protein 1 (NS1) suppresses host innate immune defense by targeting cytosolic pathogen sensor, Retinoic acid-inducible gene-I (RIG-I). In this study we show that silencing NS1 with in vitro transcribed 5'-triphosphate containing NS1 shRNA designed using the conserved region of a number of Influenza viruses not only decreased NS1 expression but also induced RIG-I activation and type I interferon expression. This strategy inhibited Influenza virus replication and also showed therapeutic potential in *in vitro* and *in vivo* experimental animal models.

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770 Optimization of cell-penetrating peptide based microRNA-146a delivery for targeting of skin inflammation

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MicroRNAs (miRNAs) are post-transcriptional gene expression regulators with potential therapeutic applications, hence, efficient cellular delivery methods are required. In the inflamed skin, keratinocytes (KCs) and Langerhan's cells (LCs) are the main cell types exposed to therapeutic targeting. miR-146a, a negative regulator of inflammatory processes, suppresses the NF-kB pathway in KCs and is upregulated in LCs as compared to inflammatory dendritic cells (DCs) suggesting that miR-146a may have therapeutic influence in inflammatory skin diseases.

We aimed to test the capacity of selected PepFect (PF), NickFect (NF) and MGPE type of cell penetrating peptides (CPPs) to deliver miR-146a mimics into human primary KCs and *in vitro* differentiated DCs as representing cell type of antigen presenting cells.

First, PF14, C22PF14, NF55, NFH52, NFH31, NFH82, MGPE9, stearyl-MGPE9 and Lipofectamine were used to deliver DyLight547-labelled control. All used CPP:miRNA and Lipofectmine complexes were internalized by KCs; however, to a lesser extent, by DCs. Second, all selected CPPs were used to deliver miR-146a. The downregulation of miR-146a target genes CARD10 and IRAK1 in KCs was detected with all tested peptides, except for MGPE-type of CPPs. Similar results were achieved with miR-146a-affected chemokines IL-8 and CCL5 when KCs were stimulated with IFN-g, a cytokine used to induce inflammatory conditions. In DCs, the downregulation of IRAK1 was detected when NF55, NFH52, NFH31 and Lipofectamine were used for miR-146a delivery, indicating that among tested peptides only NickFects are efficient in antigen presenting cells. In contrast to Lipofectamine 2000, CPP based delivery did not lead to DC activation according to CD86 and CD83 markers suggesting that NickFects have advantage in therapeutic delivery.

In conclusion, all tested CPPs delivered miRNA mimics into KCs while only NickFect type CPPs efficiently delivered miRNA mimics into DCs. Further studies will be needed to deepen into the trafficking mechanisms involved in CPP based miRNA delivery in DCs as compared to KCs as well as to test the delivery efficiency of the selected CPPs in skin inflammation *in vivo*.

771 Structural basis of thioflavin T binding between G-quadruplexes at the homodimer interface of the fluorogenic RNA aptamer Corn

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Thioflavin T (ThT) and its derivatives are intensively studied as fluorescent probes for amyloid and G-quadruplexes, and as therapeutics for different neurodegenerative diseases. The structural basis for ThT binding to biological macromolecules and cellular substructures are not well understood. Hence, for imaging, diagnostic, and therapeutic applications of ThT derivatives it is essential to evaluate how they may interact with proteins, nucleic acids and physiological or pathological protein-nucleic acids complexes. Numerous previous studies have documented that ThT and related benzothiazole dyes preferentially but sequence-non-specifically bind to G-quadruplex containing DNAs and RNAs and fluoresce brightly. At present no structural information is available to elucidate how ThT binds G-quadruplexes, and in general nucleic acids.

We characterized association of ThT and a second benzothiazole dye, thiazole orange (TO), to fluorogenic light-up RNA aptamer 'Corn' which contains two G-quadruplexes. We discovered that the structural basis of ThT fluorescence binding by G-quadruplexes differ from its binding to proteins, as well as from the binding mechanisms of highly specific interactions between G-quadruplex containing fluorogenic RNA aptamers and their cognate fluorophores. Our crystal structures likely illustrate how these fluorophores bind to any G-quadruplex containing RNA and DNA and provides a structural counterpoint to studies of ThT interacting with amyloids. Our work is of interest to scientists who study RNA structure, small molecule-G-quadruplex interactions and those who investigate cellular applications of fluorogenic light-up aptamers.

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772 High-throughput Identification of Effective Peptide Nucleic Acid Targets through a Synthetic sRNA Library

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Peptide nucleic acids (PNAs) are artificial peptide-based polymers with nucleic acid bases that have long been studied as potential antisense therapeutics. The neutrally charged backbone affords several advantages over normal antisense oligonucleotides including resistance to nuclease degradation and increased binding affinity. The field of PNA therapeutics has generally focused on only a few specific targets, but it remains to be determined in a high-throughput and global manner which oligonucleotide sequences are the most toxic or species specific candidates. However, the cost of a high-throughput, sequence-randomized PNA screen is currently prohibitive. Here, we utilize small RNAs (sRNAs) that can affect gene expression in a similar manner as PNAs. We created a synthetic sRNA library with a randomized 5' seed sequence fused to an Hfq-associating scaffold as a cost-effective proxy for screening. We began by using a GFP reporter assay to validate sRNA scaffolds by fusing all candidates to the same known seed sequence. After scaffold selection, a library of ~100,000 synthetic sRNA variants under the control of an inducible promoter was transformed into *Salmonella* Typhimurium. Using an induction-dependent differential library screen, we hope to identify lethal 5' seed sequences that will inform future PNA design. This library is also applicable to other screens for the identification of PNA sequences that activate certain cellular phenotypes.

773 High-temperature transcription generates synthetic RNA with reduced immunogenicity.

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The use of synthetic RNA for therapeutics requires that the *in vitro* synthesis process be robust and efficient. The technology used for the synthesis of these *in vitro*-transcribed (IVT) mRNAs using phage RNA polymerase is well established. Introduction of these IVT mRNAs into *in vivo* models results in an immune response, which is undesirable in therapeutic applications. Previous studies have identified double-stranded RNA (dsRNA)—a major by-product of the *in vitro* transcription process—as a trigger of cellular immune responses. It is critical to either eliminate these dsRNA by-products from the mRNA preparations or minimize their formation. Thus, a cost-effective *in vitro* transcription method that is able to directly generate large amounts of non-immunogenic IVT RNA (devoid of dsRNA by-products) is highly desired.

To address this issue, we engineered a series of RNA polymerases and discuss here the characterization of a novel methodology—high-temperature transcription—to produce large amounts of functional, non-immunogenic IVT mRNAs (lacking dsRNA by-products) using these engineered RNA polymerases. We found that the dsRNA by-products are 3'-extended and formed in an RNA-dependent manner. We further investigated the effects of RNA modifications on dsRNA formation, as modified nucleotides are routinely incorporated into synthetic mRNAs to achieve optimal protein expression and to evade the immune response.

We envision that our novel methodology will allow researchers to quickly and cost-effectively synthesize RNAs of interest that elicit little to no adverse immune responses when introduced *in vivo*.

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774 eIF3-mediated ribosome recruitment by histone H4 mRNA during translation initiation

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Eukaryotic mRNA translation initiation is a coordinated process that requires numerous initiation factors (eIFs) to trigger mRNA attachment to the small 40S ribosomal subunit, mRNA scanning and start codon selection as well as ribosomal assembly. Recent studies have unveiled a remarkable diversity of ribosome recruitment processes during the initiation of eukaryotic mRNA translation.

Our team has shown that the initiation of translation of histone H4 mRNA combined canonical (cap) and viral (no-scanning) initiation strategies (Martin et al., Mol Cell 2011, Nature Com 2016). The ribosomes are recruited by the initiation factors bound to specific structures in the histone mRNA coding region and deposited directly on the initiator AUG codon. This tethering mechanism leads to direct initiation on the AUG without scanning of the 5' untranslated region. Recent work has shown that some subunits of the eIF3 translation initiation factor ensure selective recruitment of cellular mRNAs to control their expression. The histone H4 mRNA is one of the mRNAs controlled by eIF3 (Lee et al., 2015, Villa et al., 2013). In higher eukaryotes eIF3 is composed of 13 subunits (a to m). Among them eIF3d was shown to exhibit a cap binding activity. We deciphered the eIF3-H4 mRNA interaction and analyzed his role during the translation of histone H4. Combining cross-linking and ribonucleoprotein immunoprecipitation (RNP IP) *in vivo* and *in vitro* we found that eIF3 interacted with H4 but also H1, H2A, H2B and H3 histone mRNAs. Furthermore, we have demonstrated a direct interaction between the H4 mRNA and the eIF3c, d, e and g subunits. The functional role of eIF3 subunits has been investigated *in vivo* by siRNA interference and specific analysis of histone neosynthesis after [^{35}S] pulse labelling. Results suggest that eIF3 regulates H4 mRNA translation. Combined with our previous cryo-EM results on the H4 mRNA/80S complex these data suggest that histone H4 mRNA may interact with eIF3 at both ends of the mRNA channel where the eIF3b/g/i subcomplex and the eIF3d subunits should be located. eIF3-mediated ribosome recruitment is therefore likely to play a critical role in the H4 unconventional translation initiation mechanism.

775 *Structure of the human ribosome in the classical PRE-state reveals the role of uS19 in translation fidelity*

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Translation is a crucial aspect of gene expression that is necessary for cellular activity, leading to potentially deleterious effects upon its dysregulation. In order to achieve precise control over their proteome, cells stringently regulate translation by activating/silencing specific mRNAs while preserving the fidelity of translation at the same time. While the activating/silencing of translation of specific mRNAs is carried out by ribosome/RNA-binding proteins (RBPs), the fidelity of translation is maintained mainly by ribosomes itself. Our overall goal is to understand the regulation of translational levels and fidelity by the ribosome and ribosome/RNA-binding proteins. We have determined the structure of active translating human 80S ribosome in the post-decoding pre-translocation state (classical-PRE) at 3.3 Å resolution along with the rotated (hybrid-PRE) and the post-translocation states (POST). The classical-PRE state ribosome structure reveals the existence of an archaea/eukaryote-specific function of uS19 C-terminal tail in the stabilization of A- and P-site tRNAs and decoding interactions. Analysis of the C-terminal tail of uS19 in different ribosomal conformations reveals the function of uS19 in coordinating tRNA movement during translation and provides mechanistic insights into translational dysregulation caused by multiple disease-associated mutations occurring in uS19. Additionally, analysis of inter-subunit bridges in individual ribosomal conformations suggest formation of distinct energy states due to mammalian-specific subunit rolling, and further highlights the function of uS19 tail in the context of mammalian ribosomes. Furthermore, we investigate the regulation of translation by specific RBPs that affect either translation initiation and/or elongation. To this end, we have reconstituted distinct RBP-ribosome complexes which will be used for structural studies.

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776 Thermodynamic determinants of translational frameshifting

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During translation certain mRNAs containing a 'shifty' sequence and a downstream secondary structure element stall the progression of the ribosome and induce its movement into the -1 reading frame. There are also trans-acting RNA elements that contribute to tRNA slippage. How these RNA regulatory elements act in a concerted way to produce the observed efficiencies is hampered by the lack of a model that is based on energetic contribution of individual tRNA-mRNA base pairing. Here, we combined rapid kinetics, biochemistry and Bayesian statistics to build a thermodynamic model to explain how frameshifting works on shifty messages. We also investigated how interactions of the downstream RNA with RNA regulatory elements may influence the propensity to frameshift. We show that the efficiency to frameshift on a given slippery sequence can be reproduced and can even be predicted from the free-energy differences of tRNA-mRNA base pairing in the two frames. Hence, we indicate that the efficiency of frameshifting on a slippery codon is mainly determined by thermodynamics. The downstream RNA elements with other trans-acting RNA elements impede the movement of the ribosome and generate a favorable time window so that the free energy barrier to change the reading frame can be overcome, but do not determine efficiencies per se.

777 hnRMPM is a novel hypoxic IRES trans-acting factor

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Heterogeneous nuclear ribonucleoprotein M (hnRNPM) protein belongs to the hnRNP-family of nucleocytoplasmic shuttling RNA-binding proteins that originally identified based on their association with pre-mRNAs with GU-rich manner. Recently, hnRNPM was shown to enforce the expression of CD44 antigen splice isoform (CD44s) which permits epithelial-mesenchymal transition in cancer. However, the biological functions of hnRNPM are still largely unknown. We previously identified hnRNPM as a novel IRES trans-acting factor (ITAF) of FGF9 mRNA. We subsequently showed that hnRNPM is a novel ITAF to control a subpopulation of IRES-mediated translated transcripts in hypoxic cells by deep sequencing and bioinformatic tools. Here we showed that a dramatic change of global IRES-hnRNPM interaction from normaxia to hypoxia by RIP-seq, translatome analysis and IRES database. About 50% (2706/5599) of hnRNPM-target mRNAs containing predicted IRES motif were translated in hypoxic cells. We further compared the overall translation efficiencies of three mRNA pools, hnRNPM-IRES-targets, hnRNPM-splicing-targets and eIF4E2-dependent translated-targets. The translation efficiency of eIF4E2-dependent translated-target samples is only slightly better than the hnRNPM-IRES-target samples. Taken together, our results demonstrated hnRNPM is a novel ITAF to regulate IRES-mediated translation event in hypoxia.

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778 The X-linked DDX3X RNA helicase dictates translation re-programming and metastasis in melanoma

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The X-linked *DDX3X* gene encodes an ATP-dependent DEAD-box RNA helicase frequently altered in various human cancers including melanomas. Despite its important roles in translation and splicing, how DDX3X dysfunction specifically rewires gene expression in melanoma remains completely unknown. Here we uncover a DDX3X-driven post-transcriptional program that dictates melanoma phenotype and poor disease prognosis. Through an unbiased analysis of translating ribosomes we identified the microphthalmia-associated transcription factor, *MITF*, as a key *DDX3X* translational target in melanoma cells, which directs a proliferative-to-metastatic phenotypic switch in DDX3X-depleted cells. Mechanistically, DDX3X directs *MITF* translation via an internal ribosome entry site (IRES) embedded within the 5' untranslated region of its transcript. Through this exquisite translational regulatory mechanism, DDX3X steers MITF protein levels directing melanoma metastatic potential *in vivo* and response to targeted therapy. Together these findings unravel a post-transcriptional layer of gene regulation that may provide a unique therapeutic vulnerability in aggressive male melanomas.

779 Translation of the *psbA* is regulated by mRNA secondary structure changes

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Translation in plastids is hypothesized to be altered by mRNA secondary structure changes in translation initiation region. We used high light acclimation which induces *psbA* mRNA translation encoding for D1 subunit of photosystem II in *Arabidopsis thaliana* and applied ribosome profiling together with *in vivo* RNA secondary structure analysis (SHAPE-seq and DMS-MaPseq). Our results indicate increased accessibility of translation initiation region of *psbA* in response to high light treatment likely contributing to increased translation efficiency. We also observed that in other plastid genes with weak Shine-Dalgarno sequences translation efficiency correlates with secondary structure changes in translation initiation region. Our results suggest that changes of mRNA secondary structure might be general mechanism regulating translation in plastids.

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780 Footprints in bacteria - Ribosome profiling combined with RNA-Seq of *Escherichia coli* LF82 and *Enterococcus faecalis* OG1RF in a mixed culture under aerobic and anaerobic growth-conditions

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Escherichia coli LF 82 and *Enterococcus faecalis* OG1RF live anaerobically in the human gut. Both can also grow under aerobic conditions by altering their metabolic gene expression. Here, we analysed and compared the translatome of both bacteria growing in a mixed culture under aerobic and anaerobic conditions. To predict which proteins are translated, at the moment of bacterial harvest, translation can be measured by ribosome profiling (RIBO-Seq). In this method, only the mRNA-part protected by the ribosome from RNases is sequenced.

After sequencing the total mRNA or ribosome footprints of the mixed cultures using Illumina HiSeq, the data files were mapped using Bowtie2 to each reference genome. Reads were normalised to "reads per kilobase per million mapped reads (RPKM)" for analysing up- or downregulated genes in the two strains.

Preliminary data analyses of the RIBO-Seq shows downregulation of several different sugar transporter genes in both strains under anaerobic compared to aerobic conditions (base line). However, gene regulation in metabolic pathways were more pronounced in *E. faecalis* OG1RF compared to *E. coli* LF 82. From these data we can conclude that both bacteria access different to nutrient when growing in mixed cultures. In RNA-Seq experiments several genes coding for hypothetical proteins were highly upregulated for both bacteria under aerobic compared to anaerobic conditions, but these genes were rather low translated. This indicates these genes to be somehow important (highly transcribed) but due to the low translation it might be hard to find a function. Interestingly, genes coding for flagella proteins were transcribed and translated in *E. coli* LF 82 under aerobic conditions compared anaerobic conditions, both, when growing alone or in mixed culture. Thus, gene expression of *E. coli* LF82 is hardly influenced by *E. faecalis* OG1RF when cultivated in mixed culture.

781 Genome-wide Survey of Queued Ribosomes

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Translation serves as the interface between nucleic acids and amino acids in the central dogma of life. This process is often impeded by numerous reasons, leading to ribosome pausing before the complete synthesis of full protein. Although the deceleration of ribosome movement is sensed by cells and would ultimately define the fates of the mRNAs and the synthesizing proteins, fundamental questions still remain to be addressed including where ribosomes pause in mRNAs, what kind of RNA/amino acid context causes the pausing, and how physiologically significant the slowdown of protein synthesis is. Here we surveyed the position of ribosome collision at a genome-wide level using the modified ribosome profiling technique in human and zebrafish. In both species, the collided ribosomes, *i.e.*disome, emerged at a unique proline-proline-lysine motif. Moreover, the number of ribosomes in a queue was not limited to only two, rather four or five. Among the ribosome queueing sites, XBP1, a key modulator of unfolded protein response, showed striking collisions of ribosomes thus act as a substrate for ribosome-associated quality control (RQC) to avoid the accumulation of undesirable protein in the absence of stress. Our results provide an insight into the dynamics of ribosome during elongation and the versatileness of the framework used to dissect the specific architecture of ribosomes.

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782 eIF3, an important factor for histone mRNAs translation

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The vast majority of eukaryotic mRNAs initiate translation through a canonical cap-dependent mechanism requiring a free 5' cap and initiation factors (eIF) to form a translationally active ribosome. This tightly regulated process consists of several steps and involves 12 eIFs, 40S and 60S ribosomal subunits until the anticodon of $tRNA^{Met}$ is paired with the AUG codon into the P-site of the 80S ribosome.

We have shown that translation initiation of histone H4 mRNA combines canonical features (cap-dependent translation) with viral strategy (lack of scanning and internal recruitment of initiation factors)[1]. H4 mRNA contains a double stem-loop structure called eIF4E-sensitive element that recruits the cap-binding complex eIF4F. In addition, H4 mRNA contains a three-way junction that helps ribosome recruitment and positioning on the AUG start codon.

Recent PAR-CLIP experiments showed that eIF3 plays a crucial role in the selection and regulation of some characteristic cellular mRNAs, including histone mRNAs[2]. In this work, we decipher the interactions between histone mRNAs and eIF3. To this end we performed ribonucleoprotein immunoprecipitations using HEK293 extracts and showed that all histone mRNAs are able to interact *in vivo* with eIF3. By UV crosslinking experiments, we identified four eIF3 subunits able to interact with H4 mRNA: c, d, e and g. Next we performed GST-pulldown experiments on HEK293 total RNA using the four isolated subunits produced in *E. coli*. The mRNA of the linker histone H1 was found to bind tightly the four eIF3 subunits while the octameric core histone mRNAs (H2A, H2B, H3 and H4) interacted with the three subunits c, d, and g, but not eIF3e. Our results also highlighted a marked preference of H4 mRNA for eIF3d binding.

Altogether, the data suggest that eIF3 could play a significant role during translation initiation of histone mRNAs. Further investigations will address the effects of eIF3 down-regulation on histone synthesis *in vivo*.

*Martin F., Barends S., Jaeger S., Schaeffer L., Prongidi-Fix L., Eriani G. (2011) Cap-assisted internal initiation of translation of histone H4. Mol Cell., **41**, 197-209.*

*Lee AS., Kranzusch P., Cate J. (2015) eIF3 targets cell proliferation mRNAs for translational activation or repression. Nature, **522**, 111-114.*

783 Nonsense-mediated mRNA decay factor affects aggresome formation

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Intracellular misfolded polypeptides are mainly degraded by the ubiquitin-proteasome system (UPS). When the UPS is impaired, misfolded proteins are transported to form aggresome and degraded through aggrephagy. Even though accumulation of protein aggregates is involved in neurodegenerative diseases, the detailed molecular mechanism of aggresome formation remains unclear. In this study, we find a new protein complex called CED, which contains CBP80/20-dependent translation initiation factor (CTIF)[1], eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) and dynactin 1 (DCTN1)[2]. Through the interaction between these components, misfolded polypeptides are recognized and targeted to aggresome. CTIF has already been known as nonsense-mediated mRNA decay (NMD) factor. Thus, we hypothesized that misfolded polypeptides generated by NMD could be targeted to aggresome via CED-mediated aggresomal pathway. We show that the truncated (and potentially misfolded) polypeptides synthesized from GPx1-Ter reporter mRNA harboring a premature termination codon is localized in aggresome. We also investigate whether other NMD factors are co-localized with the aggresome markers. On the basis of our observations, we propose the possibility of crosstalk between mRNA and protein quality control pathways.

References

1. Kim KM, Cho H, Choi K, Kim J, Kim BW, Ko YG, Jang SK, Kim YK. A new MIF4G domain-containing protein, CTIF, directs nuclear cap-binding protein CBP80/20-depencent translation. *Genes Dev*. 2009 July;23(17):2033-45

2. Park J, Park Y, Ryu I, Choi MH, Lee HJ, Oh N, Kim K, Kim KM, Choe J, Lee C, Baik JH, Kim YK. Misfolded polypeptides are selectively recognized and transported toward aggresomes by a CED complex. *Nat Commun.* 2017 Jun 7;8:15730..

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784 Crucial roles of ribosome ubiquitination in quality controls and gene regulations

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Ribosome stalling during translation elongation results in quality controls for mRNA (NGD: No-Go Decay) and nascent polypeptide (RQC: Ribosome-associated Quality Control). In yeast, Hel2-dependent uS10 ubiquitination and Slh1/Rqt2 plays crucial roles in both NGD and RQC within the disome. Hel2 ubiquitinates the stalled ribosomes at the endogenous mRNA and that RQC-trigger (RQT) complex promotes ribosome dissociation. Endogenous mRNA possesses powerful arrest sequence that induces the formation of the leading stalled ribosome and the following colliding ribosomes. In vitro translation of endogenous arrest-inducing sequence allows us to reconstitute the Hel2-dependent polyubiquitination of the ribosome at uS10 that is a substrate for the subunit dissociation. Together, our results provide novel insights into how stalled ribosome is dissociated into subunits to initiate RQC pathway.

The ribosome ubiquitination and RQT-dependent subunit dissociation are also crucial in Nonfunctional rRNA Decay (NRD), quality controls for non-functional ribosomes with the deleterious mutations in rRNA. In 18S NRD, sequential ribosome ubiquitination of uS3 induces subunit dissociation by Rqt2, leading to the degradation of the nonfunctional 40S subunit with the A1492C-18S rRNA, suggesting that general functions of ribosome ubiquitination and RQT-dependent subunit dissociation in quality controls for aberrant translation.

785 Ribosomal incorporation of consecutive D- and β-amino acids

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Due to their unique characteristics, peptides containing consecutive D- and/or β-amino acids are attractive scaffolds for novel peptide drugs and nanomaterials. Although ribosomal incorporation of single or non-consecutive D- or β-amino acids into peptides has previously been reported, consecutive incorporation of these amino acids had not been accomplished. This is primarily due to their incompatibility with the ribosomal translation system. Here, we devised engineered D- and β-aminoacyl-tRNAs bearing optimized T-stem and D-arm motifs for enhancing binding affinity to EF-Tu and EF-P, respectively. Combined with a reconstituted *E. coli* translation system with optimized translation factor concentrations, up to ten consecutive D- or β-amino acids could be incorporated into model peptides. Furthermore, synthesis of macrocyclic peptides consisting of D- or β-amino acids closed by a thioether bond was also demonstrated. This represents the first example of the ribosomal synthesis of peptides containing stretches of consecutive D- or β-amino acids.

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786 Oxidative stress suppresses the effect of codon choice on the efficiency of protein synthesis.

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The response of enterobacteria to oxidative stress is usually considered to be regulated by transcription factors such as OxyR and SoxR. Nevertheless, some reports have shown that under oxidative stress the levels, modification and aminoacylation of some tRNAs may be altered. In order to characterize the effects of oxidative stress on translation elongation we constructed a library of 61 plasmids, each coding for the green fluorescent protein translationally fused to a different set of four identical codons. Using these reporters, we observed that when *Escherichia coli* is cultured in M9 media supplemented with branched amino acids GFP production levels vary widely (~15 fold). Under oxidative stress caused by paraquat the levels of GFP produced by most clones is reduced and, in contrast to control conditions, the range of GFP levels is restricted to a ~2.5 fold range. Our results suggest that under control conditions the speed of translation elongation is similar to that of initiation and consequentially codon choice impacts the speed of protein synthesis. Nevertheless, under oxidative stress, elongation seems to be faster than the limiting step of translation and codon choice has only subtle effects on the overall speed of gfp translation.

787 Single molecule and ensemble analysis of 2A protein-mediated frameshifting in EMCV RNA

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Programmed -1 ribosomal frameshifting (PRF) is a translational recoding event that causes ribosome slippage along the mRNA in the -1 reading frame. This leads to the production of an alternative protein and has an effect on the lifetime of the mRNA. PRF requires specific *cis*- acting elements- a slippery sequence of eight repetitive nucleotides followed by a stable RNA structure. It is recently shown that efficiency of PRF is also affected by *trans*-acting factors, including proteins, miRNAs and metabolites. While the general mechanisms of PRF and the involvement of *cis*-acting elements in this process are well understood, the regulation of these events is still vastly understudied. Additionally, the interactions of these factors with the RNA and/or the translation machinery have not yet been completely understood.

We chose EMCV 2A protein as a model since the expression of this protein is essential for frameshifting and inhibition of PRF leads to severely reduced virulence. We investigated the interplay of the 2A protein with its RNA target by using single molecule analysis tools and microscale thermophoresis (MST). Our initial results with single molecule optical tweezers show that binding of this protein significantly increases the force required to unfold the RNA structure, which is essential for PRF. We have confirmed that the 2A protein binds directly to the EMCV frameshifting RNA by using MST. Furthermore, we have shown that the binding affinity is decreased when the RNA loop is mutated. It is unknown whether 2A binding to the EMCV stem-loop is merely an obstacle or has a specific interaction with the ribosome. Our results suggest by binding to the downstream RNA structure the 2A protein might create a roadblock for the elongating ribosome, which can only be resolved by the ribosome after shifting into the -1 frame.

We anticipate these assays to be a starting point in analyzing the translational kinetics of frameshifting and its interplay by RNA binding factors. Furthermore, recent examples of identification of such factors indicate that they play a major role in PRF regulation and understanding their mode of action will certainly uncover new fundamental principles in RNA-based gene regulation.

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788 Mitochondrial translation repression in absence of gravity

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Life on the earth have evolved in a form suitable for the gravitational force (1 G). Although the pivotal role of gravity in gene expression has been exemplified by the aging-like symptom of astronauts in space, the molecular details how mammalian cells harness the gravity, have been remained unclear. Here we show that mitochondria utilize the gravity for activating protein synthesis within the organelle. Genome-wide ribosome profiling in human cells under microgravity ($1x10^{-6}$ G) mimicked by the three-dimensional clinostat unveiled that translational efficiencies of mitochondria-encoded mRNAs are attenuated. In addition, we found the stabilization of actin filaments, which directly associate with mitochondria and are rearranged in microgravity, recapitulated the inhibition of mitochondrial protein synthesis. These results indicated the mechanistic insight how cells convert the gravitational force into biological function in mammals and provided a unique layer of gene expression controlled by the gravity.

789 Cell permeable inhibitors of cap dependent translation

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Messenger RNA (mRNA) belongs to RNA molecules that express genetic information from DNA and direct the assembly of proteins on ribosomes. At the 5' end of mature mRNA in eukaryotic organisms is present a unique structure called mRNA cap. It consist of *N*7-methylguanosine moiety linked by a 5'-5' triphosphate chain to the first transcribed nucleotide. The unusual chemical structure of the cap is essential for all stages of mRNA metabolism: synthesis, splicing, nucleocytosolic transport, intracellular localization, translation, and turnover. Moreover, the cap mRNA is responsible for 5' mRNA end protection from premature degradation and plays a crucial role in recognition of translation factors. Therefore, some chemical modifications within cap structure can increase stability of capped mRNA and enhance affinity to translation initiation factors. Importantly, synthetic cap analogs as small molecule inhibitors of cap-related processes could be considered as potential therapeutic tools. However, because of the highly polar nature of nucleotides, cap analogs are unable to be delivered to cells. To overcome this problem, we adapted ligand supported approach, which involves recognition of small molecules by cell surface receptors specifically overexpressed on tumor cells.

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790 Multi-omics approaches to study translational regulation in aggressive B-cell lymphomas

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High grade aggressive lymphomas, such as Diffuse Large B-cell Lymphoma (DLBCL) or Burkitt Lymphoma (BL), are rapidly progressing malignancies. Survival and expansion of malignant lymphocytes requires well-functioning protein synthesis machinery, which consumes substantial amounts of energy resources. As a result, imbalance between energy demand and supply exposes tumour cells to hypoxia and nutrients deprivation. B-cell receptor signalling is one of the key regulators of B-cell proliferation and cellular translation. Interestingly, translation of specific transcripts can be precisely regulated and is a strategy cancer cells employ to survive unfavourable conditions.

Ribosome Profiling (Ribo-Seq), is a high-throughput technique that aims to measure what portion of the transcriptome is actively translated and provide deep insight into translation intensity at sub-codon resolution. We assessed the utility of selected open-source bioinformatic tools for Ribo-Seq analysis comparing their performance in public and our own experimental Ribo-Seq datasets. Our optimized protocol achieved high reproducibility between biological replicates ($R^2 < 0.97$) and captured the main features of translational dynamics: accumulation of ribosomal footprints in CDS region and 3 nt periodicity of Ribo-Seq reads alignment. We integrated Ribo-Seq data with RNA-Seq, mass spectrometry, iCLIP and sequence structure analysis to obtain the full picture of mechanisms of translational regulation.

As a model of early stages of lymphomagenesis, we used a novel co-culture system that enables long-term culture and viral transduction of primary human germinal centre B cells. Overexpression of BCL6 or MYC, two transcription factors frequently deregulated in high-grade lymphomas, caused independent changes in transcriptome and translatome. Translationally regulated genes included EIF4E – translation initiation factor involved in cap-dependent translation with documented role in haematological malignancies development. Intriguingly, translational events occurring outside annotated coding regions - small Open Reading Frames (smORFs) - were enriched in cell cycle, apoptosis and stress response related genes and negatively correlated with translation of canonical ORFs. Our findings suggest that translational regulation can be reproducibly measured and may contribute to lymphoma phenotype.

791 DDX3 participates in translational control of inflammation induced by infections and injuries

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Recent studies have suggested that DDX3 functions in antiviral innate immunity, but the underlying mechanism remains elusive. We previously identified target mRNAs whose translation is controlled by DDX3. Pathway enrichment analysis of these targets indicated that DDX3 is involved in various infections and inflammation. Using immunoblotting, we confirmed that PACT, STAT1, GNB2, Rac1, TAK1, and p38 MAPK proteins are down-regulated by DDX3 knockdown in human monocytic THP-1 cells and epithelial HeLa cells. Polysome profiling revealed that DDX3 knockdown reduces the translational efficiency of target mRNAs. We further demonstrated DDX3-mediated translational control of target mRNAs by luciferase reporter assays. To examine the effects of DDX3 knockdown on macrophage migration and phagocytosis, we performed in vitro cell migration assay and flow cytometry analysis of the uptake of green fluorescent protein-expressing Escherichia coli in THP-1 cells. The DDX3 knockdown cells exhibited impaired macrophage migration and phagocytosis. Moreover, we used a human cytokine antibody array to identify the cytokines affected by DDX3 knockdown. Several chemokines were decreased considerably in DDX3 knockdown THP-1 cells after lipopolysaccharide or poly(I:C) stimulation. Lastly, we demonstrated that DDX3 is crucial for the recruitment of phagocytes to the site of inflammation in transgenic zebrafish.

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792 Study of interaction between frameshift-stimulating mRNA pseudoknots and the ribosome by single-molecule FRET

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Minus-one programmed ribosomal frameshifting (-1 PRF) occurs when the ribosome rereads a nucleotide and consequently changes the reading frame. The mechanism is employed by many RNA viruses in order to properly express their genomes. To stimulate -1 PRF, the mRNA usually contains a slippery sequence with a pattern of X-XXY-YYZ and a downstream pseudoknot. The DU177 sequence, derived from the human telomerase RNA, is folded into a pseudoknot structure with two overlapping stem-loop structures, including three major groove base triples. Previous studies have shown that the DU177 pseudoknot could function as an efficient -1 PRF stimulator. However, details about how the ribosome unwind the downstream pseudoknot are unknown. Here, we used single-molecule Förster Resonance Energy Transfer (smFRET) to elucidate the interaction between mRNA pseudoknots and ribosomes. We found that the first stem of DU177 pseudoknot was opened when the ribosome translated to the 4th codon (the pseudoknot started at position +16). In contrast, disrupting the three base triples would lead to an early open of the structure when the ribosome reached the 3rd codon. These results were consistent with the previous studies that showed the frameshifting efficiency with the mutant pseudoknot was dramatically decreased. Hence, pseudoknots containing the structure-stabilizing base triples could greatly enhance the -1 PRF efficiency.

793 Understanding the role of a dual ribosomal system in zebrafish development

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In sexually reproducing organisms, initial embryonic processes depend on maternally provided RNAs and proteins. In particular, in the absence of zygotic transcriptionthe embryo requires a large pool of maternally inherited (maternal) ribosomes to translate stored mRNAs. A prior study in zebrafish reported that maternal ribosomes contain rRNAs that differ from somatically transcribed (somatic) rRNAs in length, sequence, and genomic location (Locati et al., 2017). These findings raise the exciting possibility that a dual ribosomal system with functional differences might operate during zebrafish embryogenesis.

To investigate the biological significance of maternal and somatic ribosomes during zebrafish development, we first performed a detailed characterization of the differences in their protein composition, structure and translational state. Analysis of mass-spectrometry data and preliminary results from CryoEM revealed interesting structural and compositional differences between maternal and somatic ribosomes. Moreover, we identified several ribosome associated protein factors that correlate with the translational activity of maternal ribosomes. Our findings firmly establish the existence of different states of ribosomes during embryogenesis that are defined by specific combinations of rRNAs, associated proteins and structural differences.

In the future, we will address the functional relevance of the differentially associated proteins and of the dual ribosomal system as a whole. These studies will also investigate how maternally-provided ribosomes become translationally active after fertilization. Overall, our study provides a detailed analysis of alternative ribosomes operating during vertebrate development.

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794 Sequential ubiquitination of ribosomal protein uS3 triggers the subunit dissociation leading to degradation of nonfunctional 40S subunit

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The ribosome is a crucial platform for precise gene expression, thus is associated with multiple quality control pathways to avoid production of potentially harmful products by abnormal translation. Ribosome itself is subjected to quality control systems; in budding yeast, 18S Nonfunctional rRNA Decay (18S NRD) eliminates nonfunctional ribosomes with deleterious mutations in the decoding center of 18S rRNA. However, the mechanisms for how aberrant ribosome is recognized and eliminated remain elusive.

By affinity purification of ribosomes with mutation in the decoding center followed by deep-sequencing of ribosome-protected mRNA, we revealed that the nonfunctional ribosomes with the defect in decoding stalled at the initiation codon. Both *in vivo* and *in vitro* data showed that the nonfunctional ribosomes were mono-ubiquitinated by E3 ligase Mag2 at ribosomal protein uS3, followed by the poly-ubiquitination dependent on E3 ligases Hel2 and Rsp5. Based on the presumption that the ubiquitinated ribosome is dissociated into subunits to avoid uneconomic degradation of the normal 60S, we monitored the subunit dissociation by observation of the accumulating mutated 40S subunit under a condition where the eventual degradation of mutated 18S rRNA was partially inhibited. We found that the subunit dissociation of nonfunctional ribosome required the ubiquitination of uS3 as well as the ATPase activity of a Ski2-like helicase Slh1/Rqt2. Together, we propose that the sequential ubiquitination of stalled ribosome induces subunit dissociation by Rqt2, leading to the degradation of the nonfunctional 40S subunits.

795 Single-molecule imaging of mRNA translation during cellular stress

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Cells are frequently exposed to stress conditions that can disrupt cellular homeostasis and result in cell death. To minimize the damage and adapt to stress, eukaryotic cells employ a highly conserved signalling pathway, the integrated stress response, which mediates a reprogramming of mRNA translation in response to diverse stress stimuli. Consequently, translation of most mRNAs is inhibited to conserve energy, while a select group of stress-related mRNAs is preferentially translated to promote recovery and restore homeostasis. This preferential translation of stress-related mRNAs depends on special sequence elements called upstream open reading frames (uORF), which are short translated regions located upstream of the main coding sequence. Translation of uORF-containing mRNAs is a complex process, which can involve translation reinitiation, leaky scanning, or elongation stalling on the uORFs. How these molecular mechanisms facilitate the reprogramming of translation during stress remains incompletely understood. Here, we apply single-molecule imaging of mRNA translation in living cells to probe the translation kinetics of key stress-related mRNAs. To get insight into the function of uORFs, we obtain single-molecule measurements of translation activity at the different ORFs present in uORF-containing mRNAs. This approach also allows us to test the role of translation bursting and mRNA localization during stress.

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796 Hypusine-modified eIF5A enhances nonsense-mediated mRNA decay

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Hypusine-modified eIF5A enhances nonsense-mediated mRNA decay

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Nonsense-mediated mRNA decay (NMD) couples protein synthesis to mRNA turnover. It eliminates defective transcripts and controls the abundance of certain normal mRNAs. The translation factor eukaryotic initiation factor 5A (eIF5A) is required for cell proliferation and viral replication. It undergoes a presumptively unique post-translational modification (hypusination) in two steps, catalyzed by sequential action of the enzymes deoxyhypusine synthase and deoxyhypusine hydroxylase. eIF5A modulates the synthesis of groups of proteins, termed the eIF5A regulon, via modulation of both mRNA levels and translation (Mémin et al., Cancer Res. 2014).

We have established a connection between eIF5A and NMD in human cells (Hoque et al., Translation 2017). Expression of NMD-susceptible constructs is increased by depletion of the major eIF5A isoform, eIF5A1. NMD is also attenuated when hypusination is inhibited by RNA interference with either of the two eIF5A modifying enzymes, or by treatment with the drugs ciclopirox or deferiprone which inhibit deoxyhypusine hydroxylase. Transcriptome analysis by RNA-Seq identified human genes whose expression is coordinately regulated by eIF5A1, its modifying enzymes, and the pivotal NMD factor, Upf1. Transcripts encoding components of the translation system are highly represented, including some encoding ribosomal proteins controlled by alternative splicing coupled to NMD (AS-NMD).

These findings extend and strengthen the association of eIF5A with NMD, previously inferred in yeast, and show that hypusination is important for this function of human eIF5A. In addition, they advance drug-mediated NMD suppression as a therapeutic opportunity for nonsense-associated diseases. We propose that regulation of mRNA stability contributes to eIF5A's role in selective gene expression.

797 Ribosome ubiquitination is required for translational control during the UPR in yeast

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Ubiquitination of stalled ribosomes is crucial for quality controls for aberrant translation. However, physiological relevance of wider ribosome ubiquitination is still largely unknown. Here we report crucial roles of ribosome ubiquitination in translational control during the unfolded protein response (UPR) in yeast. A recent study showed that unfolded protein response (UPR) stimulation induces site-specific ubiquitination of the 40S ribosomal proteins including uS10 (Rps20) and uS3 (Rps3) in mammalian cells (Higgins et al., 2015). However, it remains to be unknown whether the ribosome ubiquitination plays a crucial role in UPR.

To reveal the role of ribosome ubiquitination in UPR, we performed a genetic screen to identify the putative E3 ligase that is essential for UPR in yeast and revealed that monoubiquitination of eS7A by an E3 ligase Not4 is required for translational control during the UPR. Not4-mediated monoubiquitination of eS7A facilitates translation of the spliced form of *HAC1* (*HAC1i*) mRNA thereby induction of Hac1 targets. To elucidate how the ubiquitination of eS7A is regulated during the UPR, we determined the levels of the ubiquitinated eS7A during the ER stress. We observed that the expression of Not4 was constant after the addition of Tm. However, the levels of Hel2 were gradually decreased, indicating that UPR down-regulates the E3 ligase for polyubiquitination but not the E3 ligase for mono-ubiquitination of eS7A. Furthermore, we identified that the Ubp3-Bre5 deubiquitinating complex also down-regulated in ER stress condition. These suggest that down-regulation of both Hel2 and a deubiquitinating enzyme complex Ubp3-Bre5 is crucial for maintaining the monoubiquitinated eS7A level for proper expression of Hac1. The ubiquitinaion of eS7A is also required for the upstream ORF-dependent down-regulation of *HNT1* translation. Finally, we propose that the ubiquitination of eS7A provides a pivotal regulatory platform for translational regulation upon the UPR in yeast.

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798 Regulated hnRNP K - rpS19 interaction in erythroid cell maturation

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In erythropoiesis post-transcriptional control is essential to safeguard structural and metabolic transitions during the maturation of enucleated reticulocytes to erythrocytes.

The regulation of reticulocyte 15-lipoxygenase (r15-LOX) mRNA translation by hnRNP K, which is part of a silencing complex at the 3'UTR DICE[1-3] secures the initiation of timely mitochondria degradation by the newly synthesized enzyme r15-LOX.

To elucidate how hnRNP K interferes with 80S ribosome formation, we applied three independent interaction screens: 1] DICE RNA affinity chromatography combined with hnRNP K-immunoprecipitation from cytoplasmic extracts of K562 cells, which represent a premature erythroid state[3]. 2] Enrichment of hnRNP K interacting proteins by immunoprecipitation from extracts of K562 cells induced for erythroid maturation and non-induced cells[2]. 3] Considering the decline of regulatory hnRNP K arginine dimethylation during erythroid maturation[2,4], asymmetrically dimethylated hnRNP K and non-methylated protein[5] were employed to purify differential interacting factors from RNase A-treated K562 extracts.

The three purification strategies demonstrated a differential interaction of hnRNP K with the 40S ribosomal subunit protein rpS19. Interestingly, when K562 cells are induced for erythroid maturation, the hnRNP K-rpS19 interaction decreases *in vitro* and *in vivo*. In thermal shift assays we verified that the asymmetric dimethylation of specific arginine residues affects hnRNP K binding to rpS19. Mutations in *RPS19* have been functionally linked to impaired erythropoiesis in hereditary Diamond Blackfan Anemia (DBA)[6]. Our analysis revealed a rpS19 motif, which provides a potential binding site for methylated arginines. Interestingly, amino acid W52 frequently affected in DBA[7], appears to contribute to the interaction with hnRNP K.

To examine a functional relationship of rpS19 DBA variants in erythropoiesis and hnRNP K mediated r15-LOX mRNA silencing, we have generated K562 cell lines that enable shRNA mediated reduction of rpS19 expression.

[1]Ostareck, D, et al. (1997) *Cell;* [2]Naarmann, IS, et al. (2008) *J Biol Chem;* [3]Naarmann, IS, et al. (2010) *RNA;* [4]Ostareck-Lederer, A, et al. (2006) *J Biol Chem;* [5]Moritz, B, et al. (2014) *Biol Chem;* [6]Draptchinskaia, N, et al. (1999) *Nat Gen;* [7]Gregory, LA, et al. (2007) *NAR*

799 Unveiling the molecular mechanism of function of natural anti-cancer drugs that target the eukaryotic 80S ribosome

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The ribosome is the gigantic macromolecular machinery responsible for protein biosynthesis in all kingdoms of life. In bacteria, due to its central role in cells' growth, it is one of the main target of small molecule drugs that, through competition with its functional ligands (transfer RNAs and messenger RNA), halt the translation process. The high resolution models obtained so far, either by X-ray crystallography or cryo-EM, have shown that there is a certain degree of structural conservation between bacterial and eukaryotic ribosomes, especially in the functional core. Therefore it is not unreasonable to think that a similar inhibitory mechanism might occur in eukaryotes. In the past indeed, several natural compounds derived from diverse sources, such as marine sponges and bulbs of *Narcissus* flowers for instance, have been suggested to inhibit the translation process in eukaryotes and, by consequence, been able to arrest cancer cells' indefinite growth. However, the mechanistic details of the binding and function of such compounds have remained unclear until very recently.

The X-ray structure determination of the *Saccharomyces cerevisiae* 80S ribosome provided an outstanding model for the understanding of the mechanism of protein synthesis' inhibition in eukaryotes. The crystal structure of several inhibitors bound to the yeast 80S ribosome have recently led to unveil the molecular interactions that the drugs establish within their target. The elucidation, at atomic detail, of the binding mode of such compounds represents only the first step of a multidisciplinary approach that aims to enhance the potency and specificity of these anti-cancer drugs.

Here I will present two examples of the studies we perform on such topic. In one case I will show how molecules belonging to the same family of compounds, and that bind to the same ribosomal RNA pocket within the ribosome, may exhibit different properties with regard to the pathway they inhibit. In the second example I will provide evidence of how a multi-disciplinary approach is essential to identify new chemical functional groups that can change the binding characteristics of existing inhibitors in order to develop novel drug design strategies.

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800 Self-association regulates translation repression activity of eIF4G-binding RGG-motif protein, Scd6

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Regulation of mRNA translation plays a key role in the control of gene expression. Scd6, a conserved RGG-motif containing protein represses translation by binding to translation initiation factor eIF4G1. Here we report that Scd6 binds self in RGG-motif dependent manner and self-association regulates its repression activity. Scd6 self-interaction competes with eIF4G1 binding and methylation of Scd6 RGG-motif by Hmt1 negatively affects self-association. Results pertaining to Sbp1 indicate that self-association could be a general feature of RGG-motif containing translation repressor proteins. Taken together, our study reveals a mechanism of regulation of eIF4G-binding RGG-motif translation repressors.

801 Translation of the human ABCE1 transcript is regulated by upstream open reading frames

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Short upstream open reading frames (uORFs) are *cis*-acting elements located within the 5'-leader sequence of transcripts and are defined by an initiation codon in-frame with a termination codon located upstream or downstream of its main ORF (mORF) initiation codon. Recent genome-wide ribosome profiling (Ribo-seq) studies have confirmed the widespread presence of uORFs and have shown that many uORFs can initiate with non-AUG codons. uORFs can impact gene expression of the downstream mORF by triggering mRNA decay or by regulating translation.

Based on 5'-leader sequence ribosome occupancy profiles from Ribo-seq analysis in HCT116 cells, we studied the role of 6 non-AUG and 5 AUG uORFs present in the human ABCE1 mRNA. Using a set of reporter genes expressed in HCT116 cells and luminometry assays, we have observed that there are three AUG uORFs acting in a fail-safe manner to inhibit translation from the main AUG, being this repression immune to eIF2a phosphorylation. Functional aspects and implications of this regulatory mechanism to cell physiology will be discussed.

Acknowledgements

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802 Consequences of pathogenic *Secisbp2*missense mutations (R543Q) probed by ribosome profiling

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Secisbp2 (SECIS-binding protein 2) is a RNA binding protein which interacts with SECIS (selenocysteine insertion sequence) element, located in the 3′-untranslated region of eukaryotic selenoprotein mRNA[1]. It facilitates the incorporation of the rare amino acid selenocysteine(Sec) in response to a UGA codon. In the past decade, several SECISBP2 mutations were reported in human patients[2,3,4]. It has been hypothesized that point mutations in SECISBP2 that differentially affect selenoprotein translation can reveal the functions of individual domains within SECISBP2. Nowadays, it has been widely accepted that Secisbp2 contains three distinct domains: N-terminal domain, selenocysteine incorporation domain (SID), and RNA binding domain.

Here we established neuron and liver specific mouse models carrying pathogenic missense mutations (R543Q) in the SID domain of Secisbp2. Despite the general downregulation of selenoprotein translation, no behavioral phenotype was detected. Liver-specific mutant mouse acts as a liver-specific knockout mouse[5]. Secisbp2 was not detectable in mutant mouse liver. In-vitro luciferase assay indicated Secisbp2 was thermally unstable. But this mutation completely rescues the severe neurological phenotype of neuron-specific Secisbp2 knockout mice[6]. Therefore, its effects on selenoprotein translation were characterized in detail by ribosome profiling in the cerebral cortex. Ribosomal profiling data indicated read-through of the UGA codon in selenoprotein mRNA is inefficient in *Secisbp2*^{R543Q}mutants. Furthermore, RNA sequencing data revealed a mild inflammatory reaction in the mutant mouse cortex which was accompanied by obvious astrogliosis. Overall, our observations demonstrated this mutation can affect SECIS binding affinity and therefore selenoprotein translation efficiency. Besides, this mutation still maintains low Secisbp2 expression and partial function which is tissue-dependent. Differential protein stability in individual cell types may dictate the clinical phenotype much more than alleged molecular interactions involving a mutated amino acid.

References:

[1] Copeland, P. R. EMBO J, 2000, 306-314; [2] Schoenmakers E, Journal of Clinical Investigation, 2010, 120(12):4220; [3] Dumitrescu A M, Nature Genetics, 2005, 37(11):1247; [4] Dumitrescu A M, Antioxidants & Redox Signaling, 2009, 12(7):905-920, [5] Seeher S, Biochemical Journal, 2014, 462(1):67-75; [6] Seeher S, Antioxidants & Redox Signaling, 2014, 21(6):835-49.

803 Withdrawn

804 RNA surveillance targets in protein translation therapeutics

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Our view of the biological process of protein translation is changing. Specifically, two distinct processes of evolving views are converging: the shift from seeing protein synthesis as linear, mechanical translation of codons to the emerging view of translation as an intricate regulatory system that controls where, when, how much and which proteins are synthesized in the cell, impacting gene expression no less than regulation of transcription. Concomitantly, regulatory elements of protein synthesis are emerging as valid novel drug targets, in spite of their prevalent housekeeping roles. This convergence reveals new opportunities for drug discovery in areas as varied as viral infections, fibrosis, neurodevelopmental diseases and cancer.

We have developed a novel technology of Protein Translation Monitoring (PSM), which uses tRNA, labeled as Fluorescent Resonance Energy Transfer (FRET) pairs, to report on ribosomal activity, exploiting tRNA proximity in the A and P sites of translating ribosomes. PSM provides a readout of the number of active ribosomes in situ with unprecedented temporal and spatial resolutions; it can measure global protein synthesis, using bulk tRNA, or the synthesis of specific proteins using specific pairs of isoacceptor tRNAs, to report on the synthesis of proteins enriched with this specific pair. Significantly, PSM is naturally suited for high content screening platforms. The generality of the ribosome as the universal protein manufacturing apparatus of biology means that our screens are easily adaptable for new cell types, tRNA pairs, and indications. We have successfully concluded three high content screens using 100K diverse compound libraries.

Here we describe the utilization of RNA FISH as a downstream confirmatory assay and as a tool for target identification. mRNA FISH analysis is used to characterize a compound's activity in transcription, mRNA stability, mRNA export to the cytoplasm or at translational level. We developed a robust FISH assay using a panel of mRNA inhibitors in a dose- and time-dependent manner, imaging and big data analyses. The combination of RNA surveillance with PSM is important for the discovery of novel and specific translation inhibitors.

805 5' -phosphorothiolate dinucleotide cap analogues: reagents for messenger RNA modification and potent small-molecular inhibitors of decapping enzymes

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The 5′ cap consists of 7-methylguanosine (m7G) linked by a 5′-5′-triphosphate bridge to messenger RNA (mRNA) and acts as the master regulator of mRNA turnover and translation initiation in eukaryotes. Cap analogues that influence mRNA translation and turnover (either as small molecules or as part of an RNA transcript) are valuable tools for studying gene expression, which is often also of therapeutic relevance. Here, we synthesized a series of 15 dinucleotide cap (m7GpppG) analogues containing a 5′-phosphorothiolate (5′-PSL) moiety (i.e., an O-to-S substitution within the 5′-phosphoester) and studied their biological properties in the context of three major cap-binding proteins: translation initiation factor 4E (eIF4E) and two decapping enzymes, DcpS and Dcp2. While the 5′-PSL moiety was neutral or slightly stabilizing for cap interactions with eIF4E, it significantly influenced susceptibility to decapping. Replacing the γ-phosphoester with the 5′-PSL moiety (γ-PSL) prevented β-γ-pyrophosphate bond cleavage by DcpS and conferred strong inhibitory properties. Combining the γ-PSL moiety with α-PSL and β-phosphorothioate (PS) moiety afforded first cap-derived hDcpS inhibitor with low nanomolar potency. Susceptibility to Dcp2 and translational properties were studied after incorporation of the new analogues into mRNA transcripts by RNA polymerase. Transcripts containing the γ-PSL moiety were resistant to cleavage by Dcp2. Surprisingly, superior translational properties were observed for mRNAs containing the α-PSL moiety, which were Dcp2-susceptible. The overall protein expression measured in HeLa cells for this mRNA was comparable to mRNA capped with the translation augmenting β-PS analogue reported previously. Overall, our study highlights 5′-PSL as a synthetically accessible cap modification, which, depending on the substitution site, can either reduce susceptibility to decapping or confer superior translational properties on the mRNA. The 5′-PSL-analogues may find application as reagents for the preparation of efficiently expressed mRNA or for investigation of the role of decapping enzymes in mRNA processing or neuromuscular disorders associated with decapping.

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806 ncRNA in mammalian oocyte and early embryo development

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Fully-grown mammalian oocyte is transcriptionally silent and relies on specific mechanisms involved in mRNA stabilization and translation. Crucial role in regulation of translation belongs to various non-coding RNAs in the cell. We aimed to study the involvement of long and short non-coding RNAs in protein synthesis and consequent influence on the oocyte and early embryo physiology.We found several non-coding RNAs which exhibit specific expression and localization during mouse oocyte and early embryo development. Particularly, analysis of nuclear/cytoplasmic and monosomal/polyribosomal fractions defined possible candidates involved in the maintaining and modulating of maternal mRNAs. Overexpression of a short ncRNA followed by Dual Luciferase assay detected repression of the cap-dependent translation initiation of the construct. Furthermore, we induced clustering of the FMRP protein in the cytoplasm by overexpression of specific ncRNA. In conclusion, our results indicate significant contribution of the ncRNAs to the regulation of translation of the subset of maternal mRNAs in the mammalian oocyte and embryo.

807 Detection and quantification of protein variants with N-terminal extensions containing mitochondrial targeting signals

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Most translation initiation events in eukaryotic cells follow the scanning model, which allows for leaky scanning, a mode in which more than one translation initiation site can be utilized. This increases the number of possible protein isoforms that arise from a single transcript. Protein variants with N-terminal extensions may contain additional features, including the Mitochondrial Targeting Signal (MTS) that is usually present at the N-terminus. Here, we propose a computational model for calculating initiation probability that accounts for transcriptional heterogeneity. The model is used for detection and quantification of MTS contained within N-terminal extensions that are generated by noncanonical translation initiation using non-AUG codons. Analysis pipeline is wrapped as an 'MitoCrypt' R package. Using publicly available ribosome profiling data we show that Saccharomyces cerevisiae proteome is substantially expanded via leaky scanning derived isoforms.

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808 Noncoding RNA "SINEs" up-regulate protein translation of target protein coding mRNA

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SINEUPs are antisense noncoding RNAs, in which an embedded retrotransposon SINE sequence UP-regulate translation of overlapping target mRNAs. SINEUPs contain two functional domains. First, the binding domain (BD), which is located in the region antisense to the target, provides specific sequence overlapping the mRNA. Second, the SINE constitutes the effector domain (ED) which mediates the translation enhancement. Although SINEs are massively transcribed to RNAs in eukaryotes, most of their evolutional functions, RNA structures and interactors are unknown. To adapt SINEUPs technology to a broader number of SINEs, we took advantage of a high-throughput, semi-automated imaging system to optimize synthetic SINEUP ED. We extensively screened variant of structures and multiple SINEs from other species of ED to understand the essential features of SINEs by using SINEUP-GFP as a model. We found that multiple SINEs have function of translational enhancement activities without changing expression of target mRNAs, despite of low sequence similarities and low structure similarities. To understand the mechanisms of molecular actions in culture cells, we analyzed SINEUP binding proteins by Mass spectrometry and confirmed the interaction by RNA immunoprecipitation. Our results revealed that cellular localization of SINEUPs with complex of SINEUP binding proteins are fundamental to understand the mechanisms of translation enhancement.

809 sxRNA: Modulating mRNA Regulatory Motifs Through the Binding of Structurally Interacting, microRNA

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We have a limited understanding of how post-transcritptional gene regulation is controlled. This is especially true for multi-functional genes, in which the same mRNA molecule needs to be regulated through varied processes. This indicates that a combinatorial code must exist in mRNA and is likely located in the untranslated regions (UTRs) of the message. RNA three-way junctions (3WJs) represent a major structural/functional motif commonly found in many RNA molecules such as ribosomes and ribozymes. 3WJs traditionally form when an RNA molecule folds back on itself to produce three separate helices that meet around a central unpaired region. However, when a non-coding RNA, such as a microRNA, binds to mRNA, a 3WJ can also be formed. But in this case, the interaction results from the binding of the two RNA molecules in *trans*. We have identified many examples of non-coding RNA (including microRNA) that interact with mRNA in this manner to form 3WJs. We have termed these interactions Structurally Interacting RNA or *sxRNA* for short. Many of these sxRNA interactions appear to have the potential for creating or concealing regulatory motifs targeted by RNA-binding proteins (RBPs) and can be viewed as a novel category of post-transcriptional gene regulation. The existence of sxRNA may help explain how multi-functional genes are regulated through the combination of these modular interactions, adding a level of regulatable complexity to the post-transcriptional regulatory code.

The sxRNA concept also has the potential for creating multiple new technologies centered on modulating the activity of one RNA molecule by targeting presence or absence of a second, non-coding RNA. Using this approach, we have expressed reporter proteins in a tissue specific manner based on the presence or absence of a unique microRNA. We design sxRNA "bait-RNA" sequences in which a natural RBP-motif is altered so it only forms correctly when bound in *trans* to a targeted "trigger"-microRNA. When incorporated into a mRNA molecule, the sxRNA module acts as regulatable *trans*-acting switch that activates translation when turned ON by a targeted microRNA.

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810 New Insights into the Translational Landscape of the Heat Shock Response

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The heat shock response (HSR) is a stress signaling pathway that exists in all extant species. Though its name reflects the observed induction of heat shock proteins, a class of molecular chaperones first discovered in response to thermal stress, the HSR is triggered by a variety of pressures causing protein misfolding and as such is implicated in a plethora of diseases. It exerts potent, pleiotropic effects on gene expression, protein folding, and cellular body formation. Sustained protein synthesis is required for heat shock survival and recovery, and recent work has shown that translation efficiency modifications often predominate over transcript level changes in determining protein output during adaptation to stimuli. In this study, we employed our recently developed TMT-MATRIX (Tandem Mass Tag-Mass spectrometry analysis of Active translation factors using Ribosome density fractionation and Isoptopic labeling eXperiments) approach to generate an unbiased, comprehensive blueprint of the heat shock translational architecture and translatome in human cells. TMT-MATRIX captured impartial snapshots of the cellular translation machineries under basal and heat shock conditions. Based on translational activity, we found differential utilization of translation factors, ribosomal proteins, RNA binding proteins, and previously uncharacterized translation-regulating assets. Complementary analyses of mRNA translation efficiency (TE) by ribosome density fractionation-RNA-sequencing, and protein output by TMT-pulse-SILAC (pSILAC), revealed many unexpected translationally-induced heat shock targets. Results unveiled a detailed map of the thermo-resistant translational landscape, including unexpected enrichment of eIF4H and RPS27A, reliance on hnRNPA3 and RBM25 and production of proteins such as GEM and PSMD1 which were previously unrecognized as heat shock-inducible. These findings provide empirical support for our working model of stress-specific translation machineries that generate stress-adaptive translatomes and establish global translational remodeling as a key component of the HSR. A better understanding of this phenomenon may assist in efforts to leverage our knowledge of the HSR in therapeutics.

811 Withdrawn

812 RiboTRIBE: Using RNA editing to monitor translation

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RNA translation is generally tightly regulated to ensure proper protein expression in cells and tissues. This regulation is often assayed by state-of-the-art methods like ribosome profiling and Translating Ribosome Affinity Purification (TRAP), which can accurately assess translation in many contexts. However, these biochemical assays are not ideal with limiting amounts of biological material. This is because it can be difficult to make an extract with sufficient signal or sufficient signal:noise with limiting material. Because of our interest in translation regulation within the few adult circadian neurons in *Drosophila*, we are developing a method to assay translation under these circumstances. It is based on TRIBE (Targets of RNA-binding proteins Identified By Editing), a method recently published by our lab that can bypass traditional biochemical assays and identify RBP (RNA-binding protein) targets from tiny amounts of purified RNA. TRIBE fuses an RBP of interest to the catalytic domain of RNA editing enzyme ADAR (ADARcd), which performs Adenosine-to-Inosine editing on RBP-bound RNA targets. The fusion protein is expressed in specific cells, and the targets identified by computational analysis of RNA-seq data from those cells. To extend TRIBE to the ribosome and translational regulation, we have tested several ribosome small subunit proteins fused to the ADARcd in *Drosophila*S2 cells. Our current data indicate that RPS28-TRIBE (RiboTRIBE) edits mRNA and generates a list of actively translating mRNAs. This list overlaps well with ribosome profiling targets, especially with more highly ranked targets. There is also an enriched number of editing sites in ribosome-associated mRNA comparing to total mRNA, indicating that editing occurs preferentially on polysome-associated mRNAs. The use of cycloheximide to freeze translating ribosomes causes a substantial increase in the number of RiboTRIBE targets, which is decreased by pretreating cells with the chain terminating drug puromycin. We are using a rapalog-inducible dimerization strategy to perform RiboTRIBE assays within discrete *Drosophila*neurons. In summary, our current results indicate that RiboTRIBE identifies actively translating mRNAs and will make an important addition to the translation toolkit.

813 The 30S subunit searching mechanism in translation initiation

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Translation initiation is a key step for regulating protein synthesis. As a rate-limiting step, it controls not only translation efficiency but also fidelity. During translation initiation, many mRNAs employ a purine-rich Shine-Dalgarno (SD) sequence, which is usually located 6 nucleotides upstream of the start codon (AUG), to base pair with the anti-Shine-Dalgarno sequence (aSD) on 16S rRNA of the ribosomal 30S subunit. This SD-aSD interaction allows 30S subunits to bind to the mRNA stably and search locally for an appropriate start codon to locate in the P site of the 30S subunit. Many previous studies have showed how translation initiation factors (IF1, IF2 and IF3) and initiator tRNA participate in translation initiation. However, little is known about how the mRNA recruits 30S subunits and how 30S subunits move along the mRNA to the SD sequence and start site. Here, we observe the interaction between the 30S subunit and mRNA with or without an SD sequence and structures by using single molecule Förster Resonance Energy Transfer (smFRET) to study the process of the 30S subunit binding to the mRNA, moving to the SD sequence and locating successfully at the start site.

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814 Translation Regulation by CITI Sites

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Translation of most eukaryotic mRNA is dependent on 5'-cap structure. However, some mRNAs can initiate translation in a cap-independent fashion usingIRES. Circular RNA (circRNA) is an RNA generated through back-splicing. Recently we found circRNA can function as mRNA to produce protein from IRES through cap-independent translation. Since circRNAs do not have 5' or 3' end, naturally they present a good system to measure IRES activity. Here, we developed a new circRNA-based system for transcriptome-wide screen of endogenous IRESs. Using a circRNA reporter encoding GFP, we firstly generated a library of short fragments (100-300 nt) from normalized human cDNA before the start codon, and used this library to produce millions of stably transfected clones. Subsequently we used FACS to collect green cells, purified total RNA and conducted amplicon-seq to identify sequences that drive circRNA translation. Using this system, we identified >10,000 endogenous IRES-like sequences from 6,000 genes in human transcriptome. These sites are located throughout the mRNA (including 5'-UTR, 3'-UTR, and coding region) with sequences features distinct from canonical IRES, and thus were defined as Cap-Independent Translation Initiation sites (CITI). Computational analysis reveals that CITIs at 5' and 3'-UTR have different enriched motifs, indicating they may promote mRNA translation via different mechanisms. CITIs at 5'-UTR can function as IRESs to initiate main ORF translation, however CITIs at 3'-UTR can both promote downstream ORF translation as the IRESs or enhance main ORF translation as the translation regulators. We found those genes with the highly structured 5'-UTR prefer to contain the CITIs at 3'-UTR. These 3'-CITIs recruit special proteins to unwound the hairpin structures in 5'-UTR regions during ribosome scanning, then promote translation initiation. Taking together, our data reveals a large amount of endogenous cap-independent translation regulators in human transcriptome, suggesting a greater diversity of mechanisms for translation control. The genes with different CITIs probably play diversity roles in responding to different stress conditions. These findings may also help us to better understand the relationship between translation regulation and diseases.

815 Antisense oligonucleotides that mimic pseudoknot structures are highly efficient in stimulating -1 ribosomal frameshifting

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Programmed –1 ribosomal frameshifting (–1 PRF) is stimulated by RNA structures like pseudoknots or hairpins. Previously it was shown that antisense oligonucleotides (ONs) annealing downstream of the slippery sequence that mimicking the stem of hairpin structures are capable of inducing efficient -1 PRF. Pseudoknots generally induce higher levels of frameshifting as compared to hairpin structures partly due to the formation of triple interactions between bases in loop 2 (L2) and stem 1 (S1). Based on our knowledge of the Simian Retrovirus type 1 (SRV-1) gag-pro frameshifting pseudoknot, we here designed ONs that, after binding to mRNAs, mimic pseudoknot structures. Our data demonstrate that pseudoknot-forming ONs do induce more frameshifting than duplex-forming ONs. Depending on the length of S1, this enhancement was affected by the identity of bases in L2. This finding was corroborated by testing the corresponding in cis pseudoknots, i.e. the frameshift-inducing ability of pseudoknots with longer S1 are less affected by the identity of L2 in a length dependent manner. The greater flexibility of using small ONs to study –1 PRF allows the use of non-natural modifications. For instance it was found that 2'ACE protected ONs carrying a bulky bis(2-hydroxyethoxy)methyl orthoester group at their 2' hydroxyls are fully capable of inducing frameshifting, implying functional extensions of this type of modification in gene regulation by ONs. Our findings are discussed in relation to natural frameshiftor pseudoknots and other antisense induced frameshifting studies.

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816 Structures of the antibiotic viomycin bound to the ribosome in rotated state

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During protein synthesis, one of the key steps is coupled translocation of transfer RNA and messenger RNA through the ribosome, which is target of many clinical antibiotics.Viomycin, which is a member of tuberactinomycin class antibiotics, has been shown to inhibit protein synthesis in bacteria and is widely used to fight infections of tuberculosis. Previous biochemical experiments indicated that it can block ribosome translocation by trapping it in an intermediate hybrid state1. The molecular mechanisms by which viomycin stabilize intermediate state remain unclear. Here we present a cryo-electron microscopy (cryo-EM) reconstructions of E.coli 70S ribosome bound to P/E hybrid state transfer RNA (tRNA) at 3.8-Å. Atomic model was built from the density map after fitting and refinement. The cryo-EM density map suggested five binding sites of viomycin, four of which are novel. To address the mechanisms of viomycin on the ribosome at atomic level, we solved a crystal structure of viomycin bound to rotated state ribosome in parallel at 3.1-Å resolution. Our data suggested that viomycin block translocation by inhibit inter- and intra-subunit rotation. Together, our structures provide insight into the mechanisms of how viomycin inhibit ribosome translocation.

817 Epistatic Translational Control of mtDNA-Encoded Cytochrome C Oxidase Subunits

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Small interfering RNAs (siRNAs) have been widely used to post-transcriptionally silence gene expression in higher eukaryotic cells, but it has remained unclear whether the RNAi pathway is also active within the mitochondria and the lack of such tool prevents direct perturbation of mitochondrial DNA (mtDNA)-encoded genes. However, recent studies have documented the presence of nuclear DNA (nDNA)-encoded microRNAs (miRNAs) in the mitochondria, suggesting the ability of small RNAs to enter the mitochondria.

We now use a newly developed mitochondrial Click-in strategy to demonstrate efficient entrance of exogenous siRNAs into the matrix of mitochondria and their ability to specifically target individual mtDNA-encoded transcripts. Similar to miRNAs, these siRNAs function in an Ago2-dependent, but GW182-independent manner. Our intial study showed that the stability of mitochondrial complex IV subunits are relative unstable. And siRNA against COXI, COXII, and COXIII of mitochodnrial encoded complex IV subunit are validated functionally in RNA and protein level.

Using this approach, we investigate the direct contribution of mtDNA-encoded gene products to the coordinated assembly of respiratory chain complexes, unexpectedly revealing sequential translational control of Cytochrome c oxidase subunit I (COXI) by COXII and both COXI and COXII by COXIII. These findings demonstrate a distinct active RNAi system in the mitochondria and extend the concept of mitochondrial translational plasticity previously established with imported nDNA-encoded subunits to mtDNA-encoded components to achieve epistasis of a key respiratory chain complex.

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818 Cytoplasmic pre-tRNA maturation in human cells

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Transfer RNA (tRNA) biogenesis is a complex multi-step process, which is believed to take place almost exclusively within the nucleus in mammalian cells (reviewed in [1]) ; however, the precise molecular details of tRNA biogenesis remain to be elucidated. Here, we demonstrate that in human cells many aspects of tRNA maturation can occur in the cytoplasm. RNA fluorescent in situ hybridization (FISH) for pre-tRNAs and Northern blotting with fractionated RNAs revealed that pre-tRNAs were localized mainly in the cytoplasm regardless of whether they were intron-containing or intron-less, suggesting that 5'/3' end-extended pre-tRNAs are actively exported into the cytoplasm after being transcribed. Enzymes responsible for tRNA processing, such as TSEN complex, RTCB complex, RNase P complex, RNase Z1 and CCA-adding enzyme TRNT1 were also localized in the cytoplasm or both nuclear and cytoplasmic fractions. Inhibition of RTCB ligase complex induced the accumulation of end-extended exon fragments (5'-leader-exon and 3'-exon-trailer) in the cytoplasmic fraction, suggesting that pre-tRNA splicing precedes 5'- and 3'-end removal, in contrast to that reported in yeast cells [1]. Depletion of Exportin-t or Exportin 5, the major tRNA exporters in yeast, did not affect the cytoplasmic localization of pre-tRNAs, which suggests that pre-tRNAs are exported into the cytoplasm by a different mechanism. Pre-tRNAs are efficiently pulled down with anti-La (SS-B) antibody from the cytoplasmic fraction regardless of whether they were intron-containing or intron-less, implying that 5'/3' end-extended pre-tRNAs are in a complex with La protein. In intron-containing pre-tRNAs, both unspliced and spliced pre-tRNAs (with 5'-leader and 3'-trailer) were pulled down, which suggests that La keeps binding to pre-tRNAs during splicing through both 5'-leader and 3'-trailer. Finally, knockdown of La induced the accumulation of pre-tRNAs in the nucleus, suggesting that the binding of La assists the export of pre-tRNAs into the cytoplasm. Our data suggest that cytoplasmic pre-tRNA processing can be at least an alternative pathway for tRNA biogenesis in human cells.

Reference

[1] Chatterjee K et al. BBA - Gene Regulatory Mechanisms 2018, 1861: 373-386

819 Roles of tRNAs and tRNA-derived small RNAs in Neurological Function and Development

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While numerous neurological diseases have been associated with mutations in mitochondrial transfer RNAs (mt-tRNAs), no human diseases have to date been associated with mutations in human cytosolic tRNA genes. A pivotal study in mice showed that severe neurodegeneration could be caused in part by a tRNA gene mutation leading to faulty processing of one particular cytosolic Arg-UCU tRNA -- that gene was characterized as producing the majority of Arg-UCU transcripts in the brain, demonstrating the first highly tissue-critical tRNA gene. This finding suggests that other individual tRNA genes may have unique importance in mammalian neural function. We are using a combination of approaches including analysis of genomic context, tRNA evolution patterns, epigenetic chromatin analysis, and specialized tRNA-seq analyses on human samples to assess relative abundances of full-length tRNAs, tRNA-derived RNAs (tDRs), and changes in tRNA modification profiles. Out of the full set of 500+ human cytosolic tRNA genes, we have a list of candidates that we have selected for functional characterization using tRNA expression manipulation in order to develop a better picture of their potential role(s) in neural cells. These selected tRNAs are found primarily along chromosomes 1 and 6 spanning multiple neural regulatory gene regions. Based on their genomic locations, they may play a non-translation role in the regulation of these neural genes. By utilizing both cell lines and cerebral cortical organoids, we will also be able to study recently-evolved tRNAs that may play special roles in human brain development.

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820 A tRNA half modulates translation as stress response in *Trypanosoma brucei*

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In the absence of extensive transcription control mechanisms the pathogenic parasite *Trypanosoma brucei* crucially depends on translation regulation to orchestrate gene expression. However, molecular insight into regulating protein biosinthesis is sparse. Here we analyze the small non-coding RNA (ncRNA) interactome of ribosomes in *T. brucei* during different growth conditions and life stages. Ribosome-associated ncRNAs have recently been recognized as unprecedent regulators of ribosome functions. Our data show that the $tRNA^{Thr}$ 3'half is produced during nutrient deprivation and becomes one of the most abundant tRNA-derived RNA fragments (tdRs). $tRNA^{Thr}$ 3'halves associate with ribosomes and polysomes and stimulate translation by facilitating mRNA loading during stress recovery once starvation conditions ceased. This stimulatory effect is conserved in other organisms, such as mammals and archaea. Blocking or depleting the endogenous $tRNA^{Thr}$ halves mitigates this stimulatory effect both in vivo and in vitro. Mechanistically, $tRNA^{Thr}$ halves interact with the large ribosomal subunit, in particular with 28Sα and 28Sβ rRNA. Furthermore, $tRNA^{Thr}$ 3'halves are secreted into the media and might be involved in cell-to-cell communication between parasites or even a cross-kingdom transfer to mammalian cells.

T. brucei and its close relatives lack the well-described mammalian enzymes for tRNA half processing, thus hinting at a unique tdR biogenesis in these parasites.

821 Significance of multiple, parallel primary tRNA nuclear exporters in budding yeast

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In eukaryotic cells, newly transcribed tRNAs are escorted out of the nucleus to the cytoplasm to participate in translation by the step termed primary tRNA nuclear export. Our recent work revealed that the mRNA exporter Mex67-Mtr2 heterodimer co-functions with the canonical tRNA nuclear exporter Los1 in this nuclear export process. Nuclear tRNA export by Los1 also provides an initial tRNA quality control step to generate functional tRNA, as Los1 preferentially binds to end-processed, appropriately structured tRNA. Thus, we sought to assess the fidelity of the novel Los1-independent tRNA nuclear export pathways in yeast by three different approaches. First, we document that Los1-independent export pathways are error-prone, as enhanced levels of 5'–end unprocessed, spliced, non-functional tRNA accumulate in higher levels in cells compared to wild-type cells. Second, Mex67-Mtr2 exhibits erroneous tRNA nuclear export, as 5'-end unprocessed, spliced tRNAs are detected in elevated levels in yeast cells under conditions in which Mex67 and Mtr2 are over-expressed. Third, in cells with mex67 or mtr2 mutations, the levels of aberrant tRNA are lower than wild-type cells after 2 h incubation at the non-permissive temperature, possibly because more tRNA are channeled through the high-fidelity Los1-mediated nuclear export pathway under these conditions. The question thus arises, why do cells employ multiple, parallel, but error-prone tRNA nuclear export pathways? We learned that environmental conditions can affect tRNA fidelity, likely due to the alternate use of various tRNA nuclear export pathways. We observed that elevated levels of aberrant $m^2_2G_{26}$hypomodified tRNA are detected when yeast cells are grown above 30°C, but not at 23°C. Thus, yeast cells seem to employ the Los1-independent, error prone tRNA nuclear export pathways in varying environmental conditions, which may confer a yet unknown selective advantage to the cells in those situations.

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822 Unexpected effect on selenoprotein expression by lack of i^6A modification in $tRNA^{[Ser]Sec}$

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Selenoproteins contain the amino acid selenocysteine (Sec), which is encoded by UGA. Recoding of UGA as selenocysteine requires a 3' stem loop in selenoprotein mRNAs, several protein factors, and $tRNA^{[Ser]Sec}$(UGA). $tRNA^{[Ser]Sec}$ is isopentenylated at adenosine 37 by the enzyme Trit1. Several lines of evidence suggested that isopentenylation of A37 in $tRNA^{[Ser]Sec}$ is essential for efficient recoding of UGA in selenoproteins: 1) Lovastatin treatment reduced selenoprotein expression in cultured cells. 2) Mice expressing $tRNA^{[Ser]Sec}$ carrying A37G do not express all selenoproteins. 3) Knockdown of *TRIT1* in HepG2 cells showed decreased selenoprotein expression under low selenium conditions. In order to address the role of $tRNA^{[Ser]Sec}$isopentenylation, we have generated *Trit1*-knockout mice and analysed selenoprotein translation by western-blot and ribosome profiling. Unexpectedly, selenoprotein expression in livers from *Trit1*-knockout mice was not reduced. Accordingly, UGA recoding efficiency did not change as assessed by ribosome profiling. We also examined selenoprotein expression in patient fibroblasts carrying an inactivating *TRIT1* mutation. Again, selenoprotein translation was not affected in patient cells. We can conclude that lack of isopentelylation in $tRNA^{[Ser]Sec}$seems not to affect selenocysteine incorporation.

823 Structural and functional insights into multimeric enzymes modifying tRNAs

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The translation of mRNA templates into the corresponding proteins is a highly complex choreography performed by ribosomes, mRNAs, tRNAs and translation factors. The faithful and timely biosynthesis of proteins strongly relies in part on co- or post-transcriptional modifications of all RNA species involved in translation, with methylation being the most widespread.

Methylation events are catalyzed by a wide variety of enzymes and growing evidences support that a significant number of these methyl groups are grafted by multi-subunit enzymes in eukaryotes. The importance of these proteins in cell development is currently being unraveled and mutations in many of these enzymes are emerging as potential causes of human diseases.

The Trm112 protein, which is conserved in the three domains of life, is a unique protein activating platform for several eukaryotic but also archaeal methyltransferases (MTases) responsible for rRNA, tRNA and translation factor methylation. In eukaryotes, the Bud23-Trm112 complex participates in ribosome biogenesis by catalyzing formation of m^7G at position 1575 of 18S rRNA. The Trm11-Trm112 and Trm9-Trm112 complexes modify tRNAs and hence are important for translation elongation. Trm11-Trm112 forms m^2G at position 10 on tRNAs, a modification assumed to stabilize tRNA structure while Trm9-Trm112 participates in the mcm^5U modification at position 34 of some tRNAs and hence directly contributes to decoding. Finally, the Mtq2-Trm112 complex methylates the translation termination factor eRF1 on the glutamine side chain of its universally conserved GGQ motif, which is responsible for the release of nascent proteins. The cellular importance of these Trm112-MTase complexes is supported by the strong growth defect phenotype observed upon deletion of TRM112 gene as well as the link between mutations in Trm112-associated MTases and human diseases.

Recent structural and functional results illustrating the importance of Trm112 in the activation of its various archaeal and eukaryotic MTase partners will be presented with a special emphasis on tRNA modifying enzymes.

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824 Targeting modes of Argonaute-loaded tRNA fragments

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Transfer RNA fragments (tRFs) are a class of small RNA molecules derived from mature or precursor tRNAs. Although characterized very recently, tRFs have been gradually attracting more attention as emerging regulators. They are found across a wide range of organisms and tissues in often loaded to RISC complexes in numbers comparable to microRNAs. However, their mode of action still remains to be elucidated.

We analyzed sequences of chimeras formed *in vivo* between Argonaute-loaded tRFs and their putative targets. We found the targets to correspond to various RNA types, in addition to protein-coding transcripts. In the latter, 3' UTRs were the likely primary target regions, although we observed interactions of tRFs with coding sequences and 5' UTRs as well. We also report a novel phenomenon – a large number of putative interactions between tRFs and introns, compatible with the role of Argonaute in the nucleus.

tRFs have often been discarded as sequencing noise. We performed extensive statistical analyses to test for non-randomness of the tRF fragments and their pairing with putative targets. We analyzed different types of tRFs based on their size and location in the host tRNA and found the highest resemblance to microRNAs among tRFs derived from the 3'end of a tRNA gene including either a CCA addition or the 3' trailer sequence.

We clustered tRF binding patterns and identified enriched motifs that may be responsible for tRF-target interactions. Such interaction sites appear to be primarily located on the 5' end of a tRF, often involving additional binding of the 3' nucleotides of guide tRFs, similar to microRNAs. We combined these motifs with other independent lines of functional evidence, including distributions of guide-target binding energy and frequency of conserved matches of k-mers from tRF sequence to different gene regions. Integrating these datasets, we predicted likely target binding modes for 12 tRFs.

Strikingly, our predictions of target binding regions matched every interaction site detected in a recent experimental screen[1]. This confirms the validity of our approach and opens the possibility to predict the sites and mechanisms of tRF/target interactions computationally.

1. Kuscu et al. (2018) RNA 24, 1093.

825 Intron removal from tRNA$^{Leu}_{CAA}$ genes in *S. cerevisiae*

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A part of tRNA genes in a very wide range of living organisms contain an intron inserted at the so-called canonical position, one nucleotide next to the anticodon. In yeast, total 61 tRNA genes encoding ten different tRNA species (defined by their anticodon sequence) generate intron-containing pre-tRNAs with different intron lengths, and the pre-tRNAs are spliced by the Sen complex at the mitochondrial outer membrane and cytoplasmic tRNA ligase. We constructed a complete set of *Saccharomyces cerevisiae* strains in which the tRNA introns were removed from all the synonymous genes encoding ten different tRNA species. Next, we aim to uncover the biological functions of intron-containing tRNAs by using our tRNA intronless yeast collection. Among them, the tRNA$^{Leu}_{CAA}$ intronless mutant initially displayed several mitochondrial and ribosome-related phenotypes including losing mitochondrial DNA and 5.8S rRNA reduction. To understand molecular mechanisms behind these phenotypes, we conducted ribosome profiling and RNA-seq of this mutant. Here, we report that mRNA abundance of mitochondrial targeted genes and iron-metabolism-related genes was mainly reduced, and translational efficiency of cytosolic and mitochondrial ribosomal proteins was down-regulated. These results suggest that the intron-containing tRNA$^{Leu}_{CAA}$ genes may be required for proper ribosome biogenesis and normal mitochondrial functionality.

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826 tRNA introns: turnover mechanisms and possible functions

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A subset of genes encoding tRNAs contain introns. In most organisms, tRNA introns are located 1 nt 3' to the anticodon and are removed from pre-tRNAs by the tRNA splicing endonuclease. Pre-tRNA splicing is essential in most organisms because, for at least one tRNA family, all reiterated tRNA genes contain an intron and thus, the genome cannot be decoded without tRNA splicing. It is not understood why tRNA intron possession has been conserved from archaea to vertebrates because unspliced tRNAs are not functional. One possibility is that the released introns serve biological roles under particular conditions. We study the fate of tRNA introns released upon pre-tRNA splicing. Through an unbiased screen of the budding yeast genome, we identified 2 proteins required for tRNA intron turnover of tRNA$^{Ile}_{UAU}$(Wu and Hopper 2014). We showed that the free linear tRNA$^{Ile}_{UAU}$intron is first 5' phosphorylated by the kinase activity of the tRNA ligase, Rlg1/Trl1, then the intron is degraded by the 5' to 3' exonuclease, Xrn1. Budding yeast possess 10 families of intron-containing tRNA genes. Studies of the remaining 9 families demonstrated that the intron from 1 other family, tRNA$^{Leu}_{CCA}$, is degraded similarly to tRNA$^{Ile}_{UAU}$, but 3 other families are degraded by a Rlg1/Trl1-dependent, Xrn1-independent mechanism; 2 families are degraded in a Rlg1/Trl1-independent, Xrn1-dependent mechanism, and turnover of the remaining 3 families of introns is independent of both Rlg1/Trl1 and Xrn1. Further, 1 of the 10 tRNA intron families forms circles and requires and endonuclease for degradation. Thus, surprisingly, there are at least 5 separate pathways for tRNA intron turnover in budding yeast. Although tRNA turnover of all yeast tRNA introns is very efficient under normal laboratory conditions, various stress conditions cause tRNA-specific, stress-specific accumulation of undegraded tRNA introns. Further, some of the introns possess long stretches of complementarity to particular mRNAs, raising the possibility, which we are investigating, that particular tRNA introns may function as novel noncoding RNAs for regulation in response to particular stresses and, hence, released introns may serve biological roles under some conditions.

827 Diverse mechanisms of translation inhibition by tRNA-derived stress-induced RNAs

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The survival of mammalian cells exposed to adverse environmental conditions requires reprogramming of protein translation. While stress-activated kinases target different components of translation machinery to inhibit general translation, recent data suggest that tRNA and tRNA-derived fragments also play active roles in the regulation of protein synthesis under stress. The ribonuclease angiogenin (ANG)-mediated tRNA cleavage promotes a cascade of cellular events that starts with production of tRNA-derived stress-induced RNAs (tiRNAs) and culminates with enhanced cell survival. This stress response pathway partially relies on a subset of tiRNAs that inhibit cap-dependent translation initiation and induce assembly of stress granules (SGs), RNA granules with pro-survival and cytoprotective properties. In addition to these SG-promoting tiRNAs, we have identified other subsets of tiRNAs that inhibit translation through other non-overlapping mechanisms. Here, I will overview multiple modes of translation modulation by different subsets of tiRNAs, discuss their structural properties and describe their interactions with binding partners.

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828 A perspective on noncanonical tRNA gene evolution: tRNAs for translation and tRNAs for other functions

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A number of tRNAs fold into the canonical secondary structure, known as the cloverleaf. Meanwhile, there have been accumulating reports of non-canonical tRNAs including tRNA-like sequences. Recently, we have conducted a sequence conservation analysis of the canonical tRNAs in 83 bacterial, 182 archaeal, and 150 eukaryotic species (1). During the analysis, we noticed that non-canonical tRNAs were extremely increased in eukaryotic species. In prokaryotes, more than 90% of tRNAs were canonical tRNAs, and there were only 1.8% and 7.0% of non-canonical tRNAs in bacteria and archaea, respectively. While, in eukaryotes, on average, 58.7% of tRNAs were classified as non-canonical tRNAs. Interestingly, the ratio was depended on the corresponding amino acid types of tRNA. For examples, the ratios of non-canonical tRNAs for $tRNA^{Gly}$, $tRNA^{Ala}$, and $tRNA^{Leu}$ were 77.2%, 73.3%, and 67.0%, respectively. While, the ratios of non-canonical tRNAs for $tRNA^{Asp}$, $tRNA^{His}$, and $tRNA^{Asn}$ were 23.2%, 28.0%, and 32.9%, respectively. Since the former tRNA group have four or six synonymous codons and the latter tRNA group have only two types of synonymous codons in the standard genetic codon table, we speculated that tRNAs corresponding to the degenerate or redundant genetic code may produce more non-canonical tRNAs during eukaryotic evolution.

Previously, we have characterized noncanonical V-arm-containing tRNAs (nov-tRNAs) in nematode, *Caenorhabditis elegans* (2). Interestingly, *in vitro* aminoacylation assays showed that nov-$tRNA^{Gly}$ and nov-$tRNA^{Ile}$ are only charged with leucine, which is inconsistent with their anticodons. However, a whole-cell proteome analysis found no detectable level of nov-tRNA-induced mistranslation in *C. elegans in vivo* (3). Meanwhile, we further analyzed 69 eukaryotic genome sequences and identified 253 nov-tRNAs as well as 2,415 nov-tRNA-like sequences in organisms ranging from nematodes to fungi, plants, and some vertebrates (4). The results suggested that at least some non-canonical tRNAs are evolved in a species-specific manner, although the real function of the tRNAs remain unknown.

References:

1. Tamaki, S. *et. al.* (2018) *Frontiers in Genetics* 8: 227; 2. Hamashima, K. *et. al.* (2012) *Nucleic Acids Research* 40(8): 3653; 3. Hamashima, K. *et. al.* (2015) *PLOS ONE* 10(1): e0116981; 4. Hamashima, K. *et. al.* (2016) *Molecular Biology and Evolution* 33(2): 530.

829 Kti12, a PSTK-like tRNA dependent ATPase essential for tRNA modification by Elongator

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Many RNA molecules are post-transcriptionally modified. On average, one tRNA molecule carries approximately 13 modifications that serve different purposes and are crucial for their functionality. Modifications of anticodon bases affect codon-anticodon interactions and are of particular importance for ribosomal decoding, co-translational folding dynamics and proteome homeostasis. The Elongator complex is highly conserved among eukaryotes and it conducts the 5 carboxymethyluridine (cm^5U_{34}) modification at the wobble position of 11 tRNA species. Disturbance of its activity result in a number of severe human diseases, including familial dysautonomia, intellectual disability, rolandic epilepsy and cancer. Despite recent insights into the architecture of Elongator complex, the structure and function of its regulatory factor Kti12 have remained elusive.

Here, we present the crystal structure of Kti12's nucleotide hydrolase domain trapped in a transition state of ATP hydrolysis. The structure reveals striking similarities to an O-phosphoseryl-$tRNA^{Sec}$ kinase involved in the selenocysteine pathway. Both proteins bind tRNA using a flexible C terminal domain and require $tRNA^{Sec}$ for ATPase activity. Additionally, we mapped the interaction site of Kti12 on the Elongator complex in vitro using purified proteins and revealed the influence of Elongator on the ATPase activity of Kti12. We combine mass spectrometry and in vivo yeast genetics to prove that ATP hydrolysis by Kti12 is crucial for maintaining proper cm^5U_{34}-based tRNA modification levels.

In summary, our data reveal a previously uncharacterized link between two translational control pathways that regulate selenocysteine incorporation and affect ribosomal tRNA selection via specific tRNA modifications in the anticodon region.

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830 Physiological role of queuosine tRNA modification in parasitic protist *Trypanosoma brucei*

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Queuosine (Q) is one of the most complex tRNA modifications, which is found at the first position of the anticodon of several tRNAs across bacteria and eukaryotes. Because of its presence at the wobble position, it is suggested to influence decoding of its cognate and near-cognate codons, which in turn affects the rate and fidelity of translation.

In this work, we used protozoan parasite *Trypanosoma brucei* as a model for comprehensive analysis of tRNA guanine transglycosylase (TGT), the enzyme responsible for the formation of Q modification. Unlike its bacterial counterpart, in most eukaryotes including *T. brucei*, TGT predominantly functions as a heterodimer. Using methods of molecular biology and biochemistry, we showed that both subunits are necessary for Q-tRNA formation *in vivo*. Interestingly, unlike in higher eukaryotes, TbTGT heteromer is localized to the nucleus. However, splicing of the only intron containing $tRNA^{Tyr}$ occurs in the cytosol of *T. brucei*. Hence, the spliced unmodified tRNA must undergo retrograde import to the nucleus in order to obtain Q, prior to the secondary nuclear export; highlighting the dynamic interplay between tRNA trafficking and modifications.

During the life cycle, *T. brucei* undergoes extensive metabolic and architectural remodeling, which demands rapid translational adaptation to enable survival under dynamically changing environment. In this context, we study how Q modification differentially affects global translation and consequently, the physiology of this parasite, in various life cycle stages. To further characterize physiological role of Q, we generated a knock-out of TbTGT2 and performed phenotypic *in vivo* analysis, to simulate natural parasite infection in the mammalian host. Upon infection in mice, the mutant parasites showed significantly delayed parasitemia, and prolonged survival of mice, as compared to the mice infected with WT parasites. Our data suggests that in absence of Q-tRNAs, these parasites are not able to express full spectrum of proteome required to establish a successful infection.

We hypothesize that variability in codon usage of Q codons, among individual life stages in this parasite might be correlated to the varying degree of Q modification, manifested in changes in the global proteome in a developmental stage-specific manner.

831 Exploration of Tissue-Specific Expression and Modification Patterns for tRNAs and tRNA-derived Small RNAs

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Transfer RNAs (tRNAs) are the largest, most complex non-coding RNA family universal to all living organisms. These RNAs are often disregarded as passive players in translation; however, recently it has been shown that they are involved in regulatory pathways as well. Evidence suggests that tRNAs undergo tissue-specific expression, processing, and modification, resulting in a wide variety of tRNAs and tDRs (tRNA-derived small RNAs) with the potential to regulate a myriad of expression pathways in response to cellular, and extra-cellular, environments. Furthermore, dysregulation of expression of both tRNA and tDRs have been shown in a variety of diseases. Unfortunately, eukaryotic tRNA/tDR expression and modification data is very incomplete due to difficulty in measuring both with standard high-throughput sequencing methods. As a result, we have embarked on a large NIH-funded project to use two complementary tRNA sequencing methods, ARM-seq (for tDRs) and DM-tRNA-seq (for full length tRNAs) to generate tissue-specific profiles correlating tRNA and tDR expression with modification. Both of these methods use the AlkB demethylase to remove impeding modifications, in combination with use of a specialized reverse transcriptase for DM-tRNA-seq, to generate more accurate measurements of the modification and expression levels of each transcript. To elucidate these tissue-specific tRNA/tDR profiles, these methods were applied in triplicate for brain, kidney, pancreas, and thymus *Mus musculus* samples. The computational analytic pipeline developed in the lab, tRAX (tRNA Analysis of eXpression), was used to generate normalized expression and modification values for all tRNA families, as well as multiple visual representations of these data that enable careful assessment of transcript source ambiguity. Analyzing these data has revealed a stark differential expression and modification rate of tRNA and tDRs across tissue types. For example, the pancreas samples yielded a greater variety of tDRs than the other tissues, including an Arg-TCT 5' tDR which was completely absent in both the kidney and thymus. By creating a "normal" tissue-specific expression profile across all tRNA transcripts, we can then explore deviations from these profiles in various disease states, as well as identifying potential biomarkers for abnormal cell function.

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832 Uncovering how conserved tRNA isopentenyltransferases generate variable subsets of i6A37-modified tRNAs; and a system for mitochondrial targeting and screening of point mutations in the human population for potential pathogenicity.

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tRNA isopentenyltransferases (IPTase) are conserved enzymes that add an isopentenyl group to N^6 of adenosine-37 (i6A37) in the anticodon loops of different tRNA subsets in bacteria, eukaryotic cytosol and mitochondria. Although the tRNA substrates contain the A36-A37-A38 recognition sequence, this doesn't ensure modification. Identities of i6A37 tRNAs differ among species due in part to variance of the 36-38 sequence and in part to anticodon recognition properties of the IPTases. Our new data together with prior reports suggest that IPTases and their selected tRNA substrates evolved as a translation modification code system linked to metabolism. The human and yeast IPTases are attributed with tumor suppressor and gene-silencing activities respectively, and life expectancy of *C. elegans* is specifically impacted by the mitochondrial isoform of its IPTase. Pathogenic mutations to human tRNA-IPTase (TRIT1) that decrease i6A37 on tRNAs cause mitochondrial insufficiency with resulting neurodevelopmental disease. Eukaryotes produce IPTases from single genes although their mitochondrial targeting occurs by different mechanisms. *S. cerevisiae* and *C. elegans* use alternative translation starts and a mitochondrial targeting sequence (MTS) to control IPTase distribution. Our analyses here indicate that human TRIT1 has a single translation start and predict an N-terminal MTS that we validate by GFP fusion constructs in human cells. We also examined TRIT1 for modification activity using tRNA-mediated suppression and i6A37-sensitive quantitative northern blotting in *S. pombe* in which it modifies cytoplasmic-tRNA somewhat more efficiently than mitochondrial-tRNA. Nonetheless, the MTS mutations that decrease mitochondrial targeting in human cells also decrease mito-tRNA modification in *S. pombe*. We further used this system to examine a panel of key TRIT1 mutations including ones that represent pathogenic alleles (in homozygotes) in the human population and others that have not yet been associated with disease. Finally, we show severe deficiency of TRIT1 activity specific for cy-tRNA^{Trp}CCA despite A36-A37-A38 and high activity for cy-tRNA^{Ser}AGA, and comparable activity of *S. pombe* IPTase for cy-tRNA^{Trp}CCA and cy-tRNA^{Ser}AGA. This is consistent with poor recognition of the unique anticodon loop configuration of cy-tRNA^{Trp}CCA as described for *S. cerevisiae* Mod5, but more severe. Thus, TRIT1 is another IPTase that developed substrate restriction despite A36-A37-A38 in the tRNA.

833 Overexpression of tRNA^{Gly} affects the solubility of a cyclin, altering growth of *Schizosaccharomyces pombe*

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Under stress, gene expression can be affected by changes in mRNA and protein levels. Under oxidative stress certain yeast proteins, such as Cdc13, are specifically regulated at the translational level. Cdc13 is a cyclin essential for the G2/Mitosis cell cycle transition. Under oxidative stress, the level of this protein increases without changing mRNA level. Changes in tRNA levels might explain the effect on translation of Cdc13 mRNA. The purpose of this work was to determine whether increasing tRNA levels that decode rare codons in cdc13 mRNA, affect the expression and folding of the Cdc13 and/or cell growth in *Schizosaccharomyces pombe.*

For this purpose we use, among others, the following techniques: Northern blot to asses tRNA levels of H2O2-induced oxidative stress cells and overexpressed tRNA genes. Microscopy for assessment of cell phenotype. PAGE to asses protein aggregation.

H2O2-induced oxidative stress resulted in an increased level of $tRNA^{Gly}_{UCC}$ and $tRNA^{Arg}_{UCU}$ while $tRNA^{Thr}_{UGU}$ did not change. Because $tRNA^{Gly}_{UCC}$ has a low gene dosage (decoding a rare codon), we studied the effect of the overexpression on this tRNA in the expression and solubility of Cdc13. Overexpression of the $tRNA^{Gly}_{UCC}$ gene increased aggregation of Cdc13 but did not affect the mRNA and protein levels. Additionally, overexpression of this tRNA resulted in elongated cells. However, overexpression of the high gene dosage isoacceptor $tRNA^{Gly}_{GCC}$ and another high gene dosage $tRNA^{Val}_{AAC}$, both decoding optimal codons, did not generate the elongated cell phenotype. In conclusion, these results revealed that oveerexpression of the $tRNA^{Gly}_{UCC}$ affected the solubility of Cdc13 and eventually the cell cycle.

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834 Intricate subcellular trafficking of queuosine modified tRNAs in *Trypanosoma brucei*

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Proteins and RNAs are routed across the nuclear envelope via the nuclear pore complex and require transport receptors belonging to the karyopherin family (exportins). Only a limited set of export factors, conserved in other organisms, is identifiable in trypanosomes by bioinformatics. Thus our knowledge of tRNA nuclear export in these organisms remains limited. We show here that, like in other eukaryotes, down regulation of the tRNA exporter Xpo-t is not essential in trypanosomes and resulted in neither disruption of mature tRNA export to the cytoplasm nor intron-containing tRNA accumulation in the nucleus; phenotypes commonly observed with analogous yeast mutants. Also similar to yeast, Mex67-Mtr2, the main mRNA transporter in other systems, has a role in tRNA export in *T. brucei* with one major distinction, in *T. brucei* there is a clear separation of functions between Mex67 and Mtr2. The latter still serves a general role as a tRNA and/or mRNA export factor, but down-regulation of its partner, Mex67, leads to the specific accumulation of queuosine (Q)-containing tRNAs in the nucleus. Interestingly, compared to cytosol, tRNAs fully modified with Q, are preferentially imported into the mitochondria. The absence of Q in mitochondria-imported tRNAs also leads to a decrease in de novo synthesized mitochondrial proteins implying the role of Q in mitochondrial translation and physiology. Taken together, our data highlights the significance of intracellular trafficking in determining the fate and function of tRNAs via differential modification.

835 Investigating the role of tRNA-modifying enzymes in human proteostasis

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Post-transcriptional modifications of transfer RNA (tRNA) molecules are essential to ensure its maturation process and consequent proper folding, stability and decoding. Absence of these modifications can impact multiple aspects of protein translation that are crucial for maintaining cellular proteostasis. An increasing number of tRNA modifications and/or tRNA modifying enzymes have been found deregulated in several conformational diseases, namely, neurological and metabolic disorders. However, the exact mechanisms involved in tRNA modification deregulation and proteostasis imbalance remain unclear. We hypothesize that deregulation of tRNA modifying enzymes affect translation efficiency through tRNA hypomodification, increased protein aggregation and deregulation of the integrated stress response. To test the hypothesis, we implemented high content screenings (HCS) in a stable human cell line expressing a fluorescent protein aggregation sensor (HSP27-GFP), previously developed by our team. We show that knockdown of a subset of tRNA modifying enzymes that catalyze wobble uridine modifications led to statistically significant accumulation of fluorescent foci in cells, and activation of protein quality control mechanisms. This work offers new insights about which tRNA modifying enzymes affect proteostasis, and what are the possible underlying molecular mechanisms. These results are promising as they unravel potentially novel molecular targets for protein conformational diseases.

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836 A stable tRNA-like molecule is generated from the long noncoding RNA *GUT15* in *Arabidopsis*

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The Arabidopsis *GUT15* RNA belongs to a class of noncoding RNAs that are expressed from the intergenic regions of protein-coding genes. We show that the RNA polymerase II transcribed *GUT15* transcript serves as a precursor for two stable RNA species, a tRNA-like molecule (identified in tRNA-seq data) and *GUT15*-tRF-F5, which are both encoded by the final intron in the *GUT15* gene. The *GUT15*-encoded tRNA-like molecule cannot be autonomously transcribed by RNA polymerase III. However, this molecule contains a CCA motif, suggesting that it may enter the tRNA maturation pathway. The *GUT15*-encoded tRNA-like sequence has an inhibiting effect on the splicing of its host intron. Moreover, we demonstrate that the canonical tRNA genes nested within introns do not affect the splicing patterns of their host protein-coding transcripts.

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837 Codon-specific translation reprogramming promotes resistance to targeted therapy

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Reprogramming of mRNA translation plays key roles in cancer development and drug resistance. Molecular mechanisms involved in this process remain, however, poorly understood. Wobble tRNA modifications are required for specific codon decoding during translation. Here, we found that enzymes catalyzing wobble uridine 34 (U_{34}) tRNA modification (U_{34}-enzymes) are key players of the protein synthesis rewiring during $BRAF^{V600E}$ oncogene-induced transformation and resistance to targeted therapy in melanoma. We show that $BRAF^{V600E}$ melanoma cells are addicted to U_{34}-enzymes and that concurrent inhibition of MAPK signaling and ELP3 or CTU1/2 synergizes in the killing of melanoma cells. Activation of the PI3K-signaling pathway, one of the most common mechanisms of acquired resistance to MAPK-therapeutics, dramatically increases expression of U_{34}-enzymes. Mechanistically, U_{34}-enzymes promote melanoma cells glycolysis through a direct, codon-dependent, regulation of HIF1a mRNA translation and the maintenance of high HIF1a protein levels. Therefore, acquired resistance to anti-BRAF therapy is associated with high levels of U_{34}- enzymes and HIF1a. Together, these results demonstrate that U_{34}-enzymes promote melanoma cell survival and therapy resistance by regulating specific mRNA translation.

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838 Organization of transfer RNA genes in plant genomes

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Transfer RNA (tRNA) is a molecule that plays a key role in protein biosynthesis. Beyond the central position in the translational machinery, tRNA is also associated with other cellular functions. Although tRNA genes have been widely studied in terms of structure and function, less is know about their evolutionary dynamics. tRNA genes have a tendency to be present in multiple copies in the genome. They can be spread throughout genomic DNA and arranged as individual units or within closely spaced clusters. In this study, we analyzed the distribution and organization of tRNA genes on genome-wide scale. We used genomic data from 43 model plants. The complete sets of tRNA genes in all analyzed genomes were prepared based on predictions performed by tRNAscan-SE 2.0. Our results show that larger genomes tend to feature greater number of tRNA genes. In our dataset the number of annotated tRNA genes ranges between 403 (*Dioscorea rotundata*) and 12 945 (*Triticum aestivum*). The tRNA genes were considered as clustered if presented a tRNA gene density equal or higher than 2 tRNA/kb. On average, only 10% of all tRNA genes are present as parts of tRNA clusters. The tRNA clusters sizes show significant variability between different genomes. The largest cluster identified in *Arabidopsis thaliana* contains 81 tRNA genes. Interestingly, the analysis of clusters composition indicates that the tendency to the clustered arrangement of genes is tRNA-dependent. We have performed comprehensive analysis of tRNA genes organization in plant genomes, that provides basis for further studies.

839 Modulation of translation efficiency: a new player in dendritic cell function and T cell priming

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Dendritic cells (DC) are important regulators of T lymphocyte function and they play crucial roles in the establishment of central tolerance/immunity. They are professional antigen presenting cells with the capacity to initiate immune responses though the presentation of antigens loaded into MHC-I and MHC-II molecules to the naïve T lymphocytes. For this DCs process intracellular or captured antigens for T cell priming and can mature in response to various stimuli, to produce pro or anti-inflammatory cytokines that modulate the outcome of the immune response. DCs change their gene expression pattern rapidly after activation by microbes and are key immune regulators. The control of protein synthesis during DC activation is a major determinant of proper function; tRNAs are fundamental for this process as they translate mRNA templates into corresponding proteins. Here, we show that in response to TLR stimulation by lipopolysaccharide (LPS) and polyriboinosinic:polyribocytidylic acid (poly(I:C)), RNA polymerase III (Pol lII)-dependent transcription and consequently tRNAs expression is strongly induced in DCs. This enhancement is caused by the phosphorylation and nuclear export of the Poll III repressor Maf1. The resulting enhanced tRNA expression is necessary to augment globally protein synthesis and favors translation of DC-specific mRNA. Indeed, protein synthesis regulation and tRNA abundance regulation are vital for DC activation, cytokine production and T cell priming. Therefore, we believe regulation of protein synthesis quality can affect DC fitness and immune function.

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840 A large-scale molecular evolutionary analysis uncovers a variety of polynucleotide kinase Clp1 family proteins in the three domains of life

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In many eukaryotes and archaea, transfer RNA (tRNA) genes often possess intron in the anticodon loop region, and therefore exact pre-tRNA splicing is required to produce mature and functional tRNAs. It has been reported that polynucleotide kinase (Clp1) phosphorylates the 5'-end of the tRNA 3'-exon half during tRNA splicing. It is also known that enzymes similar to the amino acid (aa) sequence of Clp1, Nol9 and Grc3 are present in some eukaryotes, and involved in pre-rRNA processing. However, the knowledge of how these Clp1 family proteins evolved and become diversified is still limited. Here, we conducted a large-scale molecular evolution analysis of Clp1 family proteins in all living organisms for which protein sequences are available in public databases. In particular, phylogenetic distribution and frequency of Clp1 family proteins were investigated on the complete genomes of bacteria and archaea. As results, a total of 3,557 Clp1 family proteins were detected in the three domains of life, bacteria, archaea, and eukaryotes: many of these proteins were from archaea and eukaryotes but the limited numbers of proteins were from restricted and phylogenetically diverse bacterial species. Domain structures of Clp1 family proteins were also different among three domains of life. Although the aa length of Clp1 family proteins ranged from 128 to 2,700 with a average of 552, large proteins with >1,000 aa were also found in 122 eukaryotes. These were novel proteins possessing the conserved Clp1 polynucleotide kinase domain as well as other varieties of functional domains for each species, and more than 80% of them were derived from either fungi or protostomia. Finally, the polyribonucleotide kinase activity of a bacterial Clp1 protein from *Thermus scotoductus* (*Ts*-Clp1) was experimentally characterized. *Ts*-Clp1 preferentially phosphorylated single-stranded RNA oligonucleotide (Km 6 nM), but single-stranded DNA oligonucleotide was also targeted for phosphorylation in the presence of higher concentration of the enzyme (Km 130 nM). Interestingly, *Ts*-Clp1 showed extremely high thermal stability up to 90 °C. Based on these findings, we proposed a comprehensive view for understanding the diversification of Clp1 family proteins and the molecular evolution of their functional domains.

841 An archaeal *in vitro* reconstitution system for pre-tRNA splicing and characterization of a putative RNA-regulating protein PF1614

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In Archaea and Eukaryotes, tRNA genes are often interrupted by intron(s), and the primary transcripts or pre-tRNAs must be processed to yield mature functional tRNAs. Here, we developed archaeal *in vitro* reconstitution system for pre-tRNA splicing using three recombinant proteins expressed from genes of a hyperthermophiles archaeon, *Pyrococcus furiosus*. The proteins are a tRNA splicing endonuclease, *Pf*-EndA (PF0266), a tRNA ligase, *Pf*-RtcB (PF1615) and a tRNA ligase-activating protein, *Pf*-Archaease (PF1552). Consequently, using an intron-containing pre-tRNA^{Met}(CAU) as a substrate, the reaction system containing the three enzymes in one tube showed complete intron removal from the substrate and self-circulation of the intron at 70 °C. However, the ligation efficiency of the fragmented exons was significantly low. The result suggested that at least another factor that acts as an RNA chaperone is required for the efficient ligation of tRNA exons under the high temperature. Since *Pf*-RtcB has been reported to interact with functionally unknown protein PF1614 by a *Pyrococcus* proteome analysis, thus we predicted it as a potential candidate factor.

To characterize PF1614 protein bioinformatically, we first performed BLASTP sequence similarity search against a reference sequence database containing the complete genome of 381 archaeal species and 17,163 bacterial species. It showed that PF1614 is not conserved in Bacteria but only in certain families in Archaea, Thermococcaceae and Methanococcaceae, which are both thermophilic. In addition, it was shown that PF1614 contain a domain found in MCM family protein, winged-helix DNA binding domain at the C-terminus. Since MCM family proteins involved in many aspects of genomic stability, we speculated that PF1614 stabilize the cleaved tRNA exon complex under the high temperature, resulting in an increase of ligation efficiency. We are now purifying recombinant PF1614, we would like to discuss the result along with the effect on *in vitro* experiment of pre-tRNA splicing reconstitution system.

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842 Dual pathways of tRNA hydroxylation ensure efficient translation by expanding decoding capability

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tRNA anticodons are heavily decorated with post-transcriptional modifications to ensure accurate and efficient decoding. 5-carboxymethoxyuridine (cmo^{5}U) and its derivatives frequently occur at the wobble position of bacterial tRNAs. These modifications allow non-Watson–Crick base pairing with guanosine and pyrimidines at the third letter of codons, thereby expanding decoding capabilities. However, the biogenesis of cmo^{5}U initiated by 5-hydroxyuridine (ho^{5}U) formation had not been elucidated, and physiological roles of these modifications remained elusive. Using reverse genetics and comparative genomics, we identified two pathways those redundantly perform ho^{5}U formation in *Escherichia coli* and other bacteria. TrhP, a peptidase U32 family protein, is involved in prephenate-dependent ho^{5}U formation. TrhO, a rhodanese family protein, catalyzes oxygen-dependent ho^{5}U formation and bypasses cmo^{5}U biogenesis in a subset of tRNAs under aerobic conditions. An E. coli strain lacking both *trhP* and *trhO* completely lost the wobble modification, showed a temperature-sensitive phenotype and decoded G-ending codons (GCG and UCG) less efficiently than a wild-type strain. Collectively, we discovered dual orthogonal tRNA hydroxylation pathways and described a role of tRNA hydroxylation in efficient decoding during translation.

843 Production, Isolation, and Use of Endogenously Modified tRNA-Derived Small RNAs from Mammalian Cells.

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Transfer RNAs (tRNAs) are cleaved during stress conditions through the activity of endonucleases, resulting in the production of distinct tRNA-derived small RNAs (tsRNAs). Stress-induced tsRNAs with isoacceptor and even isodecoder-specificity have been implicated in a wide range of biological processes, but the mechanistic details as to how these small RNAs act at the molecular level are still poorly understood. Importantly, since stress-induced tRNA cleavage affects only a few percent of all tRNAs, the actual number of potentially biologically active tsRNAs is probably very low. This has restricted addressing the biological function of tsRNAs to either bulk use of all cellular small RNAs or to experimentation with synthetic tsRNA sequences. Importantly, since stress-induced tsRNAs are derived from mature tRNAs, they likely contain post-transcriptional modifications that were already present in parental tRNAs. Therefore, when aiming to biochemically investigate tsRNA function, it is desirable to obtain tsRNAs carrying modifications from endogenous sources rather than using synthetic sequence mimics. To better define the biological activities of individual tsRNAs, we have developed various approaches for the production and preparative isolation of specific tsRNA species using chromatographic methods. By using 5' tsRNAs from tRNA-GlyGCC and tRNA-GluGUC, we show that purified tsRNAs are amenable for biochemical as well as cell biological studies.

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844 Oxidative Stress Triggers Selective tRNA Retrograde Transport in Human Cells during the Integrated Stress Response

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In eukaryotes, tRNAs are transcribed in the nucleus and exported to the cytosol, where they deliver amino acids to ribosomes for protein translation. This nuclear-cytoplasmic movement was believed to be unidirectional. However, active shuttling of tRNAs, named tRNA retrograde transport, between the cytosol and nucleus has been discovered. This pathway is conserved in eukaryotes, suggesting a fundamental function; however, little is known about its role in human cells. Here we report that, in human cells, oxidative stress triggers tRNA retrograde transport, which is rapid, reversible, and selective for certain tRNA species, mostly with shorter 3′ ends. Retrograde transport of tRNASeC, which promotes translation of selenoproteins required to maintain homeostatic redox levels in cells, is highly efficient. tRNA retrograde transport is regulated by the integrated stress response pathway via the PERK-REDD1-mTOR axis. Thus, we propose that tRNA retrograde transport is part of the cellular response to oxidative stress.

845 Species-specific T-box:tRNA-mediated synchronization of cell wall and protein synthesis in staphylococci

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In staphylococci, a structurally and phylogenetically distinct *glyS* T-box riboswitch controls transcription of the sole glycyl-tRNA synethetase, which in turn aminoacylates five $tRNA^{Gly}$ isoacceptors. A staphylococcal-specific appended stem of the terminator/antiterminator domain, termed stem Sa, distinguishes these T-boxes from their counterparts in bacilli[1,2]. Interestinglly, all five uncharged $tRNA^{Gly}$ isoacceptors modulate transcription of the *glyS* gene and at the same they serve in their aminoacylated form as substartes for both ribosomal protein synthesis and FemXAB-mediated exo-ribosomal synthesis of pentaglycine peptides which stabilize the cell wall[3]. Each tRNA ligand exhibits different affinity as modulator of the T-box-mediated transcription, which depends on both tRNA identity and binding to stem Sa and appears to be highly species-specific. Moroever, the staphylococcal stem Sa is a hotspot for binding of mainstream antibiotics which besides their main effect as protein synthesis inhibitors, can differentially affect *glyS* transcription by interefering with the T-box:tRNA interactions[4]. Extensive structural probing and *in vitro* analysis of domain-swap mutants revealed that species-specific structural differences reflect on the ability of the mutants to induce transcription in the presence or the absence of antibiotics. Moreover, deletion of the staphylococcal-specific stem Sa reduced the *in vitro* transcription ability of the wt T-box independently of the presence of antibiotics, an observation which implies that stem Sa is important for the equilibrium of transcription termination/antitermination conformations when all charged or uncharged $tRNA^{Gly}$ isoacceptors compete for either T-box riboswitch binding or are required as substartes for protein or cell wall synthesis. Taken together these results underline the important regulatory role of tRNAs in essential metabolic pathways in pathogens, beyond tranlsation.

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846 High-throughput analysis of tRNA expression in Arabidopsis thaliana

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Transfer RNAs (tRNAs) are universally conserved molecules linking the nucleotide sequence of a gene with its functional protein product. In higher eukaryotes, tRNAs specific for particular amino acids are often encoded by families of closely related genes. In Arabidopsis thaliana genome, there are over 600 regions annotated as tRNA genes. In order to validate the predictions we applied a combined computational and experimental approach to characterize expression of tRNAs in 2-week-old Arabidopsis seedlings. Analysis of the available tRNA annotations from various sources allowed to reduce the annotations to a set of unique sequences. Based on the analysis of their structural properties (conservation of tRNA-specific features) the annotated regions were divided into two groups: canonical and tRNA-like genes.

Further evaluation of the functionality of the annotated regions involved a special RNA-seq protocol designed for identification of aminoacylated molecules. The sequencing, Northern hybridization and RT-PCR results showed expression of 90% of canonical tRNA genes. Interestingly, the regions for which the expression was not detected by experimental methods originate exclusively from a large, highly repetitive serine / tyrosine tRNA gene cluster on chromosome 1.

Among the annotated genes classified as tRNA-like regions, less than 20% of unique sequences were detected as stable transcripts in sequencing results.

847 Computational analysis of tRNA gene clusters in bacterial evolution

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Function-related genes are positioned close to one another in their genome and form gene clusters. Although tRNA also form gene cluster, the detailed functions of the tRNA gene clusters have not been clarified, and the process of cluster formation remains unknown. It is reported that the bacterial phylum Firmicutes possessed genomic regions containing at least 20 tRNA genes (Tran *et al*., *Genome Biol. Evol*., 2015). In the current research, we adopted more detailed resolution and defined a tRNA gene cluster as a region where two or more tRNA genes are linked on the genome. Even under the definition, we confirmed that Firmicutes was the phylum in which the tRNA gene cluster with 10-20 tRNA genes was most frequently found in bacterial phylogeny. Furthermore, we also found that there were tRNA gene clusters with 6-10 tRNA genes in γ-Proteobacteria and some of Cyanobacteria as well as Actinobacteria. Because these phyla were separately distributed in the bacterial phylogenetic tree, the formation of tRNA gene clusters could be an independent event at the phylum level. Recently, it is argued that the translation-related genes such as tRNA genes and rRNA genes are located near the replication origin with a strong bias, especially in fast-growing bacterial species (Couturier *et al.*, *Mol Microbiol.*, 2006). Therefore, we examined whether the same correlation could be observed for the distance between tRNA gene cluster and replication origin. As a result, we found that tRNA gene cluster were present in the vicinity of the replication origin at least in Firmicutes. Based on these observations, we speculated that both the clustering of tRNA genes and the repositioning of tRNA genes in the vicinity of the replication origin maybe one of the species-specific events during bacterial evolution.

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848 Predicting tRNA gene expression using comparative genomics and DNA sequence features

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Measuring or predicting expression of transfer RNA genes is an important, unmet challenge in functional genomics. Prior studies, primarily in human and mouse cells, suggest that tRNA genes can range from highly expressed to silent. We have estimated gene expression levels of human tRNA genes based on the rich functional genomics data available for over 120 cell types, yet little to nothing is known about individual tRNA gene expression in all other mammalian species. To remedy this, we have developed a random forest classifier to predict which tRNAs are active and which are inactive, using only features in the genome sequences. We recently discovered that active tRNA genes experience mutation rates approximately 10 times greater than the genome-wide average, consistent with transcription-associated mutagenesis. Because tRNA transcription includes leader and trailer sequences that are not under strong selective pressure, these highly transcribed tRNA genes exhibit increased variation in their immediate flanking regions. We demonstrate that this variation, as well as genomic context and flanking features (proximity to protein coding genes, transcription termination sequences, CpG islands, etc.) allows for accurate inference of tRNA gene usage levels. After developing and training our model on all available human data, we predicted tRNA gene activity levels for all tRNA genes across 29 placental mammal species, most of which have no available tRNA gene expression data. To assess our performance, we compared our predictions to published functional genomic data for mouse, and find 91% agreement between predicted and measured gene activity. Additionally, we have grouped these 11,705 tRNA genes into 4,632 synteny-based ortholog sets, 1,097 of which span multiple species. We predict evolutionary transitions between active/inactive states in at least one member of 20% of these multi-species ortholog sets. By comparison, synonymous anticodon changes occured in 6% of ortholog sets, and non-synonymous anticodon mutations occurred in 5% of syntenic gene sets. Overall, our results establish that evolutionary and local genomic context analyses can be highly informative for tRNA gene expression prediction, and illuminate numerous cases of unique tRNA evolution.

849 Meta-analysis of small RNA sequencing data using missRNA reveals induction of 5′-capped tRNA halves in human cell lines.

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Small non-coding RNAs (sRNAs) play central regulatory roles in eukaryotic cells. Besides well-known classes like miRNAs or piRNAs, there is a growing number of reports describing functional sRNAs which are excised from other functional RNAs. The examples include 18 nt-long ribosome-associated RNA processed from ORF of TRM10 mRNA in yeast and tRNA-derived sRNAs observed in multiple organisms. Although most of those RNAs are revealed by cDNA sequencing, currently available computational tools for their identification are inaccurate.

Here we present a novel bioinformatic tool, missRNA (Method for Identification of Small Stable RNAs), which efficiently identifies novel small, stable RNAs from sRNA sequencing data by distinguishing them from unspecific degradation products. In contrast to currently available tools, it analyzes the distribution of both RNA ends simultaneously, resulting in superior accuracy of RNA ends estimation.

By application of our method to meta-analysis of human sRNA sequencing datasets from ENCODE project, we have identified a plethora of novel sRNA candidates. When we compared the repertoires of sRNAs among investigated samples, we have observed significant differences in content of tRNA-derived fragments. In samples derived from human cell lines, the amount of tRNA-derived reads was significantly higher (up to 70% of all reads) than in cells resembling physiological states, like neural cells *in vitro* differentiated from H1-hESC cells (up to 15 % of all reads). The observed difference was caused mostly by induction of 32 and 36 nt-long 5' tRNA halves. After a detailed analysis of cDNA libraries aimed at cloning of sRNAs with different 5' ends (all 5' end types or specific for 5'phosphate or 5' cap), we have identified a set of tRNA halves which are specifically enriched in cell line-derived cDNA libraries specific for 5'-capped RNAs. Moreover, for tRNAs which were source of observed 5' capped tRNA halves, we were able to confirm the presence of cap structure by analysis of matching CAGE-seq data, which revealed cap signatures corresponding to 5' ends of tRNAs of origin. Our observations suggest significant contribution of 5' cap to stability or processing of tRNA halves in human.

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850 Defining the tick-borne encephalitis virus (TBEV) miRNA interactome in human neurons

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Tick-borne encephalitis virus (TBEV) is a flavivirus within the *Flaviviridae* family with potential to infect the central nervous system and cause neuronal injury, meningitis and encephalitis. It is endemic to areas in Europe, Russia and Far-East Asia, which are expanding due to climate change and increased human contact with nature. Interestingly, other *Flaviviridae*, including hepatitis C virus (HCV), depend on specific cellular miRNAs for replication or manipulate miRNA activity. While control of the neurovirulent phenotype of tick-borne flaviviruses has been attempted by introducing artificial brain-specific miRNA sites, natural interactions have not been investigated. We therefore aimed to explore the TBEV-miRNA interactome.

We first characterized TBEV infection in different human neural cell lines including U87 and U251 glioblastoma, and SH-SY5Y neuroblastoma cells, and compared this to non-neural cell lines. We developed an improved method to measure TBEV virus production that we found more reliable than published assays. We observed broad TBEV tropism with rapid growth kinetics, high titres and low cytotoxicity in all cell lines tested.

To better mimic conditions in vivo, we furthermore established an efficient infection system using mature human neurons differentiated from embryonic stem cells. This system recapitulates characteristics of infection observed in other cells and therefore constitutes an ideal model to further study the action of TBEV in neurons.

We noted a highly conserved, predicted single-stranded region of the TBEV 3' UTR containing the seed site for miR-342-3p, a primarily neuronal expressed miRNA. We modulated and measured miR-342-3p activity using miRNA mimics and LNA inhibitors and miR-342-3p specific luciferase reporters. However, manipulating miR-342-3p abundance or mutating its seed site in TBEV did not change virus production in SH-SY5Y neuroblastoma or non-neural cell lines. To instead globally characterize miRNA interactions, cross-linking immunoprecipitation (CLIP) of the Argonaute (AGO) protein of SH-SY5Y-infected cells was performed and revealed other potential miRNAs binding TBEV. Data of AGO-CLIP done with TBEV-infected human neurons are currently being analysed to validate these observations. RNA-Seq transcriptome analysis of the infected neurons are also in progress. Altogether, our results could identify miRNAs affecting TBEV, thereby contributing important knowledge on virus-host interactions at the RNA level.

851 Highly efficacious antiviral protection of plants by small interfering RNAs identified *in vitro*.

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An essential component of the plant's immune system against viral infections is the RNA silencing process. DCL enzymes recognize and process dsRNAs such as viral replication intermediates into siRNAs. The guide strands of these siRNAs are incorporated into AGO/RISC complexes, which degrade ('slice') or translationally inhibit the cognate pathogen RNAs.

In response to a viral infection, the plant's RNA silencing machinery processes viral RNAs into a huge number of siRNAs. However, very few of these siRNAs actually interfere with viral replication. A reliable approach to define the characteristics underlying the activity of these immunologically effective siRNAs (*esiRNAs*) has not been available so far.

We have developed a method that uses a simple, rapid RNA silencing '*in vitro*' assay to identify *esiRNAs* that arise from pathogenic RNAs in the course of a plant immune response. We have also been able to characterize key properties of *esiRNAs* that explain why these RNAs are so active. Tests on the efficacy of such identified *esiRNAs* of a model virus achieved a virtual full protection of plants against a massive subsequent infection in transient applications. The ability to rapidly identify functional *esiRNAs* is expected to be of great benefit for all RNA silencing-based plant and crop protection measures, against viruses, but also against other pathogens.

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852 Withdrawn

853 In-gel SHAPE probing reveals structures of dengue virus UTRs

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Dengue virus is a major global pathogen with a single-stranded plus sense RNA genome. RNAs corresponding to the 5' and 3' untranslated regions (UTRs) of a selected dengue virus type 2 genome were synthesized *in vitro*. These two RNAs exist together as a mixed population of monomers and heterodimers since hybridisation of the 5' and 3' UTRs drives dengue genome circularisation *in vivo*, a process necessary for initiation of viral RNA synthesis. Our in-gel SHAPE (selective 2'OH acylation analysed by primer extension) technique was used to structurally probe individual 5' and 3' UTR conformers as well as heterodimers within this mixed population, in order to characterise structural changes that occur in the UTRs during genome circularisation. UTR secondary structures predicted using this technique showed some similarities but also significant differences to reported or predicted structures in previously published work. In particular, hybridisation between the 5' and 3' ends of the genome in the dimeric structure involved extensive additional regions of RNA, and induced conformational changes in the UTR structures that have not previously been reported. Further investigation of these structural changes is required before dengue RNA protein interactions can be fully characterised – interactions that are potential targets for the future development of antiviral agents.

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854 The number of 5′terminal guanosines modulates HIV-1 RNA destiny.

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After its production in cells, full-length HIV-1 RNA is alternately packaged into viral particles, translated, or spliced. All these processes are regulated by cis-acting signals in HIV-1's 5' leader RNA. The 5′ leader can adopt alternate conformations with distinct secondary structures. Shifting from one conformation to another can further delimit the fate and function of RNA. In our previous work we found that HIV-1 initiates transcription within a cluster of three guanosines, and transcription start site also can influence viral RNA functional partitioning. RNAs initiating with 1G, 2G and 3G are all observed in cells, but only 1G RNAs are encapsidated into virions, whereas 2G and 3G RNAs are enriched on polysomes. Here we studied determinants involved in separating HIV-1 RNA into splicing or encapsidation fates. We hypothesized that the presence of 2 or 3 Gs at the 5′ end of full-length HIV-1 RNA would favor a splicing/translation competent conformation, whereas 1G would favor a packagable fold. To test this hypothesis specific spliced transcript populations were isolated from cells expressing HIV-1 using biotinylated oligonucleotides complementary to specific splice site junctions. We observed that several spliced HIV-1 RNA subspecies are enriched in 2G/3G 5′ ends. Despite the fact that some RNA elements implicated in HIV-1 packaging lie upstream of HIV-1's initial 5′ splice site, and thus are not removed from spliced RNAs, the HIV-1 particle assembly machinery strongly favors encapsidation of only full-length RNA. To study the influence of splicing on RNA packaging we expressed pre-spliced RNAs in cells. We have found that pre-spliced RNA can be efficiently encapsidated into particles in the absence of competition with full-length RNA. We determined the transcription start site of pre-spliced RNA and found that, similar to full-length RNA, all 3 Gs are utilized to initiate pre-spliced RNA transcription. However, only the 1G RNA form was found in virions. This data is consistent with our hypothesis that variable amounts of Gs at the 5′end influence conformational changes of the whole RNA leader and predict HIV-1 RNA fates.

855 Nonreplicative RNA recombination between genomic fragments of positive stranded RNA viruses

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Some positive stranded RNA viruses, such as poliovirus and hepatitis C virus, are important human pathogens whose evolution can be driven by genetic recombination. This can possibly give rise to new viral strains carrying modified phenotypes compared to the parental ones: increased pathogenic power, modified tropism, adaptation to a new host or vector, resistance to drugs. Thus, recombination between viruses allows important evolutionary leaps and is a matter of concern in preventing the future epidemics.

To date, two mechanisms of viral recombination have been described: (i) the copy-choice mechanism that consists in a template switch of the viral RNA dependent RNA polymerase and (ii) a nonreplicative process that has not been fairly characterized so far. Nevertheless, previous studies have pointed out that the latter is not restricted to specific nucleotide sites, thus suggesting the implication of an alternative mechanism rather than the cellular splicing pathway. Moreover, it is independent of the action of viral proteins and the presence of "unconventional" RNA extremities (5'hydroxyl and 3' phosphate) greatly increases the recombination rate. These observations may suggest the implication of cellular factors that would be able to rearrange these RNA molecules.

Based on the cotransfection of *in vitro* transcripts derived from enteroviral genomes, we have developed a model in which nonreplicative recombination events restore the expression of a reporter gene. Using this tool, we have confirmed the existence of a nonreplicative recombination mechanism that is specific to RNA templates. Furthermore, sequencing of these recombination products showed that they were mostly nonhomologous presenting deletions or duplications of parental sequences. Strikingly, we also observed viral sequences that have recombined with cellular RNA. Therefore, these results suggest that this mechanism acts in a random manner and does not discriminate viral from cellular RNA.

Considering these results, nonreplicative recombination seems to represent a still relatively unknown model of genetic plasticity in enteroviruses that may be driven by cellular factors. Given the existence of chimeric recombinants between viral and cellular RNA, one might suggest that this mechanism can also promote cellular RNA recombination.

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856 Movement and accumulation of potato virus Y in infected plants.

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During the last 30 years continuously emerging PVY strains appear to replace the "old" ones as well as "older" recombinant strains. In past Europe, including Poland, was mostly populated by PVY-O. Recently this strain was almost totally replaced by PVY-N:Wi and PVY-NTN. At the beginning of the XXI century the majority of PVY population in Poland was represented by PVY-N:Wi but already in 2008 more than half were classified as PVY-NTN. In Germany the share of both strains is equal. However in many other parts of the world, for example in North America or China, PVY-O is still quite frequent. The biological reasons for replacing old strains with new ones are so far unknown. We observed that the yield of purification of PVY-N and PVY-O from tobacco inoculated with the same concentrations of the virus is usually similar, while for PVY-NTN is at least 10-fold higher. Moreover, our preliminary results indicated that in susceptible cultivar PVY-N:Wi accumulated much faster than PVY-O. The recombinant strain was also capable of infecting and replicating to a high level in moderately resistant cultivar at 21 dpi, while PVY-O was still not detectable after this time by DAS-ELISA. Application of RT-qPCR facilitated PVYO detection at 21 dpi in DAS ELISA negative plant, but its titer was much lower than a titer of PVY-N:Wi, indicating lower replication rate of "old" non-recombinant strain comparing to recombinant one. Basing on these results, we hypothesize that an increased rate of replication of new strains and their more efficient movement in the infected plant is providing them fitness advantage over old strains.

857 Molecular evolutionary analysis of hepatitis C virus non-structural protein NS5A and interferon sensitivity

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Hepatitis C virus (HCV) is a positive-sense single-stranded RNA virus with a genome of 9.5 kb. Hepatitis C is regularly treated with interferon (IFN), however, sensitivity to IFN treatment is dependent on viral load and genotypes, *i.e.* G1, 4, 5, 6 and G2, 3 with low and high sensitivity, respectively. Previous studies have determined Interferon Sensitivity Determining Region (ISDR, 40 aa) and Interferon Ribavirin Resistance Determining Region (IRRDR, 46 aa) in the nonstructural protein NS5A (approximately 450 aa) that is involved in viral replication. For example, comparison between the worldwide infectious strains G1 and G2 indicated G2 is sensitive to IFN compared to G1, caused by accumulation of mutations ISDR of G2. Therefore, the purpose of this study is to understand the evolution of HCVs by mutation accumulation in NS5A, which would lead to change of IFN sensitivity and possibly allow the emergence of new genotypes.

We obtained approximately 7,000 NS5A amino acid sequences for G1-G6 from the HCV sequence database in Los Alamos, of which 157 were extracted as representative of each subtypes. Although the entire NS5A was highly conserved, we identified genotype-specific mutations, in particular, large-scale deletions in the C-terminal half of ISDR and IRRDR in G2, G4 and G1, respectively. However, the IFN tolerant G4 also was found to contain a deletion in ISDR. We also found a G2 specific insertion sequence of 20 aa at the C-terminal side of the NS5A region that has not been considered as an IFN sensitivity-determining region. Then, we divided NS5A into five regions including the regions either for ISDR and IRRDR, and constructed amino acid sequence similarity networks for each region. Although genotypes could be distinguished using any region of NS5A, there was no critical region that discriminated IFN sensitivity. Therefore, we applied the multi-RELIEF algorithm, of which we found that the C-terminal half of ISDR and the N-terminal half of IRRDR, and other additional regions may be possible candidates for IFN sensitivity determination. These results suggest that the IFN sensitivity of HCV is not determined by a single sequence change, but is probably the result of multiple sequence changes.

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858 Transcription apparatus and 5' poly(A) mRNA leaders of the yeast cytoplasmic linear plasmids suggest their close relationship to poxviruses

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Extrachromosomal hereditary elements such as organelles, viruses, and plasmids significantly influence fitness and survival of the cell. The yeast Kluyveromyces lactis contains linear cytoplasmic DNA virus-like elements (plasmids) pGKL1,2 (VLEs) that bear genes encoding putative non-canonical two-subunit RNA polymerase (RNAP). Linear plasmids with almost identical compact genetic organization have been found in the cytoplasm of yeast species from nine genera. We employed pGKL1,2 plasmids from Kluyveromyces lactis as a model to investigate the previously neglected transcription apparatus and transcriptome of the yeast cytoplasmic linear plasmids. We showed that the two putative pGKL1,2 RNAP subunits interact in vivo, and this complex interacts with another two VLE-encoded proteins: an mRNA capping enzyme and a putative helicase. These enzymes also interact with VLE-specific DNA in vivo. We performed 5' and 3' RACE analysis of all the pGKL1,2 mRNAs and found them not 3' polyadenylated and containing frequently uncapped 5' poly(A) leaders that are not complementary to the plasmid DNA. The degree of 5' capping and/or 5' mRNA polyadenylation is specific to each gene and is controlled by the corresponding promoter region that causes plasmid RNAP slippage. We also identified transcription termination sites and found structural elements that precede the termination sites and possibly cause transcription termination. Surprisingly, translation of pGKL1,2 transcripts is independent of eIF4E and Pab1 translation factors, and is also enhanced in lsm1Δ and pab1Δ strains. We suggested a model of a primitive regulation of pGKL1,2 gene expression where the degree of 5' mRNA capping and 5' non-template polyadenylation, together with the presence of negative regulators as PAB1 and Lsm1, play an important role. We found that VLE RNAP and its promoters display high similarity to poxviral RNAP and promoters of early poxviral genes, respectively. These findings together with other our data suggest a close relationship between the yeast cytoplasmic linear plasmids and poxviruses. Part of this work has been published recently in Sýkora M et al., (2018) Transcription apparatus of the yeast virus-like elements: Architecture, function, and evolutionary origin. PLoS Pathog. 2018 Oct 22;14(10):e1007377. doi: 10.1371/journal.ppat.1007377.

859 RNA Export of Unspliced RNA as a Modulator of HIV Replication

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The HIV-1 replication cycle requires the nucleocytoplasmic export of intron-containing viral RNAs, a process that is ordinarily restricted. HIV overcomes this by means of the viral Rev protein, which binds to an RNA secondary structure called the Rev Response Element (RRE) present in all unspliced or incompletely spliced viral RNA transcripts. The resulting mRNP complex is exported through interaction with cellular factors. The Rev/RRE binding interaction is increasingly understood to display remarkable structural plasticity, but little is known about how variation of Rev/RRE sequences affect functional activity.

To study this issue, we utilized subgenomic assays of Rev/RRE function that allow a relatively high throughput of different Rev-RRE pairs. We found that Rev/RRE functional activity varied significantly (up to 24-fold) between naturally occurring viral isolates of different subtypes. The activity differences of the Rev/RRE cognate pairs tracked closely with Rev, but not with RRE activity. This variation in Rev activity was not correlated with differences in Rev steady state protein levels.

In other studies we have shown that functional variations in the RRE, but not Rev, occurred during the evolution of an infection within a patient. Thus modulation of Rev/RRE functional activity may be accomplished in different ways over the course of infection in a single patient compared to longer time scales between hosts.

Together, these data suggest that Rev or RRE sequence differences can drive substantial variation in Rev/RRE functional activity. Such variation may play a role in viral adaptation to different immune milieus within and between patients and may be significant in the establishment of latency, as well as in pathogenesis.

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860 Structural Basis for Translation Termination-Reinitiation at Overlapping Open Reading Frames in Viruses

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Most eukaryotic mRNAs have a m^7G cap, 5' untranslated region, and polyA tail which are recognized by numerous factors during translation initiation. Many viral RNAs that lack some or all of these elements contain structured RNAs that enable translation through noncanonical initiation pathways. Certain RNA viruses including norovirus and influenza B virus contain slightly overlapping open reading frames (ORFs) that are out of frame yet yield separate protein products. A termination upstream ribosome binding site (TURBS) RNA (1) embedded within the coding sequence of the upstream ORF promotes the recapture of a ribosome in a unique termination-reinitiation event. It was previously determined that a 4-6 base pair stem within the TURBS RNA as well as a region complementary to the 18S rRNA are integral elements for their reinitiation function (2, 3). However, the complete secondary and tertiary structure of TURBS RNAs as well as their mechanism for ribosome recapture remain uncharacterized. Alignment of the known TURBS RNA examples revealed that the TURBS sequences conform to two different subtypes, which were separately searched against viral sequence databases using Infernal to reveal 793 unique representatives of this RNA motif from strains of over 30 distinct viruses. Some TURBS representatives comprise a hairpin with the ribosome binding site nucleotides in the terminal loop while other examples comprise a longer stem with the ribosome binding site nucleotides in an internal loop. Chemical probing data indicate that several nucleotides outside of the previously identified stem are protected from modification and therefore might form key tertiary interactions. Conserved nucleotide positions within the ribosome binding site are protected from modification when probed in the presence of 40S subunits. Mutational analyses using chemical probing as well as dual luciferase reporters will be used to assess these predicted structures and ultimately define the intra- and intermolecular interactions necessary for TURBS-dependent reinitiation.

References:

1. Meyers, G. (2003). *J. Biol. Chem. 278*, 34051-34060.
2. Powell, M.L., et al. (2011). *PLoS One 6*, e16822.
3. Luttermann, C., & Meyers, G. (2014). *J. Biol. Chem. 289*, 11739-11754.

861 Exoribonuclease-Resistant RNAs Exist within both Coding and Noncoding Subgenomic RNA

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Many viruses produce protein-coding and noncoding subgenomic RNAs (sgRNAs) that are critical for infection. A recently discovered pathway for viral sgRNA production uses exoribonuclease-resistant RNAs (xrRNAs), discrete folded RNA elements that block the processive exoribonucleolytic degradation of RNA. XrRNAs are widespread in animal-infecting flaviviruses but had been found only in three members of the plant virus genus *Dianthovirus*. Also, xrRNAs had been found only in the 3' untranslated regions (3'UTRs) of viral RNAs, where they produce noncoding sgRNAs. The degree to which xrRNA elements exist in other viruses, the conservation of their ring-like fold, and the ability of xrRNAs to operate in diverse contexts were unknown. Using computational tools and biochemical assays, we discovered xrRNA elements pervading two large families of plant-infecting RNA viruses, the Tombusviridae and Luteoviridae, demonstrating their importance and widespread utility. Comparison of the sequences and functional requirements suggests that all adopt the characteristic ring-like fold. Unexpectedly, many of these newly discovered xrRNAs are located in intergenic regions rather than 3´UTRs, and some are associated with the 5' ends of subgenomic RNAs that encode viral proteins. This suggests that xrRNAs are involved in the production of both coding and noncoding subgenomic RNAs and can operate as part of broader mechanisms to regulate RNA levels and protein expression. These discoveries expand the potential roles for xrRNAs and suggest that xrRNAs may represent a more general strategy for RNA maturation and maintenance than previously known.

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862 Structure of segment 8 vRNA influenza virus type A in cell lysates

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Genome of influenza A virus contains 8 negative-sense single-stranded RNA and along with viral proteins form a vRNPs complex. It is known, that influenza RNA does not bind viral proteins uniformly, therefore some regions remain available for influenza replication inhibitors. The smallest, segment 8 of A/California/04/2009 has 890 nt. It encodes two non-structural proteins - NS1 and NEP/NS2. Protein NS1 is involved in early stages of infection by blocking the expression of interferon. Consequently, mutations or partial deletions in NS1 protein result in virus attenuation. Although, vRNA of segment 8 is highly studied, to date its secondary structure during influenza life cycle remains unknown.

Herein the secondary structure of segment 8 vRNA (vRNA8) is proposed based on chemical mapping in cell lysates. Cell lysates were obtained from IAV infected MDCK cells. After confirmation of presence of influenza proteins and vRNA, the *in vitro* transcribed vRNA8 was folded in cell lysate. Next, the chemical mapping with NAI and DMS reagent (or mock treated for control reaction) was made, followed by reverse transcription using two different fluorescent labeled primers. Chemical mapping reagent reacts with nucleotides within single-stranded or dynamic regions of RNA leading to reverse transcriptase drop-off. The identification of accumulated RT-stops indicate modified regions. Obtained cDNA from control and modification reaction, along with ddNTP ladders were separated by single capillary electrophoresis. The results were analyzed using ShapeFinder program and normalized. The comparison of the chemical mapping profile of vRNA8 obtained in cell lysates to *in vitro* mapping indicated some structural re-arrangements, but there are multiple preserved structural motifs. Some regions exhibit none or only partial accessibility for chemical reagents. Probably these regions could be involved in cellular and viral RNA-RNA or RNA-protein interactions. Presented results lead to new insight into vRNA secondary structure, its importance in influenza virus biology and could be used to design potentially new influenza inhibitors.

863 A model for the activation of antiviral RNA interference in *Drosophila melanogaster*

Loïc Talide[1], Simona Paro[1], Eric Aguiar[1,2], Laurent Troxler[1], Fatima Alghoul[3], Eric Westhof[3], Joao Marques[2], Jean-Luc Imler[1], Carine Meignin[1]

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Viruses represent a ubiquitous threat that every living organism has to deal with. To protect themselves, insects as well as plants largely base their innate antiviral immunity on RNA interference (RNAi). In the case of *Drosophila melanogaster*, Dicer-2 is the only described sensor of viral RNAs. However, very little is known about its *in vivo* mode of action, in particular, how this protein enters on viral templates. Indeed, replicating viral RNAs are often protected from recognition. Here, we address the sensing of Drosophila C Virus (DCV) that presents, like other viruses from the picorna-like family, a protein known as VpG covalently attached to the 5' extremity of its genomic RNA, preventing its recognition by the innate immune system.

One interesting hallmark of RNAi is that the virus-derived small interfering RNAs (vsiRNAs) produced provide a footprint of the action of the immune system. Bioinformatic analyses of the profile of these vsiRNAs in DCV infected flies revealed a specific entry site for Dicer-2. This site consists of a structured region in the 5'UTR of the viral genome, probably involved in initiation of replication. *In vitro*assays revealed that Dicer-2 is not able to cleave this region suggesting that another ribonuclease could be responsible for priming the entry of Dicer-2. Cleavage of the genome RNA template concomitantly with viral replication would generate free dsRNA extremities allowing the subsequent entry of Dicer-2. Analysis of the pattern of vsiRNAs produced in flies containing a point mutation in the evolutionary conserved Duplex RNA-activated ATPase (DRA) domain of Dicer-2 further reveals that ATPase activity is not required for the initial sensing and dicing of viral dsRNA. However, the DRA domain is essential for processive cleavage of DCV replicative form and, importantly, resistance to DCV infection.

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864 Ion exchange membrane chromatography and gel filtration as a tool to investigate plant RNA viruses

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The net charge of viral particles is one of the important factors determining their interaction with host plant proteins. It also defines the binding of viral particles to positively or negatively charged surfaces as ion exchange membranes. Such membranes can capture viral particles and in turn facilitate fast determination of their net charge in plant extract. Gel filtration can be next used for fast separation of complexes of viral particles with interacting host plant proteins from the rest of host plant proteome. To test these concepts, in this work we have investigated the ability of positively (Q) or negatively (S) charged membranes to bind plant RNA viruses using as models potato virus Y (PVY), potato virus M (PVM) and potato leafroll virus (PLRV). These viruses represent three typical groups of plant viruses differing in genome organization, the shape of particles and mechanisms of replication and translation. Particles of all investigated viruses expressed negative charge in studied conditions. However, the strength of their binding to membrane Q was different. Using PVY as a model virus, we have also developed a gel filtration on high pore beads for fast separation of host plant proteins interacting with viral particles. Both, ion exchange membrane chromatography and gel filtration were proved to be useful tools for plant virus investigation, purification as well as a mean of viruses concentration to increase the sensitivity of virus detection.

865 Regulation of RNA virus infection decoded by RNA structurome and interactome studies

Qiangfeng Cliff Zhang[1,2], Shaojun Zhang[1,2], Pan Li[1,2], Yifan Wei[1,2], Miao Mei[2,3], Lei Tang[1,2], Wenze Huang[1,2], Lei Sun[1,2], Xu Tan[3,2], Jianfeng Dai[4]

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Since its outbreak in 2007, Zika virus (ZIKV) has emerged as a global health threat that causes severe neurological conditions including microcephaly in newborns and Guillain-Barre syndrome in adults. ZIKV strains can be classified into the ancestral African lineage and the contemporary epidemic Asian lineage. Studies have identified several key amino acid substitutions that contribute to the infectivity and pathogenicity of the epidemic strains. However, more than 90% of the differences between the genomic RNA sequences of two lineages are synonymous or in the non-coding regions, suggesting regulation of infectivity at the RNA level. Here we report a comparative RNA genome structure analysis of two ZIKV strains, the MR766 strain of the African lineage and the PRVABC59 strain of the Asian lineage. The analysis identified both known and novel functional RNA structure elements. In particular, we confirmed the function of a long-range intramolecular interaction, which is specific for the Asian strains and may account for the enhanced infectivity of these strains. We also identified host proteins interacting with ZIKV genome RNA. Many of the interacting proteins are restriction or dependent factors for ZIKV infection, validated by systematic gene knock-down screening. Together, our analysis reveals the structural and interaction basis for understanding ZIKV RNA regulations and provides a rich resource for discovery of RNA structural elements and RNA binding proteins that are important for ZIKV infection.

Late Poster Abstracts

866 Toward 'RNA-Free' CRISPR-Cas9 Through Chemical Modification

Adrian Pater[1], Sunit K. Jana[2], Eman A. Ageely[1], Christopher L. Barkau[3], Elise Malek-Adamian[2], Masad J. Damha[2], and Keith T. Gagnon[1,3]

[1]Department of Chemistry & Biochemistry, Southern Illinois University; [2]Department of Chemistry, McGill University; [3]Department of Biochemistry & Molecular Biology, School of Medicine, Southern Illinois University

CRISPR (clustered regularly interspaced short palindromic repeat) RNAs and their associated endonucleases are at the forefront of biotechnology and the future of modern gene therapy. Many therapeutic applications will require making CRISPR enzymes more drug-like, especially the labile RNA cofactor that guides substrate specificity of CRISPR-associated (Cas) proteins. Chemical modification of the guide RNA can stabilize and tune Cas enzyme properties. Chemical substitution of all RNA nucleotides would establish principles for building drug-like CRISPR enzymes and provide completely synthetic guides for therapeutic development. We and others have been successful in extensively modifying CRISPR RNAs while retaining enzyme activity. These studies have revealed a need to maintain A-form-like structure and avoid bulky modifications. Importantly, several positions are resistant to RNA nucleotide substitution. To achieve 'RNA-free' CRISPR-Cas9 we are exploring diverse modifications that can retain conformational properties and 2'-hydroxyl-like contacts with Cas9 at these critical positions. These include commercially available and novel nucleotide analogs. CRISPR-Cas9 RNP assembly, catalytic activity, and genome editing in cells will be used to study the effects of chemical modification toward RNA-free CRISPR-Cas9 enzymes. Results will offer key insight into CRISPR-Cas9 engineering and enable novel therapeutic development.

867 Category: Biology and Mechanism of Transcription Leveraging the repetitive part of our genome

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Nearly two decades after the human genome was considered fully 'sequenced', we still have a very poor idea on its composition and functional elements. We know that more than half of the genome is repetitive, but in fact, two-thirds of the genome may be repetitive. Given the accumulating data derived from numerous small-scale and focused studies, the repetitive portion is clearly not 'inert' but at least some portion plays essential roles in defining the human state. Most if not all large-scale efforts have completely shied away from any functional studies, in part due to the complicated nature in identifying and assigning functional roles to any one sequence. My proposed project will attempt to tackle this seemingly intractable problem in unique ways, applying novel CRISPR technologies to functionally re-wire the human 'repeatome' to better understand functional roles in human biology. More specifically, I aim to establish a comprehensive CRISPR screen program to interrogate the repeated fraction of the human genome, based on overrepresented repetitive k-mers. In a case study, my program will allow me to identify repetitive regulators of drug resistance in chronic myelogenous leukemia (CML) cell line K562. I hypothesize that strong transcriptional activation at repeated loci will have the most pronounced impact on the cellular gene expression network. I will be required to establish new computational frameworks to study the impact of repetitive elements examined using a CRISPR perturbation screen. Collectively, this project will shed light into the essentially neglected largest part of the human in light of the cellular state during cancer. This might ultimately lead to the discovery of an entirely new class of potential therapeutic targets in cancer treatment and importantly, usher in new ways to interrogate the repetitive portion from a polygenic perturbation standpoint. Aspects of my proposal recall fundamental concepts from endogenous regulators including microRNAs and transcription factors, which elicit large scale changes through poorly understood complex genetic interactions.

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868 RNAcentral: a hub of information for non-coding RNA sequences

Blake A. Sweeney[1], Boris Burkov[1], Anton I. Petrov[1], Rob Finn[1], Alex Bateman[1], and The RNAcentral Consortium*

[1]European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Genome Campus, Cambridge, UK

RNAcentral (https://rnacentral.org) is a comprehensive database of non-coding RNA (ncRNA) sequences, aggregating >14 million sequences from >30 different databases into a single interface providing a unified text search, sequence search, and FTP archive[1]. In order to increase the amount of high-quality functional ncRNA annotations across genomes, RNAcentral performs a comprehensive sequence to genome mapping for >350 species and annotates all sequences with Rfam, which enables quality controls and the assignment of Gene Ontology (GO) terms to >10 million ncRNAs. We also regularly import GO annotations produced by model organism databases and other groups, including a set of manually curated human disease-associated miRNA annotations[2]. We incorporate miRNA-target interactions from TarBase and LncBase, as well as integrate Ensembl Compara to allow users to identify orthologs and paralogs.

RNAcentral has developed a pipeline to generate template-based secondary structures for >5 million ncRNAs. These structures are displayed in familiar, standardised layouts to facilitate structure analysis and comparison. Once deployed in production, RNAcentral will host the world's largest set of high-quality RNA secondary structures. We have also created a new sequence similarity search which is significantly faster and features a new web interface enabling easy filtering of search results using the same facets that are available for text search, such as organism and RNA type. The new search will be available as a service through an API and as a web component that can be embedded into any website. Together, these changes make RNAcentral an even more useful resource for anyone interested in ncRNA. We encourage databases wishing to join the RNAcentral Consortium to contact us and welcome feedback from the community.

REFERENCES

1. The RNAcentral Consortium. RNAcentral: a hub of information for non-coding RNA sequences. *Nucleic Acids Res.* **47**, D221–D229 (2019).

2. Huntley, R. P. *et al.* Expanding the horizons of microRNA bioinformatics. *RNA* **24**, 1005–1017 (2018).

869 Rfam: the database of 3,000+ non-coding RNA families

Ioanna Kalvari[1], Joanna Argasinska[1], Eric Nawrocki[2], Rob Finn[1], Alex Bateman[1], and Anton I. Petrov[1]

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Rfam (http://rfam.org) is the database of non-coding RNA (ncRNA) families, where each family is represented by a multiple sequence alignment, a consensus secondary structure, and a covariance model[1]. These statistical models are used to annotate nucleotide sequences with ncRNAs using the Infernal software[2]. Since its first release in 2002, Rfam has grown to 3,016 families from 22 RNA types.

To refine the recently adopted genome-centric approach, the Rfam collection of non-redundant, complete genomes was expanded by 60% to include 14,451 species from all domains of life. The latest release, Rfam 14.1, contains 226 new families[3] and introduces support for RNAcentral[4] identifiers in seed alignments that enabled us to create ~200 RNA families found in metagenomic datasets. The Rfam secondary structure diagrams now display pseudoknots using the latest version of R-scape[4] that systematically identifies pseudoknots supported by covariation. The new text search enables exploring families with manual pseudoknot annotations or with pseudoknots identified by R-scape. We plan to manually review the pseudoknot annotations and add pseudoknots to the consensus secondary structures where possible.

To speed up the creation of new families, we are implementing a new cloud-based pipeline allowing pre-approved users to build RNA families using a command line interface. To provide an additional incentive for contributing families to Rfam, we developed an integration with the ORCiD system so an Rfam family can be added to its author's ORCiD profile. The new pipeline and the documentation will be publicly available in late 2019. We invite anyone interested in contributing new families to Rfam to register their interest at https://tinyurl.com/rfam-cloud.

REFERENCES

1. Kalvari, I. et al. NAR. 46, D335–D342 (2018); 2. Nawrocki, E. P. & Eddy, S. R. Bioinformatics 29, 2933–2935 (2013); 3. Weinberg, Z. et al. NAR. 45, 10811–10823 (2017); 4. The RNAcentral Consortium. NAR. 47, D221–D229 (2019); 5. Rivas, E., Clements, J. & Eddy, S. R. Nat. Methods 14, 45–48 (2017).

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870 Cellular hypoxia and Fas pre-mRNA alternative splicing

Arvydas Kanopka[1], Inga Peciuliene[1], Gintare Januleviciute[1], Egle Jakubauskiene[1], Laurynas Vilys[1]

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Cell lines derived from human tumors have been extensively used as experimental models of neoplastic disease. Although such cell lines differ from both normal and cancerous tissue.

A striking change has been observed in alternative splicing pattern of genes and alterations in splicing factor expression under pathologic conditions especially in human cancers. Hypoxic regions have been identified within all solid tumors and their presence has been linked to malignant progression, metastasis, resistance to therapy, and poor clinical outcomes following treatment. Cellular responses to hypoxia are mediated by hypoxia inducible transcription factors (HIFs).

Our results show that Fas alternative splicing is regulated by hypoxia. In this study we show that anti-apoptotic Fas mRNA isoform formation is regulated by cellular microenvironment such as hypoxia. Also we could not find evidence that SPF45 and hnRNPA1 splicing factors are involved in hypoxia dependent Fas alternative splicing regulation, as it is shown in normoxic cells.

871 Endogenous genes exhibit different intron dispersal patterns that demonstrate independent post-transcriptional splicing

Allison Cote[1], *Sareh Bayetpour*[1], *Arjun Raj*[1]

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Splicing and other RNA processing steps are generally thought to occur at the same time as transcription. However, we observe unspliced transcripts away from the site of transcription even for endogenous genes, suggesting post-transcriptional splicing of these endogenous RNA. Localization of multiple introns within the same gene that are unspliced away from the site of transcription suggests that these introns are spliced independently of each other. We see multiple examples of introns likely being spliced not in a 5' to 3' order. For an inducible gene, not only do we see variability in dispersal distance between introns but we also see an increase in dispersal distance upon induction, suggesting an overwhelming of the splicing machinery. We also see that transcripts at the transcription site are more stretched than transcripts floating around the nucleus, suggestive of processing after the process of transcription.

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FLOOR PLANS

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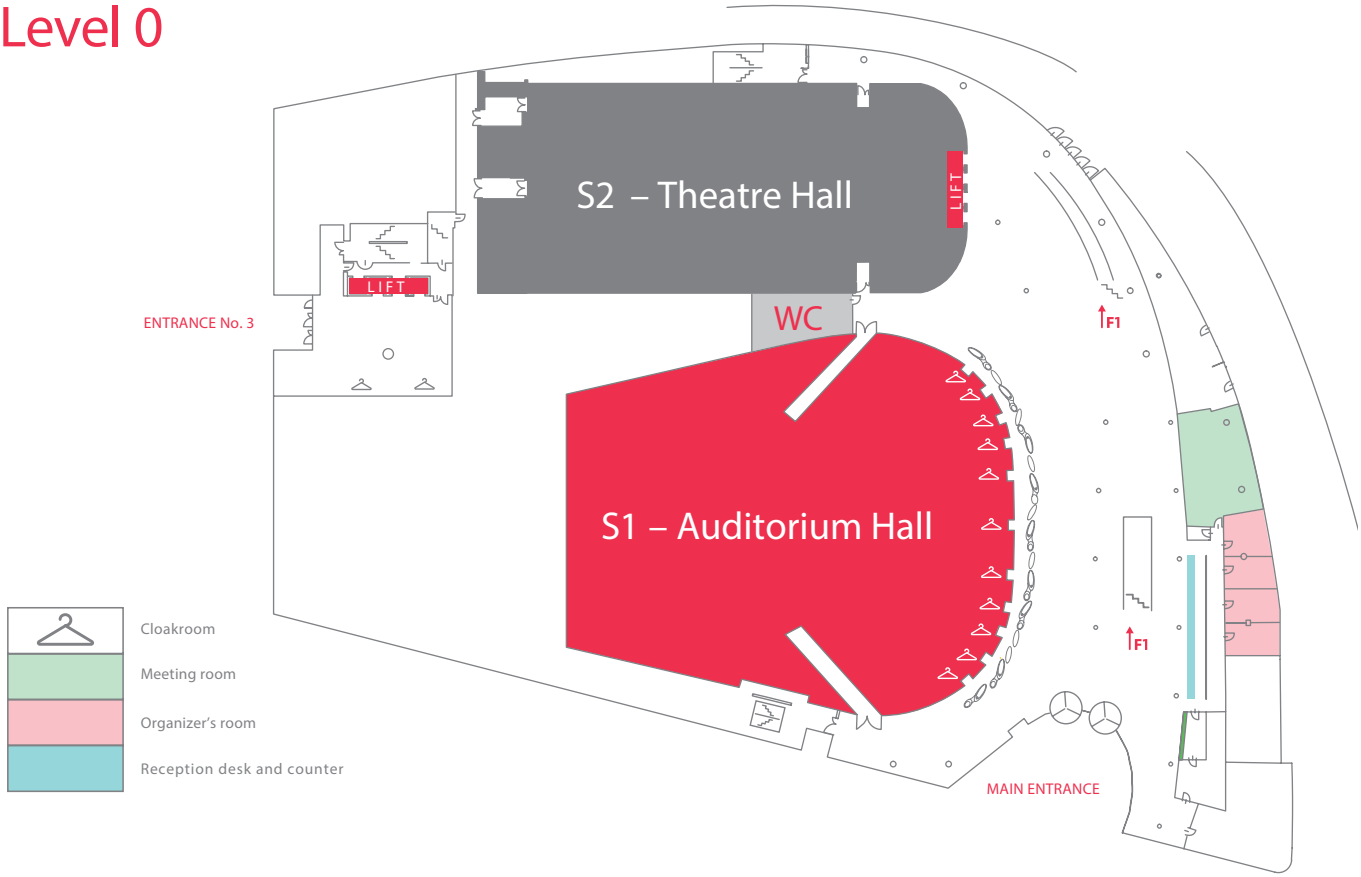
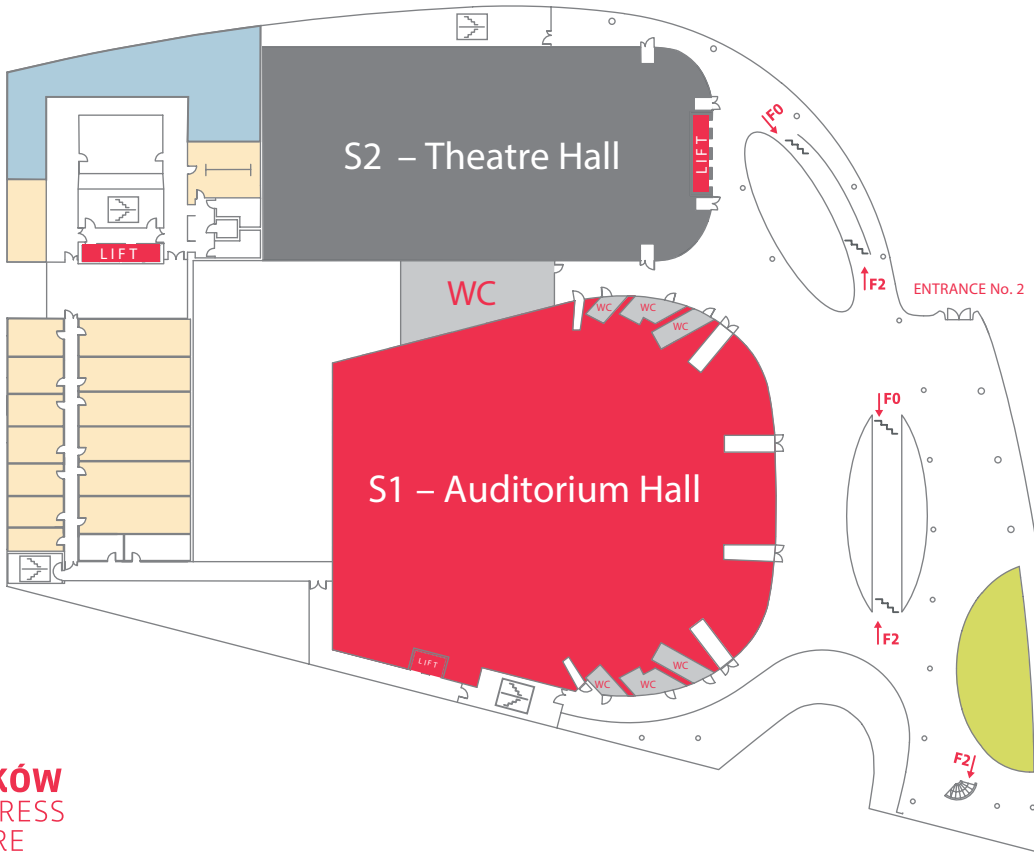


Level 1



ICE KRAKÓW CONGRESS CENTRE

Level 2

ICE KRAKÓW CONGRESS CENTRE

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ABBREVIATED SCHEDULE

TUESDAY, JUNE 11

14:00 – 20:00	Registration	Level 0 Foyer
18:00 – 20:00	Opening Session Keynote 1 (1) [Phillip Sharp]	Auditorium Hall
20:00 – 22:00	Welcome Reception	Levels 1 & 2 Foyers

WEDNESDAY, JUNE 12

07:15 – 08:45	Sponsored Seminar (page 17) [Oxford Nanopore Technologies]	Park Inn Hotel
08:00 – 19:00	Registration	Level 0 Foyer
09:00 – 11:45	Plenary 1: Mechanisms of RNA Splicing (2-11) [Magda Konarska]	Auditorium Hall
10:30 – 11:00	Coffee Break	Levels 1 & 2 Foyers
11:45 – 12:30	Keynote 2 (12) [Tom Cech]	Auditorium Hall
12:30 – 14:00	Lunch	Levels 1 & 2 Foyers
14:00 – 16:15	Concurrent 1: Regulation of mRNA Splicing (13-22) [Woan-Yuh Tarn]	Auditorium Hall
	Concurrent 2: RNA Localization & Transport (23-32) [Jeffrey Chao]	Theatre Hall
16:15 – 16:45	Coffee Break	Levels 1 & 2 Foyers
16:45 – 19:00	Plenary 2: Regulatory RNAs and RNPs (33-41) [Yukihide Tomari]	Auditorium Hall
19:00 – 20:00	Junior Scientists Social	Theatre Hall
19:00 – 20:30	Dinner	Levels 1 & 2 Foyers
19:00 – 20:30	Meetings Committee Dinner/Meeting	Level 0 Meeting Room
20:30 – 23:00	Poster Session 1 (even numbers)	Conference Hall, S4 Level 3 and Level 0 Foyer

THURSDAY, JUNE 13

07:45 – 08:45	Sponsored Seminar (page 19) [Lexogen]	Park Inn Hotel
08:00 – 13:30	Registration	Level 0 Foyer
09:00 – 11:45	Concurrent 3: Synthesis and Processing of RNA (42-51) [Karla Neugebauer]	Auditorium Hall
	Concurrent 4: RNA in Disease and Therapy (52-61) [Matthew Disney]	Theatre Hall
	Workshop 1: The tRNA World beyond Translation (62-72) Organizers: [Sebastian Glatt and Sebastian Leidel]	Chamber Hall - S3, Level 3
10:30 – 11:00	Coffee Break	Levels 1 & 2 Foyers
11:45 – 13:15	Panel Discussion: RNA Research and RNA Therapeutics: Past Insights and Future Prospects Panelists: [Tom Cech, Matthew Disney, Gideon Dreyfuss, Anastasia Khvorova, Adrian Krainer, Anna Marie Pyle, and Joan Steitz]	Auditorium Hall
13:15 – 13:30	Lunch to Go – free afternoon and evening	Levels 1 & 2 Foyers

FRIDAY, JUNE 14

07:40 – 08:40	Sponsored Seminar (page 21) [Eclipse BioInnovations]	Park Inn Hotel
08:00 – 19:00	Registration	Level 0 Foyer
09:00 – 11:45	Plenary 3: Mechanisms of Translation (73-81) [Nahum Sonenberg]	Auditorium Hall
10:30 – 11:00	Coffee Break	Levels 1 & 2 Foyers
11:45 – 12:30	Keynote 3 (82) [Maria Carmo-Fonseca]	Auditorium Hall
12:30 – 14:00	Mentoring Lunch [Nancy Greenbaum]	Park Inn Hotel
12:30 – 14:00	Lunch	Levels 1 & 2 Foyers
14:00 – 16:15	Plenary 4: RNA Modification and Editing (83-92) [Chuan He]	Auditorium Hall
16:15 – 16:45	Coffee Break	Levels 1 & 2 Foyers
16:45 – 19:00	Concurrent 5: Non-coding RNAs: Long & Short, Linear & Circular (93-102) [Julia Salzman]	Auditorium Hall
	Concurrent 6: Translation Regulation (103-112) [Andrea Berman]	Theatre Hall
	Workshop 2: Computational Modeling of RNA Structure and Complexes (113-122) Organizers: [Janusz Bujnicki, Francois Major, and Eric Westhof]	Chamber Hall - S3, Level 3
19:00 – 20:30	Dinner	Levels 1 & 2 Foyers
19:00 – 20:30	Board of Directors Dinner/Meeting	Level 0 Meeting Room
20:30 – 23:00	Poster Session 2 (odd numbers)	Conference Hall, S4 Level 3 and Level 0 Foyer

SATURDAY, JUNE 15

08:00 – 19:00	Registration	Level 0 Foyer
09:00 – 11:00	Concurrent 7: Interconnected RNA Processes (123-131) [Andrzej Dziembowski]	Auditorium Hall
	Concurrent 8: RNA Catalysis, Folding and Structure (132-140) [Rick Russell]	Theatre Hall
11:00 – 11:30	Coffee Break	Levels 1 & 2 Foyers
11:30 – 13:00	Workshop 3: Membrane-less Granules and Liquid-Liquid Phase Separation (141-148) [Dominique Weil]	Auditorium Hall
	Junior Scientists Workshop: Publishing and the Editorial Process (page 23) [Eleonora de Klerk]	Theatre Hall
	Workshop 4: Computational Methods for RNA Data Analysis (149-153) Organizers: [Eugene Yeo and Yoseph Barash]	Chamber Hall – S3, Level 3
13:00 – 14:30	Lunch	Levels 1 & 2 Foyers
14:30 – 16:45	Concurrent 9: RNA Turnover (154-163) [Oliver Mühlemann]	Auditorium Hall
	Concurrent 10: New Research Trends and Technologies (164-173) [Jernej Ule]	Theatre Hall
16:45 – 17:15	Coffee Break	Levels 1 & 2 Foyers
17:15 – 18:45	Awards Ceremony	Auditorium Hall
18:45 – 19:30	Transfer to Stara Zajezdnia on own	
19:30 – 24:00	Reception/Entertainment/Dinner/Dance	Stara Zajezdnia

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Conference concludes